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Regular Abstracts

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Opening General Session

Innate Immunity : From Flies to Humans. Jules A. Hoffmann. IBMC, University of Strasbourg, Strasbourg, France.

Insects make up some 80% of all extant species on earth and present a formidable challenge : they put one third of humanity at risk of severe diseases, through their role as vectors of pathogens. They destroy one third of human crops, adding severe strains to humans and livestock. Insects have long been known to be strongly resistant to infections. The mechanisms underlying this resistance, other than the well known process of phagocytosis, have only been addressed relatively recently. A general picture of these defences has now evolved and Drosophila is to be credited for this progress. Remarkably, the unravelling of the Drosophila antimicrobial defences has had a significant impact on understanding essential facets of mammalian immunity. It has also led to a renewed interest in innate immunity, a long neglected field in the study of antimicrobial defences in general.The presentation will review the major developments in the study of host defences in flies over the last decades. A particular emphasis will be put on the identification of effector polypeptides with various antimicrobial activity spectra, on the control of expression of the corresponding genes, on the recognition mechanisms of infecting agents and the activation of intracellular signalling cascades by these receptors. This progress will be put in parallel to that of studies performed in various laboratories on mammalian immune defences. In particular, the contribution of the Drosophila model to our present understanding of innate immunity, from sea anemones to humans, will be highlighted. Further reading : Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. Cell. 1996; 86 :973. Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA. Science. 1999; 284: 1313. Hoffmann JA. Nature. 2003; 426: 33. Hultmark D. Curr Opin Immunol. 2003; 15: 12. Ferrandon D, Imler JL, Hetru C, Hoffmann JA. Nat Rev Immunol. 2007; 7: 862. Lemaitre B, Hoffmann J. Annu Rev Immunol. 2007; 25: 697. Kemp C et al J. Immunol. 2012 in press Ganesan S, Aggarwal K, Paquette N, Silverman N. Curr Top Microbiol Immunol. 2011; 349: 25. Kawai T, Akira S. Immunity. 2011; 34: 637. Royet J, Gupta D, Dziarski R. Nat Rev Immunol. 2011; 11: 837.

Plenary Session

Molecular Mechanisms of Axon Degeneration. Marc R. Freeman. Dept Neurobiology, Univ Massachusetts Med Sch/HHMI, Worcester, MA.

Widespread axonal and synaptic degeneration is a hallmark of peripheral neuropathy, brain injury, and neurodegenerative disease. Axon degeneration has been proposed to be mediated by an active auto-destruction program, akin to apoptotic cell death, however loss of function mutations capable of potently blocking axon self-destruction remain poorly defined. We are using simple axotomy models in combination with forward genetic screening approaches in Drosophila to explore the molecular and cellular basis of axon degeneration. We recently discovered the Drosophila Toll receptor adaptor dSarm (sterile α/Armadillo/Toll-Interleukin receptor homology domain protein) promotes axon destruction, and that loss of dSarm function can cell-autonomously suppress the degeneration of severed axons for the lifespan of the fly. Notably, dSarm is dispensable for developmental neurite pruning and caspase-dependent cell death in Drosophila, indicating these events are mediated by distinct genetic programs. We have further shown that pro-degenerative Sarm1 function is conserved in mice, where transected Sarm1 null axons exhibit remarkable long-term survival both in vivo and in vitro. Our results provide direct evidence that axons actively promote their own destruction after injury and identifies dSarm/Sarm1 as a founding member of an ancient axon death signaling pathway.

Plenary Session

Genetic Approaches to Dissecting Neural Computation in the Visual System. Tom Clandinin. Dept of Neurobiology, Stanford University, Stanford, CA.

Our understanding of the complex neural circuits that underlie most visual behaviors is extremely limited. Forward genetic approaches have contributed considerably to our understanding of many aspects of biology; our goal is to adapt analogous methods to the functional dissection of visual circuitry. These methods, then, provide an entry point to examining the computational roles of specific neurons. We developed a convertible enhancer trap, the InSite system, which allows rapid replacement of genetic effectors, enabling expression patterns to be refined through intersectional approaches, and facilitating independent manipulation of gene expression in multiple cell types simultaneously. We have also developed both high-throughput, as well as single fly paradigms to examine behavioral responses to distinct visual cues, and used these in forward genetic screens to identify cell types that play critical roles in visual responses to motion and polarized light. By then imaging calcium responses in these cells, and by measuring their electrophysiological responses, it becomes possible to relate particular visual stimuli to specific neural responses, to computation and behavior. These studies have revealed deep similarities in circuit architecture and computational strategy between flies and vertebrates.

Plenary Session

The Genomics of Speciation and Pattern Evolution in (butter)flies. Chris Jiggins. Dept Zoology, University of Cambridge, Cambridge, United Kingdom.

Heliconius butterflies are a rapidly radiating neotropical genus widely used in studies of ecology, behaviour, mimicry and speciation. Closely related species typically differ in several aspects of their ecology and behaviour, and in particular their mimetic wing patterns. We sequenced the genome of Heliconius melpomene and compared it with other taxa to investigate chromosomal evolution in Lepidoptera and gene flow among multiple Heliconius species and races. Using genomic resequencing, we show hybrid exchange of genes between three co-mimics, Heliconius melpomene, Heliconius timareta and

Heliconius elevatus, especially at two genomic regions that control mimicry pattern. We infer that closely related Heliconius species exchange protective colour-pattern genes promiscuously, implying that hybridization has an important role in adaptive radiation. Furthermore, we investigate genome-wide patterns of introgression between hybridising species, H. melpomene and H. cydno by comparing genetic differentiation in sympatry and allopatry, and applying various different tests for introgression. We find a strong signal of introgression throughout the genome, and estimate that at least 25% of the genome has been shared between the Panamanian sub-populations of the two species. Furthermore, we detect patterns divergence and linkage disequalibrium that are consistent with recent or ongoing gene flow in sympatry. Introgression appears to be significantly reduced on the Z chromosome, which is consistent with observed female hybrid sterility between these populations. We also observe numerous narrow islands of divergence, which include wing patterning loci known to be under divergent selection. Overall these results show that these species have diverged and persisted despite pervasive genome sharing.

Plenary Session

Creating Gradients by Morphogen Shuttling. Naama Barkai. Weizman Institute, Rehovot, Israel.

Morphogen gradients are used to pattern a field of cells according to concentration profile of a signaling molecule. I will discuss mechanisms that buffers the shape of those gradients against variations in biochemical parameters and in the size of the patterned tissue. In particular, I will discuss the shuttling mechanism, which functions when the morphogen is produced in a broad domain. I will describe theoretical properties of this mechanisms, and present experimental evidence supporting its function in several early developmental processes.

Plenary Session

Maintenance of Niche Function and Tissue Homeostasis During Aging. Leanne Jones[1], Hila Toledano[1], Cecilia D'Alterio[1], Michael Rera[2], Christopher Koehler[1], Benjamin Czech[3], Erel Levine[4], David Walker[2]. 1) Laboratory of Genetics, Salk Inst, La Jolla, CA; 2) Department of Integrative Biology and Physiology, University of California- Los Angeles, Los Angeles, CA; 3) Watson School of Biological Sciences, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 4) Department of Physics and FAS Center for Systems Biology, Harvard University, Cambridge, MA.

Adult stem cells support tissue homeostasis and repair throughout the life of an individual. Numerous changes occur with age that result in altered stem cell behavior and reduced tissue maintenance and regeneration. Changes can be cell autonomous including changes in cell cycle progression, increased DNA damage, and epigenetic alterations. In addition, poorly understood changes to the local and systemic environments occur that result in decreased stem cell activity or alterations in commitment or differentiation potential. We have developed *Drosophila melanogaster* as a model to uncover conserved mechanisms regulating stem cell aging and explore how cellular and tissue aging impact longevity. We will compare and contrast age-related changes to germline and intestinal stem cells and present strategies to counter age-related changes in both tissues. Understanding the mechanistic basis for intrinsic and extrinsic age-related changes will facilitate stem cell based therapies to treat age-onset and degenerative diseases in older individuals.

Plenary Session

Histone Genetics in Drosophila. Jürg Müller[1*], Ana Raquel Penelly[1], Omer Copur[1], Katja Finkl[1], Herbert Jäckle[2], Alf Herzig[2]. 1) Max-Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Bavaria, Germany; 2) Max-Planck Institute for Biophysical Chemistry, Molecular Developmental Biology, Am Fassberg 11 37077 Göttingen, Germany.

We are interested in understanding the molecular mechanisms that permit cell-specific transcription and repression of developmental regulator genes to be established and maintained. To this end, we have been studying the Polycomb/trithorax system. Polycomb group (PcG) proteins control a variety of cell fate decisions in animals and plants by repressing developmental regulator genes in cells where they should not be expressed. In Drosophila, biochemical studies established that PcG proteins exist in four principal protein complexes: PRC1, PRC2, PhoRC and PR-DUB. All four complexes are bound at PcG target genes in vivo and are thought to repress their transcription by modifying chromatin. The tri-methylation of histone H3 at lysine 27 (H3-K27me3) by PRC2 and the monoubiquitylation of histone H2A (H2A-ub1) at K118 in Drosophila and K119 in mammals by PRC1-type complexes are thought to be central to the repression mechanism of the Polycomb system. We have been investigating the function of post-translational modifications on histones by performing histone genetics in Drosophila. To this end, we generated Drosophila strains in which the endogenous histone genes can be conditionally removed and replaced with transgenes encoding histone proteins with point mutations. We found that cells containing H3-K27R instead of H3-K27 fail to repress PRC2 target genes and reproduce the PRC2 mutant phenotype. This demonstrates that H3-K27 is the crucial physiological substrate that PRC2 needs to modify for Polycomb repression. However, the analysis of H2A mutants that can no longer be ubiquitylated and of other histone point mutants resulted in several unexpected findings. Progress on these studies will be presented.

Plenary Session

Information, Enhancers, and Cell Signaling: a View from the Binding Site. Scott Barolo. University of Michigan Medical School, Ann Arbor, MI.

Cell-cell signaling pathways such as Hedgehog, RTK/MAPK, Notch, BMP, and Wnt relay extracellular patterning information to transcription factors (TFs), which determine developmental cell fates by controlling gene expression. Signal-regulated TFs

bind to specific DNA sequences within enhancers of pathway target genes. Enhancers then integrate these signaling inputs with other types of information to precisely control gene expression in response to signaling.

Our lab tries to understand the cell's responses to these signals by dissecting and decoding the cis-regulatory DNA of signal-regulated enhancers. Because these pathways are extremely pleiotropic — that is, they are active in many different cell and tissue types during development and adult life — we are particularly interested in how *cis*-regulatory DNA sequences are able to interpret these "generic" signals in a context-specific manner. Simple combinatorial logic is part of the answer to this question, but upon close examination, it's clear that this is far from the whole story. In this talk, several questions will be addressed (but probably not answered):

1. Where is the complexity in animal genomes? (Hint: it's not in the number of genes or transcripts.)
2. What is the information content of a single TF binding site? Of a pair of sites? Of an enhancer?
3. Do different types of TFs carry different amounts, or distinct types, of patterning information?
4. Do enhancers really "integrate" patterning information from multiple TFs, and if so, how does this computation physically occur?

Plenary Session

"The piRNA Pathway: a Small RNA-Based Innate Immune System". Greg Hannon. HHMI, Cold Spring Harbor Lab, One Bungtown Road, Cold Spring Harbor, NY.

PIWI-family proteins and their associated small RNAs (piRNAs) act in an evolutionarily conserved innate immune mechanism that provides an essential protection for germ cell genomes against the activity of mobile genetic elements. piRNA populations comprise a molecular definition of transposons that permits them to be distinguished from host genes and selectively silenced. piRNAs can be generated in two distinct ways. Primary piRNAs emanate from discrete genomic loci, termed piRNA clusters, and appear to be derived from long, single-stranded precursors. The biogenesis of primary piRNAs involves at least two nucleolytic steps. An unknown enzyme cleaves piRNA cluster transcripts to generate monophosphorylated piRNA 5' ends. piRNA 3' ends are likely formed by exonucleolytic trimming, after a piRNA precursor is loaded into its PIWI partner. Secondary piRNAs arise during the adaptive ping-pong cycle, with their 5' termini being formed by the activity of PIWIs themselves. At least in Drosophila, piRNAs are maternally deposited and transmit an epigenetic signal essential for the effective control of at least some transposable elements. I will describe our recent efforts, which bring to bear biochemical, structural, and genetic strategies in an effort to understand how piRNAs are formed and the mechanisms by which they recognize and silence their targets.

Plenary Session

Transcription Factor Network Dynamics in Development. Ilaria Rebay, Jean Francois Boisclair Lachance, Matthew Hope, Aaron Mitchell-Dick, James Porter, Jemma Webber. Ben May Dept, Univ Chicago, Chicago, IL.

Precise spatial and temporal control of gene expression is fundamental to all biological processes. For example, even minor imbalances in gene regulatory networks can compromise the robustness and accuracy of developmental programs. A broad goal of my lab's research is to understand the dynamical properties of transcription factor networks that enable them to integrate instructions from multiple signaling pathways and to direct appropriate cell fate transitions during development. One of the projects currently underway seeks to understand the function and regulation of the Yan network, a transcriptional effector circuit that operates downstream of receptor tyrosine kinase signaling. At the core of the network are two antagonistic ETS-domain transcription factors, the repressor Yan and the activator Pointed. A regulatory web of feedback loops and interactions confers switch-like behavior to the system. Thus in response to pathway activation, attenuation of Yan-mediated repression and stimulation of Pointed-mediated transcription of common target genes triggers differentiation programs previously blocked by Yan. We are using an integrative combination of molecular genetic, genomic, biochemical and computational analysis of Yan network components and behaviors to explore how the interplay between cis-regulatory architecture, protein-protein interactions, and three-dimensional chromatin organization ensures accurate and robust output from this system. Our latest findings and models will be presented.

Plenary Session

The Role of Nuclear Pore Proteins in Developmental Gene Regulation. Martin W. Hetzer. Salk Inst for Biological Studies, La Jolla, CA.

Faithful execution of developmental gene expression programs occurs at multiple levels and involve many different components such as transcription factors, histone-modification enzymes and mRNA processing proteins. Recent findings from our laboratory suggest that nucleoporins, well known components that control nucleo-cytoplasmic trafficking, have wide-ranging functions in developmental gene regulation that potentially extend beyond their role in nuclear transport. Analysis of chromatin-binding behavior of Drosophila Nups, achieved by different methods such as immunostaining of polytene chromosomes and ChIP, revealed the presence of several NPC components at active genes and a functional requirement for their presence in transcription of their binding targets. Reducing levels of Nup98 or and a member of the Nup107/160 complex by RNA interference (RNAi) resulted in decreased levels of transcriptional activity and mRNA levels of its target genes, which included the developmentally induced ecdysone-responsive genes. Surprisingly, the NUP-chromatin contacts

were commonly found to occur in the nucleoplasm, away from the NE-embedded NPCs. Whether the unexpected role of nuclear pore proteins in transcription regulation, which initially has been described in yeast, also applies to human cells remained unknown. Recent data from our group suggest that at a genome-wide level Nup98 associates with developmentally regulated genes active during human embryonic stem cell differentiation. Overexpression of a dominant negative fragment of Nup98 levels decreases expression levels of Nup98-bound genes. In addition, we identify two modes of developmental gene regulation by Nup98 that are differentiated by the spatial localization of Nup98 target genes. Genes in the initial stage of developmental induction can associate with Nup98 that is embedded in the nuclear pores at the nuclear periphery. Alternatively, genes that are highly induced can interact with Nup98 in the nuclear interior, away from the nuclear pores. This work demonstrates that Nup98 dynamically associates with the human genome during differentiation, revealing a role of a nuclear pore protein in regulating developmental gene expression programs.

Plenary Session

Stem Cells to Synapses: Regulation of Self-Renewal and Differentiation in the Nervous System. Andrea H. Brand, Tony D. Southall, Pauline Speder, Jun Liu, Catherine M. Davidson. The Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.

Discovering how stem cells are maintained in a multipotent state and how their progeny differentiate into distinct cellular fates is a key step in the therapeutic use of stem cells to repair tissues after damage or disease. We are investigating the genetic networks that regulate neural stem cells in Drosophila. Stem cells can divide symmetrically to expand the stem cell pool or asymmetrically to self-renew and generate a daughter cell destined for differentiation. By comparing the transcriptional profiles of symmetrically and asymmetrically dividing stem cells we are identifying key regulators of the switch from symmetric to asymmetric division. The balance between symmetric and asymmetric division is critical for the generation and repair of tissues, as unregulated stem cell division can result in tumours. For example the loss of cell fate determinants, such as the homeodomain transcription factor Prospero, causes differentiating daughter cells to revert to a stem cell-like fate: they express markers of self-renewal, continue to proliferate, fail to differentiate and generate tumours. By identifying Prospero's targets throughout the genome we showed that Prospero represses genes for self-renewal and activates differentiation genes. We are characterising co-factors that act with Prospero to promote differentiation and suppress tumour formation. The systemic regulation of stem cells ensures that they meet the needs of the organism during growth and in response to injury. A key point of regulation is the decision between quiescence and proliferation. During development, neuroblasts transit through a period of quiescence separating distinct embryonic and post-embryonic phases of proliferation. We discovered that insulin signalling from a surrounding glial niche is necessary for post-embryonic neuroblasts to exit quiescence and reinitiate cell proliferation. We are investigating the systemic and local signals that regulate stem cell growth and proliferation.

Plenary Session

Neurodegeneration and Aging: Insight from Drosophila. Nancy M. Bonini. Dept Biol, 306 Leidy Labs, Univ Pennsylvania/HHMI, Philadelphia, PA.

Human neurodegenerative diseases, like Huntington's disease and the spinocerebellar ataxias, are late-onset progressive neurodegenerative disorders for which few cures or treatments are available. To develop new approaches, the Bonini laboratory has been using the fly to provide insight that we then extend to the human disease. We use the human disease gene to recreate the disease toxicity in the fly, and then take advantage of powerful molecular genetic approaches in the fly to define pathways and mechanisms. These studies have revealed multiple pathways involved in neurodegeneration, including toxicities due to RNA pathways. These processes include toxic activities of the mRNA encoding the disease proteins for repeat expansion diseases, RNA binding proteins and their altered activities, and modulation by miRNAs, including novel aspects of miRNA regulation such as 3'end trimming by the exonuclease Nibbler. A key miRNA we identified that links age-associated processes with long-term brain integrity in Drosophila is miR-34. We have extended our studies from longterm integrity of the brain to acute neural injury, with the development of a novel adult-stage injury model of the fly wing. Taken together, our approaches and findings highlight the conservation of pathways with humans, and ways to use Drosophila in order to define critical new pathways that impact integrity of the nervous system.

1

A new frontier for the Duplication Consortium: retrofitted BACs that span very large Drosophila genes and the 4th chromosome. Koen Venken[1,2*], Stacy Holtzman[3], Soo Park[4], Joe Carlson[4], Roger Hoskins[4], Hugo Bellen[1], Thom Kaufman[3]. 1) Molecular and Human Genetics, BCM, Houston, TX; 2) Biochemistry and Molecular Biology, BCM, Houston, TX; 3) Biology, IA, Bloomington, IN; 4) Life Sciences Division, LBNL, Berkeley, CA.

We generated stocks carrying molecularly defined duplications of several very large Drosophila genes using a transgenesis system based on retrofitting non-P[acman] genomic bacterial artificial chromosome (BAC) clones. Regions of the X chromosome that were previously not covered by the Duplication Consortium project were spanned by large duplications. 57 BAC clones from mapped libraries were identified and "retrofitted" to generate clones with P[acman] transgenesis functions (i.e. plasmid copy-number induction and site-specific transgenesis). Retrofitting was very efficient, and we successfully modified all 57 BACs. The modified BACs were integrated into a docking site at 65B2 using the phiC31 integrase. Transgenic flies containing duplications of between 44 kb and 212 kb were generated, exceeding the previous record of 146 kb. Hence, together with theP[acman] libraries and gap-repair procedures, BAC retrofitting allows the investigation of almost all fly genes

and chromosomal regions. Moreover, we generated a duplication kit for the 4th chromosome using this strategy. In total, 20 duplications (14 BAC and 6 CH321 clones) were generated, resulting in an overlapping tiling path that covers essentially the entire sequenced portion of the 4th. The duplications are currently tested for genetic complementation of available mutations. The new duplication lines will greatly facilitate mapping and rescue of 4th chromosome genes, and allow the manipulation of genes located on this peculiar and under-studied chromosome. All the stocks will be made available through the Bloomington Drosophila Stock Center. Information related to the stocks is available at http://flystocks.bio.indiana.edu/Browse/dp/DC-Dps.php (X chromosome duplication kit) and http://flystocks.bio.indiana.edu/Browse/dp/DC-Dps-4.php (4th chromosome duplication kit).

2

Captured segment exchange: A strategy for custom engineering large genomic regions in *Drosophila melanogaster*. Jack R. Bateman, Michael F. Palopoli, Sarah T. Dale, Jennifer E. Stauffer, Anita L. Shah, Justine E. Johnson, Conor W. Walsh, Hanna Flaten, Christine M. Parsons. Biology Department, Bowdoin College, Brunswick, ME.

Thousands of transgenic insertions carrying site-specific recombinase (SSR) recognition sites have been distributed throughout the *Drosophila* genome by several large-scale projects. Here we describe a method aimed at using these insertions to make custom alterations to *Drosophila* genomic sequences *in vivo*. Specifically, by employing recombineering techniques and a dual RMCE strategy based on the phiC31 integrase and FLP recombinase, we show that a large genomic segment that lies between two SSR recognition site insertions can be "captured" as a target cassette and exchanged for a sequence that was engineered in bacterial cells. We demonstrate this approach by targeting a 50 kb segment spanning the *tsh* gene, replacing the existing segment with corresponding recombineered sequences through simple and efficient manipulations. Given the high density of SSR recognition site insertions in *Drosophila*, our method affords a straightforward and highly efficient approach to explore gene function *in situ* for a substantial portion of the *Drosophila* genome.

3

Gene Targeting with TALENs in Drosophila. Dana Carroll[1], Kelly J. Beumer[1], Jonathan K. Trautman[1], Michelle Christian[2], Timothy J. Dahlem[3], Cathleen Lake[4], R. Scott Hawley[4], David J. Grunwald[3], Daniel F. Voytas[2]. 1) Dept Biochem, Univ Utah Sch Med, Salt Lake City, UT; 2) Dept Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN; 3) Dept Human Genetics, Univ Utah Sch Med, Salt Lake City, UT; 4) Stowers Institute, Kansas City, MO.

Gene targeting in Drosophila is getting easier and more reliable, thanks to a new class of targetable cleavage reagents. TALENs employ DNA binding domains from transcription activator-like effectors (TALEs), linked to a nonspecific cleavage domain, to make double-strand breaks (DSBs) at specific sites in chromosomal DNA. Through the action of cellular repair pathways, these targeted breaks lead to localized mutagenesis via nonhomologous end joining and to gene replacement via homologous recombination. Each TALE repeat binds a single base pair in the DNA target, thereby simplifying design in comparison to ZFNs, and there seem to be fewer context effects than with zinc fingers. Using standard embryo injection procedures to introduce the corresponding mRNAs, we have tested the function of TALENs in Drosophila. In direct comparisons, we found TALENs to be frequently (but not always) more effective than our previously described ZFNs. We have successfully knocked out several genes in which null mutations were not previously available. In addition, we have used oligonucleotide donor DNAs, in conjunction with TALEN cleavage, to introduce specific sequence changes at the target locus. TALENs promise to facilitate the manipulation of natural genomic loci in Drosophila and other organisms to a much greater degree than previous targeting reagents.

4

Pyrimidine salvaging enzyme UPRTase is active in *Drosophila* and limits the specificity of tissue specific RNA isolation by 4TU tagging. Arpan Ghosh, MaryJane Shimmel, Emma Leof, Michael O'Connor. Gen Cell & Development, Univ Minnesota Twin Cities, Minneapolis, MN.

Spatial-temporal regulation of gene expression is central to the existence of multicellular organisms. However, studying cell/tissue specific gene expression is often limited by the ability to isolate homogeneous populations of specfic cell types. Recently *T. gondii* Uracil-phophorybosyltransferase (Tg-UPRT) mediated 4-thiouracil (4TU) tagging has been described as an efficient method for tissue/cell-type specific RNA isolation (Miller, MR et. al. Nature methods 2009). Specificity and efficiency of this technique is based on the present understanding that all UPRT homologues from higher eukaryotes are inactive. Here we show that *Drosophila* UPRT homologue CG5537 (*krishah*, *kri*) is active in vivo and is essential for larval growth. Both S2 cells and larvae are capable of efficiently incorporating 4TU, and *kri*-RNAi can significantly reduce this incorporation. Additionally, loss of *kri* severely affects larval growth and gives rise to thin larvae that, in some cases, form thin pupae/pre-pupae that mostly die before reaching the pharate stage. However, developmental timing of stage transitions in the mutant larvae is not affected. Interestingly, we show that a relatively weaker *kri*-RNAi can significantly reduce 4TU incorporation without causing larval growth defects. This provides the possibility of systemically knocking down *kri* to reduce background incorporation of 4TU while using Tg-UPRT to obtain tissue specific RNA tagging. Towards this goal we have verified that the *kri*-RNAi does not affect expression of a codon-optimized Tg-UPRT construct or its ability to incorporate 4TU. Overall we provide strong evidence to show that, contrary to current understanding, the *Drosophila* UPRT homologue *kri*, is active in vivo. We also suggest ways of significantly improving the specificity and efficiency of a promising cell-type/tissue specific RNA isolation technique.

5

Sequencing mRNA from cryo-sliced *Drosophila* embryos to determine genome-wide spatial patterns of gene expression. Peter A. Combs[1], Michael B. Eisen[2,3]. 1) Biophysics Graduate Group, University of California, Berkeley, CA; 2) Department of Molecular and Cell Biology, University of California, Berkeley, CA; 3) Howard Hughes Medical Institute, University of California, Berkeley, CA.

Spatially patterned gene expression underlies animal development, yet methods do not yet exist for the genome-wide determination of spatial patterns of gene expression. Fluorescent imaging of transcripts and proteins is the gold-standard, but is relatively slow and expensive to expand to an entire genome, even when highly automated. In contrast, sequencing is fast and genome-wide, but discards spatial information by operating on homogenized tissues. Here we developed a method of rapidly determining genome-wide spatial patterns of gene expression to identify genes with previously undescribed spatial expression patterns, and to investigate the effects of mutants and other perturbations on patterned gene expression. To do this, we developed methods to sequence mRNA from single 60µm cryosections of*Drosophila melanogaster* embryos at the blastoderm stage. We identify numerous maternally deposited genes with spatial patterns, including many not yet screened in systematic *in situ* based approaches. The majority of these are localized to pole cells, although we also observe anterior localization. We also detected spatially varying usage of individual exons in transcriptional regulators, which could not have been identified in previous sequencing analyses. Finally, we compared wild-type embryos with bicoid dosage mutants, allowing us to determine concentration-dependent transcriptional responses of hundreds of Bicoid target genes simultaneously. Overall, our results fill in key gaps in knowledge that spatially homogenized approaches cannot address, and demonstrate the power of combining sectioning or anatomical dissection to provide missing spatial information to sequencing-based genomic studies.

6

Mechanical aspects of fruit fly gastrulation. Konstantin Doubrovinski[1,2], Bing He[1], Oleg Polyakov[1], Eric Wieschaus[1,2]. 1) Princeton University, Princeton, NJ; 2) Howard Hughes Medical Institute.

Epithelial morphogenesis plays a major role in embryonic development. During this process cells within epithelial sheets undergo complex spatial reorganization to form organs with specific shapes and functions. Fruit fly gastrulation serves a popular model of epithelial morphogenesis. In the course of gastrulation a subset of ventrally localized cells that constitute the mesodermal primordium constrict their apices thereby causing the tissue to bend into a crease termed ventral furrow. A number of signal transduction pathways regulating morphogenetic events that accompany gastrulation have been characterized in the past. However, physical mechanisms that underlie those morphogenetic events remain unclear. To tackle this problem we developed a novel particle velocimetry based approach for quantifying tissue deformation during the course of gastrulation. Our method involves injecting embryos with fluorescent tracer particles and tracking the motion of those particles over time. We demonstrate that the dynamics of deformation accompanying gastrulation is consistent with that of viscous flow. Specifically, we propose that surface deformation of the prospective mesoderm generated through apical constrictions causes a shearing force that brings about the motion of the cytoplasm in the interior of the tissue. We show that this simple physical description can quantitatively account for the measured velocity distribution acquired by particle tracking. In summary, our data suggests a physical mechanism through which apical constriction may translate into cell shape changes. Furthermore, our data suggests that the previously proposed physical mechanism of tissue invagination during fruit fly gastrulation may need to be revised.

7

Genomic and transcriptomic analysis of diapause—an important life history trait in *Drosophila melanogaster*. Xiaqing Zhao[1], Alan Bergland[2], Dmitri Petrov[2], Paul Schmidt[1]. 1) Dept. of Biology, University of Pennsylvania, Philadelphia, PA; 2) Dept. of Biology, Stanford University, Stanford, CA.

Diapause is a genetically determined syndrome cued by shortened photoperiod and/or reduced temperature that results in lifespan extension, delayed senescence, increased stress tolerance and reproductive quiescence. It is the primary adaptation in invertebrates to survive unfavorable seasons, and is a complex trait that links multiple processes including environmental sensing, biological rhythms and aging. In natural populations of Drosophila melanogaster, the expression of diapause is highly variable, making it possible to elucidate the genetic architecture and molecular basis of the trait. Here we exposed outbred natural populations to diapause-inducing conditions, and separated the population based on whether or not each individual fly expressed diapause. Pooled genomic DNA sequencing was performed on replicate diapause and non-diapause sets; pooled mRNA sequencing of heads and ovaries was also performed on the same populations. The transcriptome data show that many genes are up-regulated during diapause, indicating that diapause is an actively regulated process. This is especially true in heads, where the up-stream environmental sensing and neuroendocrine regulation of diapause presumably take place. The mRNAseq data are contrary to the traditional hypothesis that diapause is a passive response to adverse environments, and primarily involves a shut down or dampening of many biological processes. The genomic sequencing has identified a suite of sequence variants that are associated with the diapause phenotype. There is also a substantial overlap between genes whose sequence variation is associated with diapause incidence, and genes that are differentially expressed as a function of diapause initiation. GO enrichment analysis of the candidate genes has identified many interesting processes involved in diapause.

8

Solving navigational circuits in the *Drosophila* larva. Marc Gershow[1], Mason Klein[1], Marta Zlatic[2], Matthew Berck[1], Elizabeth Kane[1], Bruno Afonso[1], Aravinthan Samuel[1]. 1) Center for Brain Science, Harvard University, Cambridge, MA; 2) HHMI Janelia Farm, Ashburn, VA.

The transparent *Drosophila* larva, with a simple nervous system, robust behaviors, and powerful genetic tools, is an ideal model in which to relate neural structure and function. Navigation, which requires nontrivial computations to turn sensory input into motor output, is especially elucidating as the inputs can be precisely controlled and the outputs directly observed and quantified.

We developed large-scale high resolution assays to analyze tens of thousands of navigational decisions made by hundreds of larvae in response to spatially and temporally varying temperatures, light intensities, and concentrations of odors and carbon dioxide. We used these assays to determine a common navigational strategy across sensory modalities. In response to unfavorable changes, larvae interrupt runs, periods of forward movement, and initiate a series of head sweeps. During a head sweep, a favorable change increases the probability that a larva will begin a new run in the direction of its head, while an unfavorable change increases the probability that the larva will instead execute a new head sweep in a different direction. The behavior depends on temporal variation in the sensory input, even when, e.g. for light incident at an angle, a direct comparison between bilateral sensory organs might be expected.

We used our behavioral assays in an inactivation screen to identify specific neural populations involved in each navigational behavior and those implicated as part of a common navigational circuit. We recently combined our olfactory assay with optogenetic stimulation to generate a fully automated training apparatus to probe learning and developed a microfluidic apparatus to enable calcium imaging of the entire larval nervous system in concert with controlled odor presentation.

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Proper chromosome segregation and spindle assembly require both kinetochore and central spindle components in Drosophila oocytes. Sarah J. Radford, Kim S. McKim. Waksman Institute, Rutgers University, Piscataway, NJ.

Inaccurate chromosome segregation during oogenesis is a leading cause of spontaneous abortion and birth defects in humans. Proper chromosome segregation is achieved through the regulated interaction of chromosomes with a bipolar array of microtubules that constitute the meiotic spindle. The meiotic spindle in the oocytes of many organisms, including humans and Drosophila, is built in the absence of the classical microtubule-organizing centers known as centrosomes. In the presence of centrosomes, chromosome segregation depends on interactions between the kinetochore, a protein complex that assembles at the centromere, and microtubules that connect to the centrosomes, but how chromosomes interact with the spindle to ensure segregation in the absence of centrosomes remains unclear. We recently showed that Subito, a kinesin-6 family member that binds to microtubules at the central spindle, is required for proper chromosome bi-orientation in Drosophila oocytes. We report here the identification of a kinetochore component, SPC105R, that is also required for chromosome bi-orientation. Loss of SPC105R leads to loss of the microtubules that appear to end at the chromosomes, suggesting that kinetochore-microtubule interactions have been disrupted. In addition, loss of both Subito and SPC105R leads to loss of the oocyte spindle. This suggests that microtubules in the acentrosomal meiotic spindle must be stabilized through either incorporation into the central spindle or interaction with the kinetochore. Both types of interaction can facilitate chromosome bi-orientation, and both may depend on the chromosomal passenger complex (CPC), which is required for spindle assembly in Drosophila oocytes. Indeed, Aurora B, a component of the CPC, is required for both Subito and SPC105R localization. Based on these results, we suggest a model in which the CPC directs the recruitment of the proteins that stabilize the two main types of microtubules that constitute the acentrosomal meiotic spindle, resulting in the proper segregation of chromosomes.

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The oocyte-to-embryo transition requires APC/C mediated destruction of Matrimony, a POLO kinase inhibitor. Zachary J. Whitfield[1], Jennifer Chisholm[2], R. Scott Hawley[2], Terry L. Orr-Weaver[1]. 1) Whitehead Institute for Biomedical Research, MIT, Cambridge, MA; 2) Stowers Institute for Medical Research, Kansas City, MO.

The oocyte-to-embryo transition requires a cell cycle change from meiosis to mitosis. A female meiosis-specific form of the Anaphase Promoting Complex/Cyclosome, activated by the Cortex Cdc20 protein (APC^{Cort}), is essential for the completion of meiosis in the Drosophila egg. The APC/C ubiquitylates substrates to target them for degradation, and its activity is critical for anaphase onset. We investigated whether APC^{Cort} could target one or more meiotic proteins whose degradation would be necessary for mitosis to proceed properly.

To identify substrates of APC^{Cort}, we used IP/mass spectrometry to define binding partners of Cortex and quantitative mass spectrometry to identify proteins whose levels were elevated in *cortex* mutant eggs. Both approaches identified Matrimony, an inhibitor of POLO kinase during prophase of meiosis I in the oocyte. Several observations confirm Matrimony is a critical APC^{Cort} target. We found Matrimony protein levels drop drastically after completion of meiosis, and confirmed that Matrimony's protein levels are elevated in *cortex* mutant eggs by western blotting. Furthermore, Matrimony levels also are increased in eggs mutant for *morula/apc2*, a component of the APC/C, but not in *fzy/cdc20* mutant eggs, another APC/C activator. Additionally, when introduced into Kc cells, functional Cortex leads to reduced levels of Matrimony protein, consistent with Matrimony being a specific substrate of APC^{Cort}. We have identified motifs in Matrimony required for its degradation. Overexpression of Matrimony using the *UAS/GAL4* system caused a subset of embryos to exhibit mitotic defects and developmental arrest, and these are dominantly enhanced by *polo* mutations. Thus down regulation of Matrimony at the

oocyte-to-embryo transition by APC^{Cort} activity is important for adequate levels of POLO activity to ensure proper embryogenesis.

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Regulation of the asymmetric centrosome maturation cycle in neural stem cells. Dorothy A. Lerit, Nasser M. Rusan. Cell Biology and Physiology Center, NHLBI, National Institutes of Health, Bethesda, MD.

Neuroblasts (NBs) are neural stem cells that invariantly divide along an apical-basal polarity axis during asymmetric cell division to produce one self-renewing NB and one smaller ganglion mother cell (GMC) fated for differentiation. The earliest known symmetry-breaking event in NBs is the docking of a single centrosome to the cortex, which defines the apical domain and precedes the localization of the determinants that impart the stem cell fate. Comprising an inner core of two centrioles surrounded by a cloud of pericentriolar material (PCM), centrosomes serve as the microtubule-organizing centers (MTOCs) of most eukaryotic cells. Strikingly, the duplicated interphase centrosomes of NBs are asymmetric. The apical centrosome is active, or mature, because it displays high PCM levels and MTOC activity, which facilitates cortical docking. In contrast, the other centrosome is inactive until just before mitotic onset. It has been proposed that inactivation is required for this centrosome to move to the basal cortex and orient the spindle axis. However, regulation of the asymmetric centrosome maturation cycle of NBs is little understood. Using mutant analysis, we have identified a novel mechanism required to establish asymmetries in NB centrosome activity. Our data indicate *Drosophila* Pericentrin-like protein (D-PLP) functions to establish centrosome asymmetry during interphase. Using quantitative analysis and live cell imaging, we show D-PLP affects the localization of several key PCM proteins and blocks the recruitment of the master regulator of centrosome maturation, Polo kinase, to the basal-fated centrosome. Loss of D-PLP results in two active interphase MTOCs and incomplete separation of the centrosomes to the distal poles. We find some NBs and GMCs inherit an aberrant centrosome number, which can be detrimental to the cell and tissue. These data suggest differential regulation of MTOC activity is required for proper centrosome segregation and support a model where the mechanism of centrosome maturation includes the removal of negative regulators of PCM recruitment.

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The role of *corp* in apoptosis following DNA damage. Riddhita Chakraborty, Simon W. Titen, Kent G. Golic. Department of Biology, University of Utah, Salt Lake City, UT.

In most cells, an unrepaired DNA double-strand break leads to apoptosis, which serves to restrict the propagation of genetic aberrations caused by replication and rearrangement of damaged DNA. However, some cells manage to escape apoptosis and continue to divide. We are interested in understanding the genetic mechanisms that regulate a cell's life or death decision in response to unrepairable DNA damage. Through a misexpression/overexpression screen (EP) in the developing eye, we identified a gene, *companion of reaper* (*corp*) whose overexpression strongly enhanced the survival of cells in the eye that carry irreparable DNA damage in the form of a single telomere loss. We show, by TUNEL staining, that *corp* overexpression blocks apoptosis following DNA damage. Knockdown or mutation of *corp* produced the opposite effect, giving complete ablation of the eye due to massive cell death following telomere loss. Next, we studied the effect of *corp* overexpression on transmission of healed chromosomes through the germline. Following telomere loss in the male germline, a chromosome can be healed by addition of a new telomere, and recovered in offsprings at a measurable frequency. We observed that *corp* overexpression blocks the recovery of broken-and-healed chromosomes from the male germline. Thus, *corp* seems to produce opposite effects on cells that have experienced telomere loss in the soma versus the germline. This finding is, however, quite similar to the effects of *p53* mutants, which increase somatic survival of cells that have lost a telomere, but prevent transmission of broken-and-healed chromosomes in the germline. Thus, we propose that Corp is a negative regulator of p53. In support, we found that in a *corp p53* double mutant, *p53* is epistatic to *corp*. Interestingly, previous works demonstrate that the *corp* gene is a transcriptional target of *p53*, and that many of the proteins that physically interact with Corp are members of the proteasome complex. We propose that Corp promotes degradation of p53, perhaps playing a role in flies that is similar to the role of Mdm2 in mammals.

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Tissue repair through cell competition and compensatory cellular hypertrophy in postmitotic epithelia. Yoichiro Tamori, Wu-Min Deng. Biological Science, Florida State University, Tallahassee, FL.

Tissue integrity and organ size are finely maintained through removal of aberrant or damaged cells and subsequent compensatory proliferation of the surrounding normal cells, which are induced by mitogenic signals from the dying cells. Little is known, however, about the homeostasis system in postmitotic tissues where tissue-intrinsic genetic programs constrain cell division and new cells no longer arise from stem cells. Here we show that, in postmitotic *Drosophila* follicular epithelia, normal cells can kill and eliminate aberrant but viable neighbors through "cell competition," and resulting lowered cellular density triggers sporadic cellular hypertrophy to repair the tissue. This "compensatory cellular hypertrophy" (CCH) is implemented by acceleration of the endocycle, a variant cell cycle composed of DNA synthesis and gap phases without mitosis, dependent on activation of the insulin/IGF (insulin-like growth factor)-like signaling pathway. It has been shown that hyperplastic overproliferation is induced in neighboring normal cells when apoptotic cells are kept alive by the expression of baculovirus caspase inhibitor, p35, in proliferating imaginal epithlia. Although CCH was observed when sporadic apoptosis was induced in the postmitotic follicular epithelia, the “undead” cells expressing p35 induced neither overproliferation nor CCH of neighbors.

Furthermore, sporadic CCH was observed when a small group of viable cells had a growth defect. Collectively, these results led us to conclude that CCH is sporadically induced by lowered cellular density resulting from cellular growth or viability defects of some cells in the postmitotic epithelium and that apoptosis is not necessary to induce CCH. Our findings are the first identification of cell competition in a postmitotic tissue and of compensatory cellular hypertrophy induced by physical parameters, demonstrating a previously unknown strategy of homeostatic epithelial plasticity.

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The transcriptional response to tumorigenic polarity loss. Brandon D. Bunker[1], Anne-Kathrin Classen[2], Tittu T. Nellimoottil[3], David Bilder[1]. 1) Molecular and Cell Biology, University of California-Berkeley, Berkeley, CA 94720; 2) Biology II, Ludwig-Maximilians-University, Munich, D-82152 Germany; 3) Biological Sciences, University of Southern California, Los Angeles, CA 90033.

Genetic screens for growth regulators have identified *scribble* (*scrib*), *lethal giant larvae* (*lgl*), and *discs large* (*dlg*) as a distinctive class of tumor suppressor genes whose basic cellular activity is to control epithelial polarity. However, the mechanisms coupling polarity disruption to the transcriptional changes driving tumorigenesis remain unclear. To address this question, we analyzed the transcriptomes of wing imaginal discs mutant for *scrib* and *dlg*. Changes in gene expression highlighted several features associated with neoplastic transformation. Prominent amongst these was the transcriptional upregulation of the Unpaired (upd) family of JAK-STAT ligands; subsequent functional experiments demonstrated that increased JAK-STAT signaling promotes *dlg* overgrowth. To investigate the molecular pathways activating transcription upon polarity loss, we analyzed *upd3* gene regulatory elements. While previous work has identified a role for the Jun kinase (JNK) pathway in JAK-STAT activation upon polarity loss, our experiments uncovered a polarity-responsive region in the *upd3* enhancer whose activation was JNK-independent. In contrast, we found that the apical polarity regulator atypical protein kinase C (aPKC) is sufficient to drive expression of this element in a JNK-independent manner. Taken together, these results reveal how different signaling inputs elicited by polarity disruption integrate to drive mitogenic gene expression.

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Cell competition as a mechanism that can promote tumour growth through JNK activation. Luna L. Ballesteros-Arias, Verónica Saavedra, Ginés Morata. Centro de Biología Molecular Severo Ochoa, Madrid, Spain.

Drosophila endocytosis-defective cells develop tumour overgrowths in the imaginal discs. We have analysed the tumorigenic potential of cells mutant for rab5, a gene involved in endocytosis. We find that while rab5 deficient clones are subject to cell competition, a compartment entirely made by rab5 cells grows indefinitely. However, when a group of about 400 cells are simultaneously made mutant for rab5, they form an overgrowing tumour: cells in the periphery are eliminated, but those inside survive because they are beyond the range of cell competition. These results identify group protection as a mechanism to evade cell competition in Drosophila tumorigenesis. Furthermore, we find that the tumour growth depends to a large extent on the presence of apoptosis, as tested on a dronc mutant background. These results suggest that the apoptosis caused by cell competition in the periphery may act as a tumour-promoting factor, bringing about high levels of Wg signalling and inducing dMyc activity in the neighbourhood of apoptotic cells. If, in this context the activity of the JNK pathway, and hence Wg signalling, are suppressed, the apoptotic levels and the associated cell proliferation are much reduced. Moreover, tissue architecture is restored, while no signs of tumour growth are observed. We conclude that under these circumstances cell competition facilitates tumour growth through JNK activation, thus reversing its normal anti-tumour role.

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Identification and verification of genes involved in apoptosis-induced proliferation in *Drosophila*. Yun Fan[1,2], Andreas Bergmann[2]. 1) School of Biosciences, University of Birmingham, Birmingham, United Kingdom; 2) Cancer Biology, UMass Medical School, Worcester, United States.

Recent work in several model organisms has revealed that apoptotic cells are able to stimulate neighboring surviving cells to undergo additional proliferation, a phenomenon termed apoptosis-induced proliferation. This process depends critically on apoptotic caspases such as Dronc, the Caspase-9 ortholog in *Drosophila*, and may have important implications for tumorigenesis and tissue regeneration. While it is known that Dronc can induce the activity of Jun N-terminal kinase (JNK) for apoptosis-induced proliferation, the mechanistic details of this activation are largely unknown. It is also controversial if JNK activity occurs in dying or in proliferating cells. Signaling molecules of the Wg and Dpp families have been implicated in apoptosis-induced proliferation, but it is unclear if they are the only ones. To address these questions, we have developed an efficient assay for screening and identification of genes that regulate or mediate apoptosis-induced proliferation. We have identified a subset of genes acting upstream of JNK activity. We also demonstrate that major JNK activation occurs autonomously in apoptotic cells. Finally, in a pilot screen we identified signaling by the EGFR pathway as important for apoptosis-induced proliferation. Interestingly, requirement of EGFR signaling is also verified in another regenerative assay we developed further suggesting a link between apoptosis-induced proliferation and tissue regeneration. These data thus underscore the importance of genetic screening and promise an improved understanding of the mechanisms of apoptosis-induced proliferation.

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HIF- and non-HIF-Regulated Hypoxic Responses Require the Estrogen-Related Receptor in *Drosophila*. Keith D. Baker[1],

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Low-oxygen tolerance is supported by an adaptive response that includes a coordinate shift in metabolism and the activation of a transcriptional program that is driven by the hypoxia-inducible factor (HIF) pathway. The precise contribution of HIF-1α in the adaptive response, however, has not been determined. Here, we investigate how HIF influences hypoxic adaptation throughout Drosophila development. We find that hypoxic-induced transcriptional changes are comprised of HIF-dependent and HIF-independent pathways that are distinct and separable. We show that normoxic set-points of carbohydrate metabolites are significantly altered in sima mutants and that these animals are unable to mobilize glycogen in hypoxia. Furthermore, we find that the estrogen-related receptor (dERR), which is a global regulator of aerobic glycolysis in larvae, is required for a competent hypoxic response. dERR binds to dHIFα and participates in the HIF-dependent transcriptional program in hypoxia. In addition, dERR acts in the absence of dHIFα in hypoxia and a significant portion of HIF-independent transcriptional responses can be attributed to dERR actions, including upregulation of glycolytic transcripts. These results indicate that competent hypoxic responses arise from complex interactions between HIF-dependent and -independent mechanisms, and that dERR plays a central role in both of these programs.

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Loss of the *Drosophila* nuclear receptor dHNF4 recapitulates Maturity Onset Diabetes of the Young 1. William E. Barry, Carl S. Thummel. Department of Human Genetics, University of Utah, Salt Lake City, UT.

Nuclear receptors are ligand-regulated transcription factors that play critical roles in metabolism. Mutations in one of these, *HNF4α*, lead to an inherited form of diabetes called Maturity Onset Diabetes of the Young 1 (MODY1). MODY1 is characterized by impaired glucose-stimulated insulin secretion and hyperglycemia that develop during early adulthood. Several studies have attempted to model MODY1 in mice through tissue-specific disruption of *HNF4α*, yet none of these reported diabetes or reduced glucose-stimulated insulin secretion. Thus, it remains unclear how HNF4 regulates glucose homeostasis. In contrast, loss-of-function mutants for the single *Drosophila HNF4α* ortholog, *dHNF4*, faithfully recapitulate the MODY1 disease phenotype. *dHNF4* mutants are sensitive to dietary sugar, dying as early adults. These animals display glucose intolerance, hyperglycemia and accumulate sorbitol and fructose, all of which are hallmarks of diabetes. dHNF4 protein localizes to the insulin producing cells in the adult brain and *dHNF4* mutants display the hallmark symptom of MODY1 - a defect in glucose-stimulated secretion of *Drosophila* insulin-like peptides (DILPs). RNA-seq analysis of *dHNF4* mutants identified a*Drosophila* homolog of *Glucokinase* (*GK*), *Hexokinase C* (*HexC*), as one of the most down-regulated genes. In mammals, GK is required in pancreatic beta cells and the liver for proper insulin secretion and the uptake of circulating glucose. In addition, *GK* mutations in humans lead to MODY2, a disease related to MODY1. Current efforts are focused on genetic rescue experiments to test the hypothesis that*HexC* is a critical downstream target of dHNF4. Taken together, our findings establish a model to understand the molecular mechanisms by which HNF4 regulates glucose homeostasis, and how mutations in this receptor contribute to MODY1. Our data also suggest that MODY1 and MODY2 may be linked through HNF4 regulation of *GK* expression. Finally, we have shown that nutrient sensing for DILP secretion differs between larval and adult stages, where glucose is sufficient to trigger secretion in adults.

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Central regulation of lipid metabolism and starvation response by a histone acetyl-transferase. Nina Moderau, Ingo Zinke, Michael J. Pankratz. Molecular Brain Physiology and Behavior, LIMES Institute, University of Bonn, 53115 Bonn, Germany.

Histone acetylation plays a key role in chromatin remodeling and regulates many processes, including development and cell proliferation. Through this epigenetic modification chromatin is decondensed and allows the transcriptional machinery to access the genes. We identified Enoki mushoom (Enok) a MYST Histone Acetyl-Transferase (HAT), as an epigenetic metabolic regulator. Enok autonomously controls neuronal stem cell division as well as growth, feeding behavior and nutritional homeostasis, especially lipid catabolism. Immunofluorescence microscopy reveals that Enok is expressed during second and third larval stage in oenocytes in periphery, a special type of glial cells and neuronal stem cells in the CNS. These tissues play important role in nutrient storage, metabolism and growth control. Animals with tissue-specific overexpression of Enok protein showed no change in lipid content and lipid depletion under starvation conditions. Transcriptional analysis by microarrays and qPCR suggests that Enok controls the activity of genes involved in lipolysis and β-oxidation. Altogether, we demonstrate that Enok controls lipid metabolism in the periphery, as well as in the CNS, and regulates the global starvation response. We provide a model for the transcriptional regulation of lipolysis and β-oxidation genes by Enok on an epigenetic level.

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Control of ovarian stem cells by adipocytes in response to diet. Alissa Armstrong[1], Leesa Sampson[1], Kaitlin Laws[1], Robert Cole[2], Daniela Drummond-Barbosa[1]. 1) Biochemistry and Molecular Biology, JHU School of Public Health, Baltimore, MD; 2) Mass Spectrometry and Proteomics Facility, JHU School of Medicine, Baltimore, MD.

Adult stem cells play key roles in tissue homeostasis and damage repair; however, it is unclear how whole-body physiology influences stem cell lineages. Our past work showed that *Drosophila* ovarian stem cell lineages respond to diet via multiple

nutrient-sensing pathways. For example, insulin-like peptides, ecdysone and Target of rapamycin (TOR) act on or within ovarian cells to control germline stem cell (GSC) maintenance and proliferation. Other adult tissues are also sensitive to diet and nutrient-sensing pathways, suggesting potential crosstalk; however, the role of multi-organ communication in the stem cell dietary response is largely unknown. Mammalian adipose tissue and the *Drosophila* fat body are sensitive to nutrients and have energy storage and endocrine roles; therefore, our studies focus on how nutrient sensing within adipocytes remotely impacts adult ovarian stem cells and their progeny. We find that inhibiting either insulin or TOR signaling specifically in adult adipocytes reduces GSC number and egg production. We also observe specific effects of different nutrient-sensing pathways within adipocytes on the ovary. Blocked insulin signaling in adult adipocytes increases dying vitellogenic egg chambers. In contrast, inhibiting TOR signaling within adipocytes does not affect vitellogenesis; instead, ovaries accumulate mature oocytes. These data suggest that nutrient sensing within adipocytes remotely controls ovarian stem cells and their progeny, refining their response to diet. In search of fat body factors that transmit dietary status to the ovary, we performed a quantitative proteomics comparison between the fat body from flies fed yeast-rich versus yeast-free diets, identifying over 50 putative secreted proteins altered by diet. We are currently performing functional analyses of these candidates for potential roles in oogenesis. This work will illuminate how inter-organ communication modulates adult stem cell lineages upon dietary changes.

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Social interactions drive organism non-autonomous regulation of lifespan through pheromone perception. Christi Gendron[1], Tsung-Han Kuo[2], Zachary Harvanek[1], Ingrid Hansen[2], Scott Pletcher[1,2]. 1) Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Work from our laboratory has clearly demonstrated that sensory perception of important ecological cues is sufficient to alter *Drosophila* physiology and aging. To exemplify, we have shown that odorants from live yeast can limit the longevity-extending benefits of dietary restriction. We have also demonstrated that the perception of CO_2 limits fly lifespan. However, the neuronal mechanisms underlying these effects are currently unknown. To identify additional sensory functions that impact aging and to better understand the mechanisms through which they operate, we asked whether the perception of pheromones can significantly modulate health and lifespan in the fly. To avoid the confounding effects of mating, we used genetic tools to replace the cuticular pheromones of individual animals with those expressed by the opposite sex. We discovered that exposure of male flies to female pheromones, without mating, results in a significant decrease in lifespan, fat storage, and stress resistance. The effect of pheromones is robust across several laboratory and wild-caught strains, and is completely reversed following pheromone removal. To identify the sensory receptors responsible for the observed effects, we performed a candidate screen of known taste and odorant receptors. We identified a known pheromone receptor as well as a select group of receptor neurons that are required for the phenotypes described above. Furthermore, we are beginning to map the neural circuits involved. This is the first report demonstrating that the perception of pheromones from the opposite sex is sufficient to alter physiology and lifespan. Our work also defines a framework for the study of organism non-autonomous effects (i.e., the ability of a genotype of one individual to modulate the lifespan of another), and it paves the way for a mechanistic investigation of the effects of social interactions on health and longevity.

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A Role for Drosophila p38 MAP Kinase in Protein Homeostasis. Alysia D. Vrailas-Mortimer[1,2], Amelia M. Burch[1], Subhabrata Sanyal[1,2,3]. 1) Cell Biology, Emory University, Atlanta, GA; 2) Center for Behavioral Neuroscience, Atlanta, GA; 3) Center for Neurodegenerative Disease, Emory University, Atlanta, GA.

One hallmark of aging is the formation of protein aggregates in the brain and musculature, which is often magnified in a disease state such as Alzheimer's disease, though the significance of these aggregates or how they contribute to a disease state is not precisely understood. These protein aggregates can be cleared through several mechanisms, such as Chaperone Assisted Selective Autophagy (CASA) a specialized form of autophagy that utilizes a BAG3-HspB8-Hsp70 chaperone complex to target specific protein substrates for degradation through the lysosome. In Drosophila, age-dependent protein aggregation is delayed in long-lived mutant strains suggesting that either preventing protein aggregation or efficient resolution of aggregates plays an important role in aging. We have previously reported that the p38 MAPK (p38K) is a regulator of lifespan and oxidative stress. We have found that inhibition of p38K leads to increased protein aggregation, whereas over-expression of p38K coincides with a reduction in the number of protein aggregates throughout life and is protective against oxidative stress induced aggregation. These data suggest that p38K may play an integral role in general and damage induced protein homeostasis. Furthermore, the Drosophila Protein Interaction Map project has shown that the CASA complex member HspB8 binds to p38K. We therefore hypothesize that p38K may play an integral role in regulating CASA throughout the aging process. We have found that p38K co-localizes with CASA complex members in the muscle and our preliminary data suggests that p38K genetically interacts with select members of the CASA complex to regulate lifespan. To determine if p38K is required for the clearance of CASA specific substrates, we are testing if p38K modifies the phenotypes of protein aggregation disease models such as spinocerebellar ataxia 3 and the Alzheimer's disease Aβ model.

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***In vivo* interaction proteomics reveal a novel role of p38MAPK in controlling proteostasis in ageing *Drosophila* muscle.** Vladimir Belozerov[1,2], Anne-Claude Gingras[2], Helen McNeill[2], John McDermott[1]. 1) Department of Biology, York University, Toronto, ON, Canada; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON,

Canada.

Several recent studies suggest that systemic ageing in metazoans is differentially affected by functional decline in specific tissues, such as skeletal muscle. In *Drosophila* longevity appears to be tightly linked to the regulation of myoproteostasis, and the formation of misfolded protein aggregates has been shown to be a hallmark of senescence in ageing muscle. Similarly, defective myoproteostasis is described as an important contributor to the pathology of several age-related degenerative muscle diseases in humans, e.g. inclusion body myositis. p38 MAP kinase plays a central role in a conserved signaling pathway activated by a variety of stressful stimuli. In ageing *Drosophila* muscle p38b/Mef2/MnSOD pathway was shown to control muscle function and longevity by modulating ROS. Concomitant with declining motor functions in ageing p38b mutant flies, we observed enhanced accumulation of detergent-insoluble protein aggregates in flight muscles, suggesting deregulation of myoproteostasis. To define the molecular mechanism of p38MAPK-mediated regulation of protein turnover we used affinity purification and mass spectrometry (AP-MS) to identify proteins interacting with a kinase-dead mutant of p38b (acting as a substrate trap) in ageing flight muscles. One of these substrates, dRack1, is of particular interest as it interacts with the ribosome, and may serve as a link between p38MAPK signaling and translational regulation. Using *in vitro* kinase assays and mass spectrometry we show that dRack1 is indeed a substrate of p38b. Further genetic and biochemical tests position dRack1 downstream of p38b, and demonstrate that dRack1 inhibits translation in ageing muscle in response to p38b signaling. Finally, we used AP-MS to examine protein interaction network formed by RACK1 in human cells, and identified a novel complex of RACK1 with known translational repressors, providing a likely mechanism of p38MAPK/RACK1-mediated control of proteostasis.

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A metabolic adaptation in muscle mediates the protective effects of dietary restriction in Drosophila. Subhash D. Katewa, Kazutaka Akagi, Matthew J. Laye, Pankaj Kapahi. Buck Institute for Research on Aging, Novato, CA.

Dietary restriction (DR) is a robust environmental intervention that slows aging in various species. We recently showed that upon DR, *Drosophila melanogaster* shift their metabolism towards increasing fat turnover, which is required for various responses to DR. Inhibition of fatty acid synthesis or oxidation genes specifically in the muscle tissue reduced spontaneous activity and inhibited lifespan extension upon DR. Reducing spontaneous activity of the flies by physical or genetic manipulations also reduced the DR dependent lifespan extension. Now, we report that *d4E-BP* (Drosophila eIF-4E binding protein), a downstream target of the TOR pathway mediates the DR dependent increases in fat turnover and spontaneous activity. Muscle specific over-expression of *d4E-BP* increased spontaneous activity, enhanced fat metabolism in fat bodies and was sufficient to increase lifespan in a nutrient dependent manner. The regulation of fat metabolism in a distant tissue such as fat bodies by muscle indicates involvement of myokines- muscle specific secreted factors. Together these results suggest a critical role of muscle specific *d4E-BP* in regulating whole body physiology, metabolism and aging upon DR.

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Recent and strong adaptation in Drosophila melanogaster is driven primarily by soft selective sweeps. Nandita Garud, Philipp Messer, Erkan Buzbas, Dmitri Petrov. Stanford University, Stanford, CA.

Adaptation is typically thought to proceed by the rapid increase in frequency and ultimate fixation of a single adaptive allele. This process results in the signature of a hard sweep, specified by the presence of one haplotype bearing the adaptive allele at high frequency in the population. However, not all modes of adaptation necessarily lead to the presence of a single common haplotype. For instance, in some cases, adaptation might involve subtle changes in frequency at a large number of sites, leaving no signatures of selective sweeps. In other cases, adaptation might drive multiple haplotypes to high frequency, generating signatures of soft sweeps. Such soft sweeps can occur when adaptation involves standing genetic variation, where the adaptive allele was already present in the population prior to the onset of positive selection, or when multiple de novo adaptive mutations arise in the population independently on different haplotypes and sweep through the population simultaneously. Here we developed a haplotype statistic (H12) that identifies both hard and soft sweeps with similar power. We further developed a second statistic (H2/H1) that can determine whether a given sweep identified with H12 was hard or soft. We used these statistics to carry out a genome scan for adaptation in the North Carolina population of D. melanogaster sequenced by DGRP. We found evidence of pervasive haplotype structure suggestive of abundant, recent, and strong adaptation in this population. Interestingly, when we applied our H2/H1 statistic to the 50 most prominent peaks in the scan, we were able to reject the hard sweep hypothesis in every case. On the other hand, the vast majority of the peaks are compatible with a simple model of soft sweeps from multiple de novo mutations. We conclude that recent adaptation in North American populations of D. melanogaster has led primarily to soft sweeps either because it utilized standing variation or because short-term effective population sizes are on the order of billions or larger rather than on the order of millions, as suggested previously.

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Population and metabolic genomics of five geographically dispersed fully-sequenced population samples of *Drosophila melanogaster*. Andrew G. Clark[1], J. Roman Arguello[1], Margarida Cardoso Moreira[1], Jian Lu[1], Cornelia J. Scheitz[1], Anthony J. Greenberg[1], Sean R. Hackett[1,2], Julien F. Ayroles[1,3], Srikanth Gottipati[1], Lawrence G. Harshman[4], Jennifer K. Grenier[1]. 1) Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY; 2) Grad Program Quant and Comp Biology, Princeton Univ, Princeton, NJ; 3) Dept of OEB, Harvard Univ, Cambridge MA; 4) School of Biol Sciences, Univ Nebraska, Lincoln, NE.

In order to assess the role of geographic subdivision on the genetics of complex traits in *Drosophila melanogaster*, 92 inbred

lines were established by sib mating isofemale stocks from Beijing, Ithaca, Netherlands, Tasmania and Zimbabwe. Genomic DNA from these lines was sequenced to approximately 12x coverage, aligned to the reference sequence and SNPs were called with the GATK pipeline. SNP genotype calls were validated with double-digest GBS and deep (100x) whole-genome sequencing of a subsample. Small indels, copy number variants (including novel genes) and inversions were also discovered and validated. Many metabolic phenotypes have been quantified in nested and well replicated designs of the inbred lines and a partial F1 diallel, including kinetics of 22 enzymes, whole-transcriptome microarrays, respirometry, flight performance, metabolites, and lipidomics. Hierarchical and generalized linear models find extensive geographic differences in genetic architecture of key metabolic traits as well as widespread genotype x environment interaction. The sequence data were of sufficient quality to provide useful inference of demographic parameters including effective size in each sample, founding times and migration rates (inferred by approximate Bayesian computation). A surprising result is the degree of both phenotypic and genome sequence differentiation of the Beijing lines, and the strength of evidence for back migration into Africa. There are large differences in distributions of transposon abundance, and even the lipidomics data show a strong signature of population differentiation. These lines augment the DGRP reference panel by adding a geographic dimension to genomic variation and adaptation.

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Parallel selection on copy-number variants across continents and species in *Drosophila*. Daniel R. Schrider[1,2], Matthew W. Hahn[1,2], David J. Begun[3]. 1) Department of Biology. Indiana Univesity, Bloomington, IN; 2) School of Informatics and Computing. Indiana Univesity, Bloomington, IN; 3) Department of Evolution and Ecology. University of California. Davis, CA.

Regions of the genome that vary in copy-number within a species, referred to as copy-number variants (CNVs), have been shown to have important functional and evolutionary consequences in a variety of organisms. However, the importance of copy-number variation to adaptation in *Drosophila* is largely unknown. In order to address this question, we examine pooled sequence data from opposite ends of two latitudinal clines in *D. melanogaster* and *D. simulans*. Because extensive gene flow occurs across each of these clines, regions exhibiting strong differentiation in allele frequency across a cline are candidates for local adaptation. This strategy has been used to identify single nucleotide polymorphisms and transposable element insertions that may be experiencing spatially varying selection. We extend this approach to the problem of identifying CNVs differing in allele frequency between two pooled samples. We examine pooled *D. melanogaster* whole genome sequences from the ends of the latitudinal cline along the East Coast of the United States, and also from the ends of the cline along eastern Australia. We find hundreds of highly differentiated CNVs in each of these clines that represent strong candidates for spatially varying selection. Furthermore, we find that many of these CNVs are differentiated in both continents and in the same direction with respect to distance from the equator. Because this overlap is not expected if these CNVs are not under selection, we have especially high confidence that these CNVs are involved in local adaptation. Finally, we perform the same analysis on *D. simulans* pooled sequence data from these two clines, finding similarly high numbers of differentiated CNVs, again with many exhibiting the same pattern of differentiation across continents. Several genes were found to reside within differentiated CNVs in both species. Together, these results show that copy-number changes are a major contributor to local adaptation in *Drosophila*.

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Interpreting faster-X evolution in light of expression breadth and adaptation. Richard Meisel. Cornell University.

Accelerated rates of molecular evolution can be driven by increased mutation rate, relaxed selective constraints, or higher rate of adaptive substitutions. Comparative and population genomics studies often measure the total divergence between genes (k), and the fraction of amino acid substitutions fixed by positive selection (α). This has revealed that narrowly expressed genes are more divergent than genes broadly expressed across many tissues ($k_{narrow} > k_{broad}$), and X-linked genes tend to be more divergent than autosomal genes ($k_X > k_A$). The faster evolution of narrowly expressed genes can be attributed to relaxed constraints permitting more neutral fixations and/or more adaptive substitutions. The faster evolution of X-linked genes (the faster-X effect) has been hypothesized to be a result of the exposure to selection of X-linked recessive beneficial mutations in hemizygous males, which leads to more adaptive substitutions on the X chromosome. Comparisons of polymorphism and divergence between X-linked and autosomal genes (the McDonald-Kreitman test and its derivatives) have indeed revealed a higher frequency of substitutions fixed by positive selection in X-linked genes relative to autosomal genes ($\alpha_X > \alpha_A$). To simultaneously address the effects of X-linkage and expression breadth on divergence and adaptation, we tested for a relationship between the faster-X effect and gene expression profiles in Drosophila melanogaster within a McDonald-Kreitman framework. We find that, while faster-X divergence ($k_X > k_A$) is only observed amongst narrowly expressed genes, faster-X adaptation ($\alpha_X > \alpha_A$) is limited to broadly expressed genes. The faster-X adaptation in broadly expressed does not translate to faster-X divergence because the total number of substitutions in these genes is small. This low substitution rate can be attributed to increased constraints on broadly expressed genes, which has been shown to increase α by decreasing the rate of neutral divergence. We therefore conclude that faster-X divergence is driven by relaxed selective constraints, and the specific type of faster-X evolution (divergence versus adaptation) depends on the constraints on the gene set under consideration.

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Neofunctionalization of young duplicate genes in Drosophila. Raquel Assis, Doris Bachtrog. Integrative Biology, University

of California, Berkeley, Berkeley, CA.

Gene duplication is a key mechanism by which novel phenotypes arise. There are two major hypotheses for how this occurs. According to the neofunctionalization hypothesis, one gene copy acquires a novel function, while the other retains the ancestral function. In contrast, the subfunctionalization hypothesis proposes that there is a division of functions between duplicate genes, such that each copy performs a subset of the ancestral functions. To disentangle these two processes in Drosophila, we studied the phenotypic evolution of recent gene duplicates in D. melanogaster by using gene expression profiles as a proxy for function. Comparison of gene expression profiles in D. melanogaster and D. pseudoobscura revealed that ancestral genes tend to have conserved and broadly expressed functions, whereas new copies frequently have novel tissue-specific functions. Moreover, we found that new copies evolve significantly faster at the sequence and expression level than ancestral copies, which evolve at similar rates to single-copy genes. In particular, new genes that are testis-specific and/or male-biased evolve the fastest at the sequence and expression levels and display evidence of positive selection. Thus, our findings are consistent with the neofunctionalization hypothesis and suggest that the origin of novel phenotypes by neofunctionalization in Drosophila is driven by strong positive selection on young duplicate gene copies.

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Signatures of correlated evolution predict new members of a protein network required for *Drosophila* female post-mating responses. Geoffrey D. Findlay[1], Nathaniel L. Clark[1,2], Jessica L. Sitnik[1], Charles F. Aquadro[1], Mariana F. Wolfner[1]. 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA.

Mating induces long-term changes in *Drosophila melanogaster* females, including decreased receptivity to courtship, increased egg production, and efficient sperm storage. These changes are caused primarily by a seminal fluid protein, sex peptide (SP), which females receive from the male and store for several days. Five additional seminal fluid proteins (Sfps) are required for SP to be stored in females and to act over the long term. To discover new members of this "SP network," we used a new comparative genomic method to detect correlated changes in the rates of protein evolution across the*Drosophila* phylogeny. The logic was to discover new proteins within the SP network by virtue of their shared evolutionary selective pressures. We first confirmed that the known network proteins showed correlated evolutionary rates. We then used each member of the network to computationally query hundreds of male and female reproductive proteins for correlated evolution and used RNAi to functionally evaluate potential candidates. RNAi tests of 16 top candidates identified three male Sfps and two female-expressed proteins that are each required for the long-term effects of SP on fertility and female receptivity. Molecular genetic analysis showed the three new male proteins are required for the transfer of other network proteins and for SP storage in mated females. The two female proteins, in contrast, act downstream of SP storage. Our results provide the first demonstration that signatures of correlated evolution can be used prospectively to predict new members of a protein network. In addition, they have expanded our knowledge of the male-derived portion of the SP network and identified new female regulators of SP action.

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The role of the Drosophila meiotic MCM proteins in crossover formation. Kathryn P. Kohl, Corbin D. Jones, Jeff Sekelsky. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Meiotic recombination increases genetic diversity and aids the proper segregation of homologous chromosomes through the formation of crossovers (COs). To prevent aberrant crossing over the formation of COs is highly regulated. One method of regulation is through the use of anti-CO helicases, which unwind inappropriate recombination intermediates, and pro-CO proteins that prevent these helicases from unwinding intermediates destined to form COs. The Msh4-Msh5 complex has been shown to antagonize the anti-CO helicase Sgs1, the S. cerevisiae Bloom syndrome helicase BLM ortholog. All metazoan genomes have Msh4 and Msh5 except Drosophila and Glossina morsitans (tsetse fly). We identified a novel complex of mini-chromosome maintenance proteins (mei-MCMs) that functionally replace Msh4-Msh5 in flies (Kohl et al. Science 2012). Two of these proteins, MEI-217 and MEI-218, are encoded on one dicistronic mRNA and are structurally predicted to contain MCM N- and C-terminal domains, respectively. The third complex member, REC, was found to be under strong positive selection prior to the split of Glossina from Drosophila, suggesting natural selection drove the repurposing of REC into an antagonist of DmBLM. COs are nearly absent in mei-MCM mutants, but removal of DmBLM in these mutants restores CO formation, supporting the hypothesis that the mei-MCMs promote crossing over by blocking the anti-CO activities of DmBLM. We are now examining the role of the mei-MCMs in CO interference. We created flies with mutations in REC's Walker A and Walker B ATPase motifs. We found that mutation of the Walker A motif allowed normal chromosome disjunction but increased the number of double and triple COs. The Walker B mutant showed high levels of non-disjunction and very few COs. However, the residual COs were distributed normally - a surprising result since mei-MCM mutants are characterized by an altered CO distribution. We also created a MEI-218 mutant with proper chromosome segregation but increased multiple COs, further suggesting a role for the mei-MCMs in CO interference.

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Contact-mediated long distance signaling by Drosophila cytonemes. Sougata Roy, Thomas B Kornberg. Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA.

How cells communicate with each other at long distances is key to understanding how cells cooperate to form organized

tissues during development and why cells in various disease states lose or escape normal controls. Although much progress has been made identifying signaling molecules that mediate these communications - proteins such as Hedgehog, Wingless, Decapentaplegic (Dpp, a BMP homolog), Fibroblast Growth Factor and Epidermal Growth Factor - the mechanism by which these proteins move with target specificity and in regulated amount through and across tissues remains unproven. Several proposed models postulate that some form of diffusion moves these signaling proteins through extracellular spaces. My work has investigated a different "direct delivery" mechanism whereby specialized filopodia (cytonemes) transfer signaling proteins between cells at sites of direct contact (Roy and Kornberg, Sci Signal. 2011; 4:pt8). Cytonemes are types of filopodia first identified in the Drosophila wing imaginal disc that were proposed to be involved in long distance signaling during development. My work shows that same group of cells emanate different types of cytonemes that can be distinguished by their specific response to different signaling ligands depending on the presence or absence of different signaling protein receptors in them (Roy et al. Science. 2011; 332:354-358). I then show, using the GRASP GFP reconstitution method, that cytonemes make direct contact with target cells, and also show that contacting cytonemes exchange, receive and transport morphogen molecules from target cells to recipient cells in receptor dependent manner. Finally, I show that genetic conditions that reduce cytoneme-mediated contacts also reduce signal transduction. These findings establish that non-neuronal cells can make direct long distance contacts for signal transduction and support the model of cytoneme-based movement of signaling proteins as a novel and essential mechanism for cell-cell communication.

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Ubquitination of Costal 2 by the Ubr3 E3 ligase is required for proper Hedgehog signaling. Tongchao Li[1], Nikos Giagtzoglou[2], Junkai Fan[7], Jianhang Jia[7], Sinya Yamamoto[1], Wu-Lin Charng[1], Manish Jaiwal[2], Hector Sandoval[2], Vafa Bayat[1,5], Bo Xiong[1], Ke Zhang[3], Gabriela David[1], Andy Groves[1,2,4], Hugo Bellen[1,2,3,4,6]. 1) Program in Developmental Biology; 2) Department of Molecular and Human Genetics; 3) Program in Structural and Computational Biology & Molecular Biophysics; 4) Department of Neuroscience; 5) Medical Scientist Training Program; 6) Howard Hughes Medical Institute, Neurological Research Institute at Baylor College of Medicine, Houston, Texas; 7) Markey Cancer Center, University of Kentucky, Lexington, KY.

Hedgehog (Hh) signaling affects cell proliferation, cell differentiation and wound healing. Loss of Hh signaling leads to developmental disorders including holoprosencephaly, craniofacial defects, polydactyly and skeletal malformations whereas aberrant activation of Hh signaling causes polydactyly, multiple cancers including basal cell carcinoma (BCC), medulloblastoma, and rhabdomyosarcoma. Here we report the isolation of the *Drosophila* homolog of *UBR3*, a member of the UBR superfamily of E3 ubiquitin ligases, from a forward genetic screen aimed at identifying genes that regulate organogenesis of sensory organs. *ubr3* homozygous mutants are first instar larval lethal. Mosaic clones show that loss of *ubr3* causes a loss of Hh signaling at the boundary of anterior/posterior compartments of the developing eye imaginal and wing discs. Ubr3 functions as a novel regulator of Hh by promoting the activation of the pathway. Loss of ubr3 results in the upregulation of Costal2 (Cos2), a Kinesin-related motor protein that negatively regulates Hh signaling. Cos2 is necessary and sufficient to suppress Hh signaling, indicating the importance of controlling correct protein levels of Cos2. We show that Ubr3 binds to the amino terminal motor domain of Cos2 with its UBR domain and that it polyubiquitinates Cos2, promoting its degradation. In summary, we identified a novel E3 ligase that acts as a positive regulator of Hh signaling revealing a critical regulatory mechanism to control the protein levels of Cos2.

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Contribution of Ihog and Boi to the Hedgehog receptor in Drosophila. Darius Camp[1,2], Haitian He[1], Don van Meyel[1], Frédéric Charron[2]. 1) Centre for Research in Neuroscience, McGill University, Montreal, Quebec, Canada; 2) Institut de recherches cliniques de Montréal, Montreal, Quebec, Canada.

Hedgehog (Hh) proteins are secreted molecules that elicit intracellular signaling vital for tissue development in both vertebrates and invertebrates. Misregulation of the Hh signaling pathway is responsible for many human congenital defects and cancers. Reception of Hh at the cell surface has long been thought to be mediated by Ptc, a 12-pass transmembrane protein, which ordinarily inhibits the pathway when Hh is absent. Binding of Hh to Ptc is thought to inhibit Ptc and thereby initiate transduction of the pathway. The interaction between Hh and Ptc is also believed to be essential to sequester Hh and thus limit its spatial range of influence. In Drosophila, we and others have found that additional factors at the cell surface play an important role in the reception of Hh: Ihog and Boi are two functionally redundant type 1 transmembrane proteins of the immunoglobulin superfamily that are required for pathway activation and capable of binding both Hh and Ptc. This raises the possibility that Ihog/Boi and Ptc form a complex required for the reception of the Hh signal. However, the mechanism underlying the requirement for Ihog and Boi in the inhibition of Ptc is not known. Our work aims to better understand the Hh receptor complex by using genetic approaches to clarify the involvement Ihog/Boi and Ptc. In one model, Ihog and Boi are proposed to be important for trafficking Ptc to the plasma membrane and for high affinity Hh binding. This model predicts that cells mutant for both Ihog and Boi will be unable to bind and sequester Hh, and will fail to inhibit Ptc. We tested this model in the developing wing disc, where Hh plays an important role in anterior-posterior patterning. Our results show that Ihog and Boi, unlike Ptc, are dispensable for the sequestration of Hh. Our findings are consistent with a central role for Ptc in binding Hh in vivo. Further experiments to probe the role of Ihog and Boi in receiving and transducing the Hh signal are ongoing.

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The Formin Frl functions in Planar Cell Polarity Signaling in Drosophila. Andreas Jenny[1], Saw-Myat Maung[1], Gretchen Dollar[1], Cathie Pfleger[2]. 1) Dept Molec & Dev Biol, Albert Einstein Col Med, Bronx, NY; 2) Department of Oncological Sciences Mount Sinai School of Medicine, New York, NY.

The non-canonical Fz/Planar cell polarity (PCP) pathway regulates establishment of polarity within the plane of an epithelium to generate diversity of cell fates, asymmetric, but highly aligned structures (e.g. stereocilia in the inner), or to orchestrate the directional migration of cells during convergent extension during vertebrate gastrulation. In *Drosophila*, PCP is essential to orient actin wing hairs and to polarize the ommatidia in the eye by coordinating the movement of groups of photoreceptors during ommatidial rotation. Thus, common themes in PCP dependent processes are cytoskeletal rearrangements and cell migration processes. PCP is governed by Wnt signals through Fz to elicit nuclear responses and cytoskeletal changes mediated by Rho Kinase (Rok). Loss of *rok* causes ommatidial rotation and neural tube defects in flies and fish, respectively, yet how Rok is regulated and its targets during PCP remain largely unknown. In a genome wide screen to identify novel Rok substrates we identified the formin Frl, the single fly FMNL (Formin related in Leukocytes/ Formin-like). Formins catalyze actin polymerization and are thus compelling candidates to regulate cytoskeletal changes downstream of Rok. To investigate Frl function, we took a loss of function approach and found that knock-down of or mutations in *frl* cause PCP defects in the eye, consistent with a role for Frl in PCP signaling. Furthermore, dominant negative *frl*genetically interacts with *cdc42* and *rhoA*, suggesting that Frl integrates signals from different Rho family GTPases. We are addressing how Frl acts inPCP signaling. In particular, we test if Frl acts together with Daam, a formin that was previously shown to link RhoA to Dsh during PCP signaling in Xenopus, but has no PCP phenotype in flies. Our findings bring us closer to a better understanding of how the PCP signal is transduced to the cytoskeleton and suggest that FMNL homologs might function during PCP signaling in vertebrates.

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Frizzled induced Van Gogh phosphorylation regulates PCP signaling. Lindsay Kelly, Marek Mlodzik. Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY.

A great deal of work has focused on how individual cells within an epithelium adopt a defined polarity. However, the process by which polarity is coordinated between cells is poorly understood. Competing hypotheses propose that cells polarize in response to a long-range diffusible signal or through a cell-cell relay mechanism. Because the core planar cell polarity (PCP) protein complexes that signal across cell membranes are asymmetric, it is difficult to assess which interaction is more important for the transduction of polarity information or the instructive long-range signal(s). It has previously shown that the core PCP protein, Van Gogh (Vang) functions as a Frizzled (Fz) receptor in signal receiving cells to sense Fz activity levels. Our preliminary data suggest that a novel pathway exists downstream of Vang that functions to interpret and relay polarity information to neighboring cells. We have also observed that Vang is phosphorylated in response to Fz signaling and identified that a single residue substitution of Vang Y341F generates a phosphorylation defective mutant. In vivo, this mutant fails to localize normally and does not rescue Vang function in PCP. We are using a combination of of genetic and biochemical approaches to determine the kinase(s) that phosphorylate Vang Tyr341. Identification of this kinase will provide an important entry point into the presumed downstream signaling cascade.

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CG9723 is required for spermatogenesis in *Drosophila*. Robyn Rosenfeld[1,2], Helen McNeill[1,2]. 1) Samuel Lunenfeld Research Institute, Mount Sinai hospital, Toronto, ON, Canada; 2) Molecuar Genetics, University of Toronto, Toronto, ON, Canada.

In Drosophila, testes development is a complex process regulated by the interplay between germline stem cells and somatic stem cells. Through a screen to identifying regulators of growth, we found a novel gene, CG9723, that is essential for proper testes development. CG9723 was previously uncharacterized and encodes a multi-pass transmembrane protein of 450 amino acids with a highly conserved domain of unknown function (DUF2215). Antibody analysis indicates that CG9723 is a nuclear membrane protein, with high expression in the apical tip of the testes that declines basally. We generated a CG9723 null allele through ends-out gene targeting and found that homozygous mutant flies display male sterility and lethality phenotypes. Flies lacking CG9723 have small testes that lack proper cyst structure and rarely produce late stage sperm cells. The hub of the testis, the niche for the stem cells, and stem cells directly surrounding the hub appear normal. However, after this stage, the organization and morphology of the cells are abnormal. The testis contains both somatic and germ cells. By expressing CG9723 within different compartments of CG9723 null flies, we determined that CG9723 is required in the somatic cells of the testis. Interestingly, there are significantly more early somatic cells in CG9723 null males compared to wild type. The nuclear membrane structure in mutant flies is relatively normal, suggesting that CG9723 is essential for proper signalling. Interestingly, CG9723 homologues are highly expressed in mammalian spermatogenesis, suggesting that CG9723 may play a conserved role in sperm development.

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Exosomes, secreted from secondary cells of the male accessory glands, fuse with sperm and female epithelia to modulate reproductive function. Laura Corrigan[1], Shih-Jung Fan[1], Carina Gandy[1], Aaron Leiblich[1], Rachel Patel[1], Siamak Redhai[1], John Morris[1], Freddie Hamdy[2], Clive Wilson[1]. 1) Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom; 2) Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom.

Reproduction involves more than just the transfer of gametes from males to females: it is also dependent upon secreted

signals that are transported in the seminal fluid during mating, and influence sperm and female behaviour. The accessory glands of *Drosophila* males, along with the seminal vesicles and ejaculatory duct, secrete components of the seminal fluid. The accessory gland secretes specific factors that activate sperm, promote sperm storage and modulate female post-mating behaviours. Each gland contains approximately 40 specialised secondary cells, which selectively grow as flies age and mate. Here we show that these cells secrete exosomes, membrane-bound nanoparticles formed by vesicular budding inside the late endosomal multivesicular body (MVB). These intraluminal vesicles are released as exosomes when the MVB fuses with the plasma membrane. Intracellular secretory compartments of secondary cells are huge, 2-10μm in diameter, allowing us to visualise intraluminal vesicle formation, exosome secretion and fusion in living and fixed tissue. We show that exosome production and secretion is regulated by mating and that exosomes are transferred to females during mating, where they fuse with sperm and specific epithelial cells of the female reproductive tract. Blocking secondary cell exosome production suppresses post-mating effects on female behaviour, suggesting that these exosomes act as vehicles to transfer important signals from males to females and providing novel *in vivo* evidence that exosomes have important physiological roles in reproduction. Exosomes produced by the prostate also fuse with sperm *in vitro* and transfer signalling tools that stimulate motility. Our data suggest that this mechanism may be conserved in higher eukaryotes, while raising the possibility that male exosomes can also reprogramme female cells.

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Drosophila genome-wide RNAi screen identifies novel genes involved in Sindbis virus entry. Debasis Panda, Patrick Rose, Sheri Hanna, Beth Gold, Sara Cherry. Microbiology, University of Pennsylvania, Philadelphia, PA.

Alphaviruses are a large class of insect-borne human pathogens which exhibit a broad host range in nature. Little is known about the host factor requirements for alphaviruses and thus we performed a genome-wide RNAi screen in Drosophila cells which validated 96 genes that impacted infection of Sindbis virus (SINV), the prototypical alphavirus. This led to the identification of Natural Resistance-Associated Macrophage Protein (NRAMP, Divalent Metal Transporter (DMT1)), as an entry receptor for SINV in insects and the mammalian homolog NRAMP2 as a receptor in vertebrates. NRAMP is the major iron transporter in cells, and thus NRAMP expression is tightly regulated: either iron deficiency (a major public health concern) or excess causes human disease. Further studies revealed that Endoplasmic Reticulum Associated Decay genes along with the proteasome promote viral infection in vitro and in vivo at the level of entry. Furthermore, we found that depletion of dSEC61A and dPSMD11 significantly reduced NRAMP protein levels, decreasing both SINV infectivity and reducing NRAMP-dependent iron transport, suggesting a role for these genes in iron metabolism. Altogether, our study reveals new genes involved in SINV infection and also sheds light onto novel modes of NRAMP regulation. The identification of genes and pathways involved in NRAMP stability are critical for our understanding of alphavirus pathogenesis as well as iron metabolism.

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TAK1-dependent Ubiquitin Chain Editing Regulates IMD Signaling. Li Chen, Uday Aggarwal, Boae Choi, Neal Silverman. Med/Div Infectious Dis, Univ Massachusetts Med Sch, Worcester, MA.

The humoral immune response in Drosophila is characterized by the robust and rapid induction of a battery of antimicrobial peptides (AMPs) immediately upon infection. Through the IMD pathway, AMP gene expression is induced upon sensing DAP-type peptidoglycan (PGN), common to the cell wall of Gram-negative and certain Gram-positive bacteria. The IMD pathway drives AMP expression through the activation the transcription factor Relish, an NF-κB precursor protein. Signal transduction leading from PGN recognition to Relish activation requires both proteolytic cleavage and K63-polyubiquitination of IMD. However the molecular mechanisms regulating polyubiquitination in the IMD pathway remain unclear. Here, we demonstrate that upon stimulation by PGN, the cleaved IMD protein is rapidly K63-polyubiquitinated at lysine residues 137 and 153, by the E3 ligase DIAP2 and two E2 (ubiquitin conjugating) enzymes: Effete (a Drosophila Ubc5 homolog), and a complex of Uev1a and Bendless (the Drosophila Ubc13 homolog). Furthermore, ubiquitination of IMD leads to activation of the kinase TAK1, which, in turn, is required for the phosphorylation of IMD at T162 and S164. TAK1 is not only required for IMD phosphorylation, but also for the removal of K63-polyubiquitin and subsequent conjugation with K48-polyubiquitin. These data suggest that the TAK1-dependent phosphorylation of IMD protein is crucial for the ubiquitin editing of IMD. Upon stimulation, TAK1 is activated and, in a feedback loop, triggers the phosphorylation and subsequent transition from K63- to K48-polyubiquitination of IMD. Once conjugated with K48-chains, IMD is degraded by the proteasome, a critical event to down-modulate the immune response.

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JAK/STAT pathway in autophagic control of intracellular mycobacteria. Claire Péan, Sharon W.S.Tan, Marc Dionne. CMCBI, Kings College London, London, United Kingdom.

The Jak/Stat pathway has been extensively studied during the past 20 years and the function of each Jak, Stat and Socs protein has been analyzed in all cell types involved in the inflammatory response in mammals. However, despite a broad literature on Jak/Stat in innate immunity, we still do not understand the in vivo consequences of these signals, especially in the control of infection with pathogens.

In human cells, part of the difficulty in understanding how the pathway regulates inflammation is the presence of complex compensatory mechanisms between the different Jak and stat proteins. In flies, there is only one Jak, one stat and a few cytokines. In the Dionne lab, we use Drosophila melanogaster as a model to study in vivo activation of the Jak/stat pathway

upon mycobacterial infection.

We show that, in Drosophila, blockade of Jak/Stat signalling is beneficial for the host upon mycobacterial infection. Loss of upd3 and inhibition of Stat92E or dome in hemocytes all improve survival and decrease bacterial growth and hemocyte death. Strikingly, we find that Jak/Stat signaling inhibits autophagy gene expression in hemocytes in vivo, partly by activating expression of a transcriptional repressor. We also show that promoting autophagy in phagocytes can reduce bacterial numbers, indicating that in flies, as in mammals, autophagy plays a role in killing intracellular mycobacteria.

We thus show a mechanism by which Jak/stat inhibits autophagy gene expression and demonstrate that this inhibition is detrimental to the survival of the host.

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Vertical transmission of a Drosophila endosymbiont via co-option of the yolk transport and internalization machinery. Jeremy K Herren, Juan C Paredes, Fanny Schupfer, Bruno Lemaitre. Global Health Institute, EPFL, Lausanne, Vaud, Switzerland.

Spiroplasma is a diverse bacterial clade that includes many vertically transmitted insect endosymbionts, including Spiroplasma poulsonii, a natural endosymbiont of Drosophila melanogaster. These bacteria persist in the hemolymph of their adult host and exhibit efficient vertical transmission from mother to offspring. Here, we identify the mechanism that underlies their vertical transmission, showing that these bacteria use the yolk uptake machinery to colonize the germline. We show that Spiroplasma reach the oocyte by passing through the intercellular space surrounding the ovarian follicle cells and are then endocytosed into oocytes within yolk granules during the vitellogenic stages of oogenesis. Mutations that disrupt yolk uptake by oocytes inhibit vertical transmission of Spiroplasma and lead to an accumulation of these bacteria outside of the oocyte. Impairment of yolk secretion by the fat body results in Spiroplasma not reaching the oocyte and a blockage of vertical transmission. We propose a model in which Spiroplasma first interacts with yolk in the hemolymph to gain access to the oocyte and then uses the yolk receptor, Yolkless, to be endocytosed into the oocyte. Co-option of the yolk uptake machinery appears to be a powerful strategy for endosymbionts to target the germline and achieve vertical transmission. This mechanism may apply to other endosymbionts and provides a possible explanation for endosymbiont host specificity.

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Intersection of *Drosophila* innate immunity and epidermal wound response in the serine proteolytic pathway. Michelle T. Juarez, Rachel A. Patterson, William McGinnis. University of California, San Diego, La Jolla, CA.

After injury to the animal epidermis a variety of genes are transcriptionally activated in nearby cells to regenerate the missing cells and facilitate barrier repair. The range and types of diffusible wound signals that are produced by damaged epidermis and function to activate repair genes during epidermal regeneration remains a subject of very active study in many animals. In *Drosophila* embryos, we have discovered that serine protease function is locally activated around wound sites, and is also required for localized activation of epidermal repair genes. Conversely, the serine protease trypsin is sufficient to induce a striking global epidermal wound response without inflicting cell death or compromising the integrity of the epithelial barrier. Genetic analyses combined with the trypsin treatment have placed serine protease activity downstream of a hydrogen peroxide response signal and upstream of a well-characterized pathway that regulates the transcriptional response to epidermal wounds genes (*grainy head, Flotillin-2, Dual oxidase, Src42A*). We used the trypsin wounding treatment as an amplification tool to more fully understand the changes in the *Drosophila* transcriptome that occur after epidermal injury. By comparing our array results with similar results on mammalian skin wounding we can see which evolutionarily conserved pathways are activated after epidermal wounding in very diverse animals. Our innovative serine protease-mediated wounding protocol allowed us to identify 8 additional genes that are activated in epidermal cells in the immediate vicinity of puncture wounds, and the functions of many of these genes suggest novel genetic pathways that may control epidermal wound repair. Additionally, our data augments the evidence that clean puncture wounding can mount a powerful innate immune transcriptional response, with different innate immune genes being activated either in epidermal cells in the immediate vicinity of wounds; in all epidermal cells; in the developing fat body; or in multiple tissues.

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The dynamics of tolerance and resistance in heterogeneous environments. Virginia Howick, Brian Lazzaro. Cornell University, Ithaca, NY.

Defense against pathogenic infection comes as a combination of resistance and tolerance. Resistance is the host's ability to limit pathogen burden, whereas tolerance is the host's ability to limit the health or fitness effects of that burden. This distinction recognizes that the fittest host may not have the most aggressive immune system. Studies of animal defense have focused almost completely on resistance to infection, while ignoring potential tolerance mechanisms. Using outbred genotypes derived from the *Drosophila* Genetic Reference Panel, we have dissected the relative contributions of resistance and tolerance to defense across dietary environments. We measured pathogen load, survival, and change in fecundity over five days after infection with *Providencia rettgeri*. Using a mathematical framework that allows for quantitative definitions of both resistance and tolerance, we were able to measure variation in both components and the relationship between them. It is possible to infer evolutionary costs associated with each strategy, and we note that the relative balance changes across dietary environment and over time. Our quantitative definitions of resistance and tolerance recognize that infection status is not a dichotomous state, but a continuum that may yield different defense strategies in different contexts. We have also provided a

framework for understanding the evolutionary constraints and trajectories of host defense, as well as how the collection of defense strategies a host employs could influence host-pathogen co-evolution and the transmission of infectious disease.

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Investigating the Host-Pathogen Interaction: Tolerance in Perspective. Kyung Han Song, David Schneider. Dept of Microbiology and Immunology, School of Medicine, Stanford University, Stanford, CA.

Hosts can protect themselves from infections using two different mechanisms; The first is "resistance," and it reduces microbe load. The second is "tolerance", which helps the host to endure the damage caused by infection. Tolerance is measured by plotting the dose response curve of host health versus microbe load across a population. In practice, researchers have used two points (or sometimes only one point) to define tolerance curves and thus we know nothing about the shape these curves and have had to assume that they are straight. We plotted the full length of tolerance curves for a Drosophila infection and found that they have a useful shape that suggests new types of analyses. We use an infection model in which we challenged fruit flies with Listeria monocytogenes, which grows in the fly and produces a lethal outcome. These tolerance curves are best fit with a sigmoidal model. This lets us apply analyses used to study drug action and we can now monitor the slope of the curve (Hill coefficient) and the median inhibitory dose of bacteria (IC50) and the range of the response (both maximum and minimum health). We applied this model to a variety of mutant flies and bacteria. The tolerance curves for flies with resistance or tolerance phenotypes have very different shapes from each other. Bacterial virulence mutants showed shifts in the IC50 while the shape of the curve remained constant. This approach provides a more complete representation of host-pathogen interactions and lets us treat infections in a more quantitative manner.

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Evolutionary change in fatty acid synthase expression underlies ecological divergence and reproductive isolation in a pair of Australian Drosophila species. Henry Chung[1], David Loehlin[1], Kathy Vacarro[1], Heloise Dufour[1], Jocelyn Millar[2], Sean Carroll[1]. 1) HHMI and Laboratory of Molecular Biology, University of Wisconsin, Madison, WI; 2) 2Department of Entomology, University of California, Riverside, CA.

Evolutionary changes in traits during ecological adaptation may contribute to reproductive isolation and speciation if they also play a role in mating. However, the genes underlying the production of such dual traits and the functional evolutionary changes within them have largely not been identified. Methyl-branched cuticular hydrocarbons (CHCs) of insects are potentially one such trait. These compounds can protect animals from desiccation but also have roles in sexual signaling, as in *Drosophila serrata*, a fruit fly widely distributed in Australia. Its rainforest-restricted sibling species, *D. birchii*, in contrast, produces low amounts of methyl-branched CHCs, and is extremely sensitive to desiccation. Here, we identify a fatty acid synthase gene, *mFAS* (*CG3524*), that is responsible for the production of methyl branched CHCs in *Drosophila* oenocytes, and show that *mFAS* expression is undetectable in *D. birchii* oenocytes. We demonstrate that transgenic RNAi-mediated knockdown of *mFAS* in *D. serrata*dramatically reduces desiccation resistance, as well as mating success. We suggest that ecologically-influenced changes in the expression of *mFAS* in the evolving *D. birchii* lineage have contributed to the reproductive isolation between the two species.

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Evolution of *miR-92a* underlies natural variation in the naked valley in *Drosophila melanogaster*. Saad Arif[1], Sophie Murat[2], Isabel Almudi[1], Maria Nunes[1], Diane Bortolamiol-Becet[3], Naomi McGregor[1], James Currie[1], Matthew Ronshaugen[4], Elio Sucena[5], Eric C. Lai[3], Christian Schlötterer[2], Alistair McGregor[1]. 1) Oxford Brookes University, Oxford, United Kingdom; 2) Institute for Population Genetics, Vetmeduni Vienna, Vienna, Austria; 3) Sloan-Kettering Institute, New York, NY, USA; 4) Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom; 5) Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Identifying the genetic basis of phenotypic change is essential to understanding how gene regulatory networks evolve and ultimately the genotype-to-phenotype map. While a range of mechanisms lie at evolution's disposal, it is possible that particular mechanisms, and even specific nodes in GRNs are targeted. Characterising the basis of natural variation in phenotypic traits is a powerful approach to identify the underlying genetic mechanisms, and thus the routes of evolution. *Drosophila melanogaster* subgroup species display a portion of trichome-free cuticle on the femur of the second leg called the 'naked valley'. It was previously shown that the Hox gene *Ultrabithorax* (*Ubx*) is involved in the naked valley variation between *D. melanogaster* and *D. simulans*. However, the size of the naked valley varies considerably among populations of *D. melanogaster* ranging from a small patch of 4000 um2 up to 40,000 um2. We investigated the genetic basis of this intra-specific variation in the naked valley in *D. melanogaster* and found that neither in *Ubx* or *shavenbaby* (*svb*), which underlies the evolution of larval trichomes, is responsible. Instead, we found a novel mechanism for the evolution of trichome patterns, where expression differences in *miR-92a* underlie changes in naked valley size in *D. melanogaster* via the differential down regulation of *shavenoid*. Therefore, our results show that morphological evolution can be caused by natural variation in microRNA expression and suggests that such changes in microRNAs may play a prominent role in fine scale morphological change within and between species.

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The power of a multivariate approach to genome-wide association studies. David Houle[1], Jessica Nye[1,2], Eladio Marquez[1],

William Pitchers[3], Alycia Kowalski[3], Ian Dworkin[3]. 1) Dept Biological Science, Florida State Univ, Tallahassee, FL; 2) Dept. of Genetics, North Carolina State Univ, Raleigh, NC; 3) Dept. of Zoology, Michigan State University, Lansing, MI.

Genome-wide association studies (GWAS) are almost invariably conducted on one phenotypic trait at a time, despite the fact that organisms present integrated patterns of variation. We demonstrate that a multivariate GWAS has increased power, and gives more interpretable results than a set of univariate analyses. We measured the shape of *Drosophila melanogaster* wings in the Drosophila Genome Reference Panel (DGRP) using the automated Wingmachine system. We analyzed data with 59 degrees of freedom that captures the size of the wing and the location of all the wing veins. Over 22,000 wings from 165 DGRP lines were measured in two different labs. We analyzed the data by MANOVA, which determines both the direction of the phenotypic effect in the 59 dimensional space, and the statistical significance of the effect. Inferred effects were very consistent across labs. After eliminating SNPs in strong gametic disequilibrium (GD) with nearby SNPs, we found 2711 of 1.5 X 10^6 SNPs had a significant effect at a false discovery rate of 5%. Causal inferences in the DGRP lines are greatly hampered by random disequilibrium between SNPs across the entire genome. SNPs with minor allele frequencies less than 10% are almost certain to be correlated at greater than $r^2>0.8$ with at least one SNP elsewhere in the genome - usually on a different chromosome. Simulations show that this random GD effect alone can explain the tendency of small MAF SNPs to have large estimated effects. We have validated hits by comparing of the effect vectors of RNAi knockdowns for several implicated genes including *ds* and *dpp*. The multivariate vector of phenotypic effects makes informative validation much easier as it is very unlikely that similar directions of effects will be generated with no causal connection. Similarly, when two effect vectors for SNPs not in GD are similar in direction this is very likely to be due to similar mechanisms of development.

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The severity of a mitochondrial-nuclear incompatibility depends upon the developmental thermal environment. Kristi L. Montooth, Luke A. Hoekstra, Mohammad A. Siddiq. Dept Biol, Indiana Univ, Bloomington, IN.

Energetic performance can create a dynamic context for the effects of mutations. Given that protein-protein and RNA-protein interactions between mitochondrial and nuclear genomes underlie energetic performance in eukaryotes, we expect that the effects of many mitochondrial mutations will be conditional on variation in the nuclear genome. Furthermore, in ectotherms, the phenotypic effects of these mitochondrial-nuclear interactions may be conditional on the thermal environment, because temperature impacts rates of biological processes and can place high demand on energy use. I will present data that demonstrate strong thermal-dependence of the phenotypic effects of a mitochondrial-nuclear genetic interaction. We have previously found that a mitochondrial-nuclear incompatibility between a single nucleotide polymorphism in the *D. simulans* mt-tRNA-Tyr and a non-synonymous polymorphism in the nuclear-encoded *D. melanogaster* mt-Tyr-tRNA synthetase encoded by *Aatm* severely affects development and reproduction via compromised mitochondrial protein translation (C.D. Meiklejohn, M.A. Holmbeck, M.A. Siddiq, D.N. Abt, D.M. Rand and K.L. Montooth *manuscript in review*). Remarkably, a shift in developmental temperature from 25°C to 16°C masks these deleterious effects, while a shift to 28°C results in male and female sterility. Mitochondrial-nuclear epistatic effects on development time, pupation height and reproduction - traits that are associated with energetic state - are all worse when temperature accelerates the rate of life. I will present these results in the context of what we have recently learned about the molecular evolution and population genomics of *Drosophilid* mitochondrial versus nuclear genes relative to humans, mammals and other invertebrates.

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A Drosophila Model to Investigate Natural Variation Effect in Response to Expression of A Human Misfolded Protein. Bin He[1], Michael Ludwig[1], Soo-Young Park[2], Pengyao Jiang[1], Cecelia Miles[3], Levi Barse[1], Desiree Dickerson[1], Sarah Carl[1], Graeme Bell[2], Martin Kreitman[1]. 1) Department of Ecology & Evolution, The University of Chicago, Chicago, IL; 2) Department of Medicine, The University of Chicago, Chicago, IL; 3) Biology Department, Augustana College, Sioux Falls, SD.

Identifying the genetic variants and the underlying molecular mechanism for disease variability is crucial in both complex and Mendelian disease. However, its progress has been hampered by the mapping resolution and further experimental challenges in human, leaving many basic questions unanswered: what types of variants? how do they act and interact? Here we present a novel approach to the genetic investigation of a complex disease trait, featuring high mapping resolution and experimental tractability in a *Drosophila* model of human disease. The approach uses natural genetic variation in *Drosophila* to screen for modifying loci in a sensitized disease background, created by expressing a mutant (disease-causing) form of human proinsulin in the developing eye imaginal disc, causing neuro-degeneration in the eye that mimics the β-cell death in human patients. Crossing this transgenic line to a panel of 178 inbred lines of *D. melanogaster* resulted in a continuous distribution of the disease phenotype. GWAS in 154 sequenced lines identified multiple loci, with the strongest signal fine-mapped to a 400bp region in the intron of the gene *sulfateless* (*sfl*). RNAi knock-down of *sfl* enhanced the eye phenotype in a mutant-proinsulin-dependent manner; the same approach identified two more genes in the Heparan Sulfate Proteoglycan (HSPG) pathway, to which *sfl* belongs, strongly suggesting a previously unknown link between HSPG and cell response to misfolded protein. Finally, we used pyro-sequencing to show evidence of allele-specific expression associated with the *sfl* intronic variants, revealing the potential mechanism of the non-coding variants in regulating the host-gene expression.

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From missing genotypes to negative epistasis. Russ Corbett-Detig, Jun Zhou, Daniel Hartl, Julien Ayroles. OEB, Harvard University, Cambridge, MA.

Negative epistasis, or genetic incompatibilities resulting from interactions between loci, is believed to be an important force in speciation. To date the majority of "speciation genes" that have been characterized affect hybrids of ancient speciation events. In most cases it is unclear if these loci could have contributed to the initial genetic isolation of taxa. Here we present an alternative approach that aims to identify incompatibiliies segregating within a species. Specifically, we scanned panels of Drosophila melanogaster recombinant inbred lines for inter-chromosomal linkage disequilibria. In total, we identified eightteen pairs of incompatible haplotypes, and conservatively estimate that any two haploid genomes have one in three chance of harboring a pair of incompatible alleles. Building on the genome scan, we showed that one pair of incompatible alleles causes almost complete male sterility. Our results indicate that natural populations are segregating many cryptic incompatible alleles. This suggests that postzygotic isolating barriers exist prior to divergence between populations and that speciation may be a highly polygenic phenomenon.

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Adaptation to hypoxia in experimentally evolved Drosophila melanogaster: convergent and highly polygenic. Aashish R. Jha[1,2], Christopher D. Brown[2], Dan Zhou[3], Gabriel H. Haddad[3], Kevin P. White[1,2,4]. 1) Human Genetics, The University of Chicago, Chicago, IL; 2) Institute of Genomics and Systems Biology, The University of Chicago, Chicago, IL; 3) Department of Pediatrics, University of California an Diego; 4) Ecology and Evolution, The University of Chicago, Chicago, IL.

Adaptation to low oxygen (hypoxia) has fascinated biologists from multiple disciplines. Despite years of research the comprehensive genetic architecture of hypoxia tolerance remains elusive. We implemented experimental evolution followed by whole-genome sequencing approach in Drosophila melanogaster to investigate the role of natural variation in adaptation to hypoxia. Significant allele frequency divergence between replicate hypoxia-tolerant populations and normoxic controls were observed at ~3000 polymorphic loci distributed throughout the genome; however, scans for reduction in gene diversity showed selective sweeps were rare. This suggests adaptation to hypoxia occurred almost exclusively from the natural standing variants via soft sweeps and heterozygosity based tests are poorly suited to identify polygenic adaptation occurring from standing variation. The differentiated variants were harbored by ~1400 genes. Filtering these genes based on evolutionary conservation and differential gene expression identified ~600 positively selected genes that are involved in various development processes including respiratory and tracheal systems development, several metabolic processes and neuron generation. Genes in Wnt and Cadherin pathways were significantly enriched and many genes have known functions in Notch and EGFR pathways. Most notable positively selected genes included Drosophila homologs of EPAS1, PPARA, and GCH1, the classic O2-sensing genes under selection in high-altitude Tibetans. Human orthologs of many positively selected genes in our Drosophila populations have known functions in cancer. This suggests that adaptation to hypoxia is convergent and highly polygenic and Drosophila melanogaster can be an excellent model system to understand genetic pathways and networks involved in cancer.

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The spatial distribution of tension on E-cadherin in migrating border cells. Danfeng Cai[1], Li He[2], Jessica Sawyer[3], Denise Montell[1]. 1) Department of Biological Chemistry, Johns Hopkins University, Baltimore, MD; 2) Department of Genetics, Harvard Medical School, HHMI, Boston, MA; 3) Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC.

Cell migration involves constant interactions between the cell and its environment. While great progress has been made in identifying the molecules that mediate these interactions, tools for measuring mechanical forces in tissues without perturbing them have been lacking, limiting our ability to probe how biochemical signals and mechanical forces feed back on one another during morphogenesis. In order to overcome this obstacle, we developed a FRET-based sensor to measure tension across E-Cadherin molecules in vivo with high spatial resolution and without perturbing the system. We have used this E-Cadherin tension sensor to examine the spatial distribution of forces during collective border cell migration in the Drosophila ovary.

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Real-time analysis of the dynamics of coordinated epithelial plasticity. Lara C. Skwarek, David Bilder. Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

During development, morphogenetic rearrangements often result from regulated changes in both individual and collective cell behavior. One such example is the remarkable cellular transformation known as epithelial-mesenchymal transition (EMT). During EMT, epithelial cells dramatically alter polarity, change shape and acquire mesenchymal characteristics, often without cell division. Such programs of cellular plasticity are essential for normal development and are also hallmarks of tumor metastasis. Strikingly, we still lack a comprehensive knowledge of the integrated mechanisms involved in coordinating such plasticity *in vivo*, leaving a large gap in our understanding of these important cellular events during both development and disease. To address this I have been studying the final steps of wing development, during which a bilayered epithelium comprising the immature wing disassembles, allowing for rapid maturation of the adult wing. Though this process differs from classical EMT events, it nevertheless requires coordination of the disassembly and ultimate death of an epithelium with secretion of components required for wing maturation. Using a combination of high resolution live imaging and genetic manipulation I have observed that regulated loss of E-cadherin and adherens junction components precedes dramatic shape changes within the epithelium. In addition, preliminary results indicate that knocking-down known regulators of plasticity with RNA interference causes defects in wing maturation. This system is particularly suited for studying the early stages of

epithelial cell transition, and together with an unbiased forward genetic screen, these studies will identify new mechanisms regulating the cell biology of epithelial transition and cellular plasticity.

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Robo2 shapes Slit-dependent muscle repulsion by altering the association of Slit to tendon cell surface. Elly Ordan[1], Marko Brankatschk[2], Frank Schnorrer[3], Talila Volk[1]. 1) molecular genetics, Weizmann Institute of Science, Rehovot, Israel; 2) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 3) Muscle Dynamics,Max-Planck-Institute for Biochemistry, Munich,Germany.

Every segment of the Drosophila embryo contains a highly reproducible pattern of muscles, which is the result of muscle specification, fusion, migration, and attachment. The information regarding the control of muscle migration and targeting to tendon cells is limited. The Robo-Slit pathway has emerged in a number of studies concerning muscle pattern formation. This study focuses on Robo2-mediated Slit signaling in muscle migration. We found that Robo2, although expressed by the Slit-secreting tendon cells and not by muscles, was essential for proper migration of a subset of muscles. Robo2 and Slit genetically interact in the context of muscle migration, and overexpression of Robo2 by the ectoderm induced Slit cleavage. Moreover, consistent with a functional significance of Slit cleavage we found that uncleavable Slit, inserted by homologous recombination into the slit locus did not rescue the effects of Slit loss of function on muscle migration. However, membrane-bound, uncleavable Slit did rescue slit phenotype, suggesting that Slit cleavage is essential for its association with the tendon cell membrane. Consistent with this idea, membrane-bound uncleavable Slit is primarily detected on tendon cells membrane and can partially rescue the muscle phenotype of robo2. Live imaging has shown that the muscles migrate in close proximity to Slit- and Robo2-expressing cells but avoid entering their expression domain. Based on these findings, we propose a model whereby tendon-cell-expressed Slit is secreted, trapped by Robo2 and undergoes cleavage, which is necessary for Slit tethering to the membrane. The membrane-tethered Slit is then presented to the migrating muscle and serves as a short-range repellent of the migrating muscle.

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Regulation of Hippo signaling by EGFR-MAPK signaling through Ajuba. Venu Reddy Bommireddy Venkata, Ken Irvine. Waksman Institute, Piscataway, NJ.

Epidermal growth factor receptor signaling plays an important role in growth control, and inappropriate activation of EGFR signaling has been implicated in several cancers. Likewise, the recently discovered Hippo signaling plays a crucial role both in controlling normal growth during development, and when dysregulated contributes to tumorigenesis. Here, we identify and characterize a conserved link between these pathways. We find that EGFR activates the Hippo pathway transcription factor Yorkie, and demonstrate that Yorkie is required for the influence of EGFR on cell proliferation in both glial cells and wing imaginal discs of Drosophila. We determine that EGFR regulates Yorkie through the Ras-MAPK branch of EGFR signaling. Genetic and biochemical experiments implicate the Ajuba LIM protein Jub as the key target of EGFR-Ras-MAPK signaling within the Hippo pathway, as Jub is epistatic to EGFR and Ras for Yorkie regulation, Jub is subject to MAPK-dependent phosphorylation, and EGFR-Ras-MAPK signaling enhances Jub binding to the Yorkie kinase Warts, and to the scaffolding protein Salvador. We further show that an EGFR-Hippo pathway link is conserved in mammals, as activation of EGFR or RAS results in activation of the Yorkie homologue YAP, and EGFR-RAS-MAPK signaling promotes phosphorylation of the human Ajuba family protein WTIP, and also promote WTIP binding to the Warts and Salvador homologues LATS and WW45. Our observations implicate the Hippo pathway in EGFR-mediated tumorigenesis and identify a novel molecular link between these two pathways.

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Src controls tumorigenesis through JNK-dependent regulation of the Hippo pathway. Masato Enomoto[1], Tatsushi Igaki[1,2]. 1) Division of Genetics, Kobe University Graduate School of Medicine, Kobe, Japan; 2) PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan.

Cell-cell interactions within the tumor microenvironment play crucial roles in epithelial tumorigenesis. However, the mechanism by which each genetic alteration contributes to oncogenic cell-cell communication is poorly understood. Here, we show that the oncoprotein Src regulates tumor microenvironment by JNK-dependent regulation of the Hippo pathway. Clones of cells with elevated Src expression activate the Rac-Diaphanous (Dia) and Ras-MAPK pathways, which cooperate to cause intracellular accumulation of F-actin, thereby leading to activation of the Hippo pathway effector Yorkie (Yki). Simultaneously, Src activates the JNK pathway, which antagonizes the autonomous Yki activity and causes propagation of Yki activity to neighboring cells, thereby inducing overgrowth of surrounding tissue. Blocking JNK signaling in Src-expressing clones cancels the propagation of Yki activity and leads to autonomous tumor overgrowth. Our findings unveil a mechanism of Src-induced tumorigenesis through JNK-dependent switch of Yki activity and would help understand how oncogene Src regulates tumor microenvironment *in vivo*.

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The Hippo Pathway targets the Cdh1/fzr inhibitor Rae1 to regulate mitosis and establish organ size homeostasis. Maryam Jahanshahi[1], Kuangfu Hsiao[2], Andreas Jenny[3], Cathie Pfleger[1]. 1) Department of Oncological Sciences, Mount Sinai School of Medicine, New York, NY; 2) Fishberg Department of Neuroscience, Mount Sinai School of Medicine, New

York NY; 3) Department of Molecular and Developmental Biology, Albert Einstein College of Medicine, Bronx NY.

The Hippo Tumor Suppressor pathway serves as a master regulatory axis which coordinates proliferation, growth, and apoptosis to establish and maintain appropriate organ size. It is well established that loss of pathway components promotes cell division, cell death resistance, and tumor-like overgrowth in both Drosophila and vertebrates. Loss of Hippo Pathway activity is also implicated in initiation and progression of a range of cancers including colorectal cancer, liver cancer, melanoma, lung cancer, leukemias, and ovarian cancer. Therefore the Hippo pathway has an essential role in organ size regulation and tumorigenesis. Although it is clear how the pathway promotes cell death resistance, crucial targets responsible for the distinct functions of restricting growth and restricting cell proliferation and specific effectors responsible for coordinating organ size and proliferation remain largely unknown. We have identified the Cdh1-inhibitor Rae1 at the nexus within the Hippo Pathway integrating proliferation and organ size. Exogenous Rae1 increases both cell proliferation and organ size. Rae1 is required in vivo for S-phase entry and mitotic progression and is phosphorylated and degraded upon activation of Hippo signaling. We propose a model that Hippo signaling promotes Cdh1-Anaphase Promoting Complex/Cyclosome activity by relieving its Rae1-mediated inhibition. Importantly, Rae1 reduction compromises survival of Hippo-deficient tissue indicating synthetic lethality and a requirement for Rae1 reminiscent of oncogene/non-oncogene "addiction". The "Rae1 addiction" of tissue upon loss of Hippo pathway activity further implicates Rae1 in tumorigenesis and suggests that Rae1 may represent a therapeutic target for cancers in which Hippo signaling is dysregulated.

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dCORL is required for dSmad2 activation of Ecdysone Receptor expression in the Drosophila mushroom body. Stuart J. Newfeld[1], Michael Stinchfield[1], Kazumichi Shimizu[2], Mayu Arase[3], Janine Quijano[1], Tetsuro Watabe[3], Kohei Miyazono[3], Norma T. Takaesu[1]. 1) Sch Life Sci, Arizona State Univ, Tempe, AZ; 2) Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan; 3) Department of Molecular Pathology, University of Tokyo, Tokyo 113-0033, Japan.

CORL proteins (fussel in humans) are related to Sno/Ski oncogenes but their developmental roles are unknown. We cloned dCORL and show its expression is restricted to distinct subsets of cells in the central nervous system. We generated a deletion of dCORL and noted that homozygous individuals rarely survive to adulthood. Df(4)dCORL adult escapers display mushroom body defects and Df(4)dCORL larvae are missing Ecdysone Receptor (EcR-B1) expression in mushroom body neurons. This is phenocopied in dCORL-RNAi and dSmad2-RNAi clones in wild type larvae. Further, constitutively active Baboon (Type I receptor upstream of dSmad2) cannot stimulate EcR-B1 mushroom body expression in Df(4)dCORL larvae demonstrating a formal requirement for dCORL in dSmad2 signaling. Studies of mCORL1 revealed that it binds specifically to Smad3. Overall the data suggest that dCORL facilitates dSmad2 activity upstream of EcR-B1 in the mushroom body. The conservation of neural expression and strong sequence homology of all CORL proteins suggests that this is a new family of Smad co-factors.

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Steroid-induced microRNA let-7 acts as a spatio-temporal code for neuronal cell fate in the developing Drosophila brain. Mariya M. Kucherenko, Halyna R. Shcherbata. MPRG of Gene expression and signaling, Max Planck Institute for biophysical chemistry, Goettingen, Germany.

Cell fate decisions are determined by an activation and repression of lineage-specific genes. In this context microRNAs (miRNAs), small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level, are important factors that maintain the balance between stem cell self-renewal, proliferation and differentiation during embryonic development and adult life. We found that in the post-embryonic Drosophila brain, cell fate of late-born neurons in the mushroom body, a brain region critical for olfactory learning and memory is regulated by the miRNA let-7 that is expressed in response to developmentally regulated steroid pulses. More specifically, ecdysteroid-induced miRNA let-7 controls the neuronal switch from α'/β' to α/β neurons that happens at the prepupal to pupal stage, which is one of many changes occurring at this developmental transition regulated by ecdysone signaling. let-7 is required cell autonomously for proper differentiation of the last-born α/β neurons and its deficiency leads to α/β lobe morphological defects that affect olfactory learning and memory. The cellular effect of steroid-hormone-induced let-7 expression is a modulation of levels of the cell adhesion molecule Fasciclin II (Fas II) in differentiating neurons partially via a posttranscriptional regulation of the transcription factor Abrupt (Ab) that we show to be a key factor for establishing α'/β' neuron identity. The differential adhesion hypothesis helps to explain how neurons that express different levels of cell adhesion proteins cluster and form complex internal brain structures, e.g. Drosophila mushroom bodies. Taken together, our data demonstrate that the miRNA let-7 is a steroid hormone-dependent cell fate determinant serving as a temporal code along with spatially controlled lineage cues to specify neuronal cell fate.

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miRNome analyses reveal K box miRNAs function in mediating class specific dendrite morphogenesis. Srividya Chandramouli Iyer[1], Myurajan Rubaharan[1], Ramakrishna Meduri[1], Shruthi Sivakumar[1], Francis Aguisanda[1], Suhas Gondi[1], Atit Patel[1], Eswar P R Iyer[1], Diane Bortolamiol-Becet[2], Eric C. Lai[2], Daniel N. Cox[1]. 1) School of Systems Biology, Krasnow Inst. Adv. Study, George Mason University, Fairfax, VA; 2) Sloan-Kettering Institute, Dept. Developmental Biology, New York, NY.

While microRNAs (miRNAs) have recently emerged as critical post-transcriptional modulators of gene expression in neuronal development, very little is known regarding the roles of miRNA-mediated regulation in the specification of cell-type specific dendritic complexity. The dendritic arborization (da) sensory neurons of the *Drosophila* PNS offer an excellent model

system for elucidating the molecular mechanisms governing class specific dendrite morphogenesis and for exploring miRNA-mediated control of this process. To facilitate functional analyses of miRNA regulation in da neurons, we have conducted whole-genome miRNA expression profiling as well as mRNA expression profiling of three distinct classes of da neurons, thereby generating a comprehensive molecular gene expression signature within these individual subclasses of da neurons. To further validate the role of the significantly expressed miRNAs in directing dendritic architecture, we conducted a genome-wide UAS-miRNA phenotypic screen using live-image confocal microscopy to directly assess the effect of over/mis-expression of individual and clustered miRNAs on neurons of varying dendritic complexity. Through this approach, we have identified numerous miRNAs with previously unknown functions in dendritic development, including the K box family of miRNAs. Both gain-of-function and loss-of-function analyses, via miRNA sponge transgenes, reveal that K box miRNAs repress the expression of genes required to restrict dendritic branching complexity in da neuron subclasses. Moreover, we have implemented an integrative bioinformatic analysis approach involving inverse correlation between miRNA and mRNA expression profiling data in combination with existing target prediction algorithms to identify putative target of miRNAs in regulating da neuron dendritic development.

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Piwi Is Required in Multiple Cell Types to Control Germline Stem Cell Lineage Development in the Drosophila Ovary. Xing Ma[1,2]. 1) Stowers Institute for Medical Research, Kansas city, MO; 2) Department of Anatomy and Cell Biology, University of Kansas, Medical Center.

Background: The piRNA pathway plays an important role in maintaining genome stability in the germ line by silencing transposable elements (TEs). In the Drosophila ovary, escort cells (ECs) physically interact with differentiated germline stem cell (GSC) progeny and promote their differentiation by preventing BMP signaling. Although piRNAs are known to be produced in Drosophila ovarian somatic cells, their biological function remains poorly defined. Results: Using genetics and cell biology approaches, we demonstrated that Piwi, a key piRNA pathway component, functions in multiple cell types to control GSC maintenance and differentiation. EC-specific knockdown of piwi causes a reduction in EC number and accumulation of GSC-like cells in which BMP signaling activity is elevated. In the piwi knockdown ECs, TE transcripts increase significantly and consequently DNA damage is also elevated. Interestingly, simultaneous knockdown of chk2, encoding a key checkpoint regulatory kinase, can rescue the GSC lineage differentiation defect caused by piwi knockdown, indicating that DNA checkpoint activation is the cause for the germ cell differentiation defect. Although Piwi is proposed to function in the niche for maintaining GSCs, niche-specific piwi knockdown only causes moderate GSC loss phenotype. Surprisingly, germ cell-specific knockdown of piwi but not aub and armi, results in complete germ cell loss, indicating that Piwi is required intrinsically to control early germ cell development in a piRNA-independent pathway. Conclusions: Our results therefore have revealed novel functions of Piwi in ECs to promote germ cell differentiation and in early germ cells for their maintenance. We propose that Piwi is required in ECs to promote germ cell differentiation by maintaining genome stability and.

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Role of the nuclear pore in piRNA biogenesis and speciation. Swapnil Parhad[1], Jie Wang[2], Zhiping Weng[2], William Theurkauf[1]. 1) Program in Molecular Medicine,Univ Massachusetts Med Sch, Worcester, MA; 2) Program in Bioinformatics and Integrative Biology, Univ Massachusetts Med Sch, Worcester, MA.

Crosses between recently diverged species, which often carry distinct transposon families, can lead to hybrid lethality or sterility. *Drosophila nucleoporin 160kDa (Nup160)* has been implicated in hybrid incompatibility between *Drosophila melanogaster* and *Drosophila simulans*. The gene appears to be evolving rapidly under positive selection, which is often linked to a host-pathogen interaction. Piwi-interacting RNAs (piRNAs) repress transposons in the germline, several piRNA pathway genes appear to be evolving under positive selection, piRNA mutations lead to sterility and developmental arrest during early embryogenesis, and the piRNA machinery appears to be organized around nuclear pores. These observations led us to speculate that *Nup160* and the transposon silencing machinery may be co-evolving in response to transposon invasion. To test this hypothesis, we have used transgenes expressing either *D. melanogaster* Nup160 (DmNup160) or *D. simulans* Nup160 (DsNup160) to rescue null mutants in the *D. melanogaster Nup160* locus. We find that ubiquitous expression of DmNup160 rescues the lethal and sterile phenotypes associated with *Nup160* null mutants. By contrast, expression of DsNup160 restores viability, but not fertility, and the resulting females produce eggs that fail to hatch due to mitotic division failure and developmental arrest during early embryogenesis. DsNup160 can therefore support housekeeping roles for the nuclear pore during *D. melanogaster* development, but is defective in a germline specific function. Small RNA sequencing shows that expression of DsNup160 leads to a global suppression of piRNA ping-pong amplification and significantly reduces expression of major chromosome 4 cluster, which produces piRNAs to telomeric transposons. These observations suggest that co-evolution of piRNA genes and *Nup160* generates species specific interactions between nuclear pores and transposon silencing machinery that may contribute to hybrid incompatibility.

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Modeling Spinal Muscular Atrophy point mutations in *Drosophila melanogaster*. A Gregory Matera, Kavita Praveen, Ying Wen. Department of Biology, Univ of North Carolina, Chapel Hill, NC.

Spinal Muscular Atrophy (SMA) is a prevalent childhood neuromuscular disease. In its most common form, SMA causes death by the age of two years. The disease is caused by loss-of-function mutations in the *survival motor neuron 1 (SMN1)* gene. SMN

is an essential protein and has a well-characterized role in the biogenesis of small nuclear ribonucleoproteins (snRNPs), which are core components of the spliceosome. Numerous additional functions for SMN have been put forth in the literature, however, no convincing link has been made between any putative SMN function and the disease etiology. We have studied the consequences of SMN loss in the *Drosophila* model system by generating a series of transgenic flies that exclusively express mutant forms of SMN that mimic mutations identified in human SMA patients. Null mutants in *Smn* die as larvae, have significant locomotor defects and reduced levels of minor-class snRNAs. Surprisingly, despite these reductions, minor-class intron splicing in *Smn* null mutants is unperturbed. In addition, transgenic expression of low levels of a wild-type or an SMA patient-derived mutant dSMN rescued the larval lethality and locomotor defects, however, snRNA levels were not restored. These data provide genetic evidence that non-snRNP related functions of SMN may be critical to SMA pathology. We have also generated flies carrying twelve additional SMA patient-derived *Smn*point mutations. These mutants vary in severity, recapitulating the full range of severity observed in humans. We are currently using these animals for RNA-seq and proteomic analyses to understand the differential effects of these mutations. These new SMA models will be important tools in identifying functions of SMN that are etiologic for SMA.

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A conserved RNA processing pathway coordinates striated muscle development. Aaron N. Johnson[1,3], Mayssa M. Mokalled[2], Kenneth D. Poss[2], Eric N. Olson[3]. 1) Department of Integrative Biology, University of Colorado Denver, Denver, CO; 2) Department of Cell Biology and Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC; 3) Department of Molecular Biology, UT Southwestern Medical Center at Dallas, Dallas, TX.

Striated muscle differentiation requires the coordinated expression of arrays of genes involved in sarcomere formation and contractility. Although muscle structural genes are dispersed throughout the genome, sarcomeric proteins are somehow simultaneously assembled at specific subcellular locations through a largely unknown post-transcriptional mechanism. In a genetic screen for regulators of muscle development in Drosophila, we identified the RNA binding protein Hoi Polloi (Hoip). Remarkably, numerous sarcomeric proteins fail to be expressed in *hoip* mutant embryos even though the transcriptional activator of muscle structural genes, Mef2, is expressed at normal levels. Hoip physically interacts with multiple sarcomeric mRNAs and is required for their processing and nuclear export. In addition, the human Hoip orthologue NHP2L1 rescues muscle defects in *hoip* embryos, and knockdown of endogenous *nhp2l1* in zebrafish blocks skeletal muscle differentiation. Thus, sarcomeric RNAs transcribed from dispersed genomic loci are co-regulated by a conserved regulatory network that directs the precise assembly of the contractile apparatus during myogenesis.

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Identification of a novel splicing factor required for proper myotendenous junction formation and maintenance in *Drosophila*. Kate M. Rochlin[1,2], Mary Baylies[1]. 1) Dept Dev Biol, Sloan-Kettering Inst, New York, NY; 2) Weill Cornell Biomedical University New York, NY.

The anchoring of the musculoskeletal system links the force-producing muscles to the skeletal system of the organism, allowing stability and movement. In *Drosophila*, the connection between muscles and the exoskeleton occurs via tendon-like cells that develop in the ectoderm. A number of cross-regulatory interactions are required for targeting muscles to tendons and the subsequent formation of the myotendinous junction (MTJ). Key MTJ pathways rely on rapid changes in protein and isoform expression. However, how this splicing is regulated, either temporally or in a tissue specific manner, is unclear. In a screen to find new genes required for muscle morphogenesis, we uncovered a novel predicted member of the SR family of splicing factors, which are pivotal regulators of all aspects of mRNA metabolism. We named this gene *missed connections (mcx)* based on its muscle mis-attachment phenotype. Mcx is expressed in the embryonic musculature and localizes to nuclear speckles, consistent with the subcellular localization of other splicing factors. Mutations in *mcx* fail to form robust MTJs and show changes in localization of crucial attachment site proteins that undergo alternative splicing regulation such as tropomyosin and integrins. The distribution of other proteins known to localize at the MTJ is also affected. We propose that splicing via Mcx is essential to regulate expression and isoform switching of critical proteins required for MTJ formation and maintenance. *mcx*Identification of a novel splicing factor required for proper myotendenous junction formation and maintenance in Drosophila is conserved and expressed in mammalian muscle. Since mammalian skeletal muscle also requires alternate splicing and changes in splicing patterns have been linked to muscle disease, we predict our work will define fundamental mechanisms of splicing regulation critical for muscle biology in all organisms.

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Synaptic endosomes as sorting stations for synaptic vesicle proteins. Valerie Uytterhoeven, Ine Maes, Sabine Kuenen, Jaroslaw Kasprowicz, Katarzyna Miskiewicz, Patrik Verstreken. Center for human Genetics, KU Leuven, Center for the Biology of Disease, VIB, Leuven, Vlaams-Brabant, Belgium.

Neuronal terminals that are located far away from their cell bodies largely operate as independent units during long periods of stimulation. While many proteins in vesicle fusion and reformation are characterized, it is not known how synaptic terminals replace dysfunctional proteins and lipids and incorporate fresh ones to protect against synaptic ageing. Recently, we uncovered a protein we named Skywalker (Sky) that controls the sorting and degradation of dysfunctional proteins. Sky contains a TBC domain that is commonly found in GTPase Activating Proteins (GAPs). Our work indicates that Sky accelerates the GTPase activity of Rab35, a member of the Rab GTPase family that controls specific vesicle trafficking events, in vitro and

in vivo at synapses. Furthermore, in sky mutants or in animals with constitutive active Rab35, newly formed synaptic vesicles are forced to travel excessively via an endosomal compartment at the nerve terminal. At these stations, dysfunctional, ubiquitinated synaptic vesicle proteins are recognized and sorted for degradation in the lysosome. As a consequence, vesicles that leave the endosome in sky mutants or in animals that express GTP-bound active Rab35, harbor a larger percentage of functional proteins in their membranes and as a result, sky mutants (or active Rab35) display increased neurotransmitter release. Hence, Sky controls synaptic ageing and loss of Sky function results in a more performant synaptic release apparatus. Sky defines a novel molecular mechanism that is used in neurons to control ageing and synaptic plasticity and our ongoing work is geared towards further elucidating the synaptic Sky-pathway using genetic modifier screens based on electrophysiology as well as using yeast two hybrid interaction screens.

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Cold avoidance and cold sensing in the *Drosophila* larva. Mason Klein[1,2], Ashley Vonner[1,3], Marc Gershow[1,2], Elizabeth Kane[1,3], Bruno Afonso[1], Paul Garrity[4], Aravinthan Samuel[1,2]. 1) Center for Brain Science, Harvard University, Cambridge, MA; 2) Department of Physics, Harvard University, Cambridge, MA; 3) Program in Biological and Biomedical Sciences, Harvard Medical School, Boston, MA; 4) Department of Biology, Brandeis University, Waltham, MA.

Response to temperature to reach environmental conditions conducive to survival and prosperity is a universally important behavior in all animals. Using the *Drosophila* larva as a model system, we connect the activity of cold sensing neurons to behavioral cold response. In particular, we identify a previously uncharacterized group of neurons with a unique morphology in each dorsal organ ganglion (DOG) that respond specifically to cooling. We map projections of these cold sensing neurons to the larval antennal lobe (LAL), where they innervate a region distinct from that of the olfactory receptor neurons also found in the DOG. We use *in vivo* 3D confocal imaging to monitor calcium activity while modulating temperature. The sensitivity to cooling and activation thresholds of these neurons are consistent with quantitative analysis of larval navigation on linear spatial temperature gradients, where crawling larvae also respond specifically to cooling. Further, laser ablation of the antennal nerve connecting the DOG to the central brain demonstrates that DOG neurons are required for cold avoidance behavior. These results point toward a more complete neuronal circuit understanding of temperature sensorimotor transformation in the larva with potential applications to higher organisms.

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***Drosophila* taste receptors reveal combinatorial and cross-modality functions.** Erica Freeman[1], Alice French[2], Zev Wisotsky[3], Frédéric Marion-Poll[2,4], Anupama Dahanukar[1,3,5]. 1) Bioengineering Graduate Program, University of California, Riverside, CA; 2) INRA, Physiologie de l'Insecte: Signalisation et Communication, Versailles, France; 3) Neuroscience Program, University of California, Riverside, CA; 4) AgroParisTech, Départment Sciences de la Vie et Santé, Paris Cedex 05, France; 5) Department of Entomology, University of California, Riverside, CA.

Drosophila melanogaster use a highly diverse group of gustatory receptors (Gr) to taste the chemical world and determine the palatability of potential food sources. The 68 receptors of this family are expressed in complex combinatorial patterns in taste neurons. Of these, eight belong to a sub-family of putative sugar receptors, at least four of which have been directly linked to the detection of sweet compounds by genetic analysis. Here we use an ectopic expression system to identify that each sweet Gr protein serves as a determinant for recognition of unique but overlapping subsets of sweet tastants. Together with analysis of available Gr mutants, the ectopic response profiles suggest a model in which receptors act in combinations of two or more Gr subunits, each contributing to ligand recognition and specificity. Interestingly, we discover that sweet Grs are directly inhibited by bitter alkaloids, and individual Gr proteins display specificity for bitter antagonists. Recordings from *Drosophila* confirm that alkaloids can inhibit sugar responses of sweet taste neurons in a manner that is independent of their excitatory activity on bitter taste neurons. A comparison of sweet neuron responses in two species of mosquitoes, *Anopheles gambiae* and *Aedes aegypti*, suggests that such mechanisms of sweet receptor inhibition by bitter alkaloids may be evolutionarily conserved. Our results reveal combinatorial mechanisms for sweet and bitter ligand recognition by sweet taste receptors, and lay the foundation for further investigation of Gr function in *Drosophila* and other insects.

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Evolved changes in pheromone production underlie differences in larval social behaviors between closely related *Drosophilids*. Joshua D. Mast, David L. Stern. Janelia Farm Research Campus, HHMI, Ashburn, VA.

Both the genetic and neurobiological mechanisms underlying the evolution of behavior are not well understood. Species in the *melanogaster* subgroup provide an opportunity to explore these mechanisms. While still closely related and amenable to traditional genetic analysis and techniques, these species occupy different ecological niches and have a variety of divergent behavioral traits. For example, we have found that larval social signaling in this species group has evolved. *D.melanogaster* and *D.sechellia* larvae are attractive to other larvae, while *D.simulans* larvae are not. We have identified both a novel attractive larval pheromone whose production has evolved between these species, and a single pair of gustatory neurons in *D.melanogaster* that are required to respond to this compound. By comparing the bouquet of compounds produced by larvae in these species, and then screening these molecules for attractive activity in behavioral assays, we identified a fatty acid monene pheromone. This attractive pheromone is produced by both *D.melanogaster* and *D.sechellia* larvae, but not by *D.simulans*. The attraction to this pheromone in *D.melanogaster* larvae is

not affected by silencing chemosensory neurons expressing genes required in the adult fly to detect sex pheromones, namely *Or83b, Gr66a* and *Gr33a*. Rather, attraction is abolished by silencing a pair of gustatory neurons we identified in a targeted silencing screen using the Rubin fragment GAL4 collection.

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A sexually dimorphic flight muscle functions in the generation of Drosophila male courtship song. Troy Shirangi, David Stern, James Truman. JFRC/HHMI, Ashburn, VA.

Insects often utilize multiple acoustic signals to organize social interactions. *Drosophila melanogaster* males, for example, court females by vibrating a wing to produce two types of songs: trains of pulses and bursts of continuous tone called sine song. Currently, it is not known how the Drosophila nervous system generates the individual song types. Moreover, the neuromuscular mechanisms that generate courtship song have not been elucidated. Here, we identify a thoracic motoneuron in *Drosophila melanogaster* whose inactivation ablates sine song yet leaves pulse song unaffected. This motoneuron innervates a single, male-enlarged flight muscle, hg1, whose sexually dimorphic development is required specifically for maximal sine song amplitude. Furthermore, we demonstrate that males lacking sine song court females less effectively than do normal males. These results define hg1 and its motoneurons as a critical motor unit controlling sine song, provide insights into how the individual components of Drosophila song are generated, and set the stage to decipher the upstream neurons in the circuitry for sine song.

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Drosophila melanogaster flies communicate using substrate-borne vibrations during courtship. Caroline C. G. Fabre[1*], Berthold Hedwig[2], Graham Conduit[2], Peter A. Lawrence[2], Stephen Goodwin[3], José Casal[2]. 1) Department of Zoology, Cambridge University and Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, United Kingdom; 2) Department of Zoology, Cambridge University, Cambridge, United Kingdom; 3) Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, United Kingdom.

Courtship in Drosophila melanogaster consists of a series of stereotyped actions by the male to first assess the female's suitability and then elicit her acceptance of copulation, which is signaled by her ceasing to walk. The male and female communicate via vision, air-borne sounds and by pheromones, but it remained unclear what cues trigger female immobility. We describe a further component of Drosophila courtship behaviour that has, surprisingly, been overlooked. We show by video recordings and laser vibrometry that the abdomen of the male vibrates rhythmically ("quivers") to generate substrate-borne vibrations that have a repetition rate of about 6 pulses per second. We present evidence that the female stops walking and becomes receptive mainly because she senses these vibrations and not, as had previously been suggested, as a response to the air-borne song produced when the male extends and flutters one wing. We also show that the neural circuits expressing the sex determination genes fruitless and doublesex are required for the quivering behaviour. Moreover, we show that these abdominal quivers and associated vibrations, as well as their presumed effect on female receptivity, are conserved in other Drosophila species. Substrate-borne vibrations are an ancient form of communication that is widespread in invertebrates and vertebrates. We are now also investigating the neuromuscular circuitry responsible for the generation of these substrate-borne signals and the sensory systems needed for their reception.

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Juvenile hormone acts through *Methoprene tolerant* to modulate female receptivity and sex pheromones in *Drosophila melanogaster* . Julide Bilen[1], Jade Atallah[2], Reza Azanchi[1], Joel Levine[2], Lynn Riddiford[1]. 1) Janelia Farm Research Campus HHMI, Ashburn, VA; 2) Department of Biology, University of Toronto, Ontario, Canada.

In 1966 Manning suggested that juvenile hormone (JH) was necessary for the normal maturation of female receptivity to a courting male in *Drosophila melanogaster*, but this role has been little studied. JH is secreted by the corpora allata (CA), starting just before adult eclosion. To determine the role of JH in maturation of female receptivity, we genetically ablated the CA by expressing diphtheria toxin at the late pupal stage. Comparison of the time course of the receptivity of allatectomized and control females showed that CA ablation significantly delayed the onset of female receptivity. Application of the JH mimic (JHM), methoprene, to these allatectomized females not only restored the normal timing of receptivity but also higher doses caused a precocious onset of receptivity. To determine whether JH modulated female attractiveness, we examined male courtship behavior with a decapitated female that had minimal rejection behaviors. The allatectomized females were less attractive than intact control females. The application of JHM rescued female attractiveness. Assays of the cuticular hydrocarbons showed that JH affected the female-specific, sex pheromone diene blend. In Drosophila the JH receptor is encoded by two duplicated genes, *Methoprene-tolerant* (*Met*) and *germ cell expressed* (*gce*). We found that a null allele of Met caused a similar delay in female receptivity. Paradoxically, the loss of function of Met increased female attractiveness apparently by increasing the long chain sex-specific dienes. In contrast, the loss of function of *gce* had no effect on either receptivity or attractiveness in the females. Together these findings suggest that JH acts through Met to modulate female receptivity and sex pheromone synthesis.

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The neurobiological basis of personality in flies. Benjamin L. de Bivort[1,2,3], Jamey S. Kain[1], Sean M. Buchanan[1], Julien Ayroles[3], Chelsea Jenney[1], Sarah Zhang[1]. 1) Rowland Institute, Harvard University, Cambridge, MA; 2) Center for Brain Science,

Harvard University, Cambridge, MA; 3) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Flies exhibit personalities (persistent, idiosyncratic behavioral tendencies) just like humans. Whereas considerable progress has been made in identifying the molecular and neurobiological bases of averaged, population-level behaviors, mechanisms underlying individual-to-individual variation in behavior are largely unknown. We developed a suite of high-throughput ethological rigs capable of characterizing the behaviors of many flies, individually. Focusing on the simple behaviors of phototaxis and locomotor handedness, we find profound levels of behavioral heterogeneity. These idiosyncrasies constitute fly personality since they persist throughout the flies' lifespan. Interestingly, in all cases tested, idiosyncratic behaviors of parent flies were not inherited by their progeny. Using the *Drosophila* transgenic and pharmacological toolkits, we have identified several molecular and circuit determinants of the magnitude of behavioral variability. Specifically, the White pathway and serotonin suppress phototactic personality, and neural activity in small field neurons of the protocerebral bridges suppresses personality with respect to locomotor handedness. The implication of neurotransmitters and specific neural circuits as regulators of behavioral diversity raises the intriguing possibility that flies can dynamically modulate their population-level behavioral diversity, perhaps as an adaptive response to environmental cues. Lastly, we have conducted a genome-wide association study to identify genetic loci regulating the magnitude of personality. This was only possible because we observed variation (across lines) in the degree of behavioral variation (within lines). Preliminary results implicate a number of genes preferentially expressed in the brain, consistent with the effects of pharmacological and targeted circuit manipulation.

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Multi-parametric analysis of CLASP-interacting protein functions during interphase microtubule dynamics. Jennifer B Long[1], Maria Bagonis[1], Laura Anne Lowery[1], Haeryun Lee[1,2], Gaudenz Danuser[1], David Van Vactor[1]. 1) Cell Biology, Harvard Medical School, Boston, MA; 2) Pohang University of Science and Technology, Pohang, Gyungbuk, KOREA.

Regulation of microtubule (MT) dynamics is critical to many aspects of development, from control of cell proliferation to morphogenesis. MTs are key effectors downstream of various signaling pathways and are subject to tight regulation in response to intrinsic and extrinsic cues, often through transient interactions with a variety of microtubule-associated proteins. The MT plus-end tracking protein (+TIP) Orbit/MAST/CLASP, known to be involved in mitotic spindle formation, cell motility and axon guidance, mediates multiple dynamic cellular behaviors and interacts with numerous cytoplasmic proteins. While the action of some CLASP interactors on MT dynamics have been examined, a comprehensive survey of the proteins in the CLASP interactome as MT dynamic regulators has been missing. Ultimately, we are interested in understanding how CLASP collaborates with functionally linked proteins to regulate MT dynamics. Through an additional genetic screen of nearly 12,000 transposon insertion strains, we expanded the previously identified CLASP interactome from 24 to 118 interactors. We then utilize multi-parametric analysis of time-lapse MT+TIP imaging data acquired in Drosophila S2R+ cells to assess the effects on individual microtubule dynamics for RNAi-mediated depletion of 48 gene products previously identified as in vivo genetic CLASP interactors. While our analysis corroborates previously described functions of known CLASP-interactors, its multi-parametric resolution reveals more detailed functional profiles (“fingerprints”) that allow us to precisely classify the roles CLASP-interacting genes play in MT regulation. Using this data, we identify subnetworks of proteins with novel yet overlapping MT regulatory roles, and also uncover subtle distinctions between the functions of proteins previously thought to act via similar mechanisms.

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Dynamic myosin phosphorylation is required for pulsed contractions during apical constriction. Claudia G Vásquez, Adam C. Martin. Biology, Massachusetts Institute of Technology, Cambridge, MA.

The formation of tissue layers, such as germ layers, during gastrulation, is critical for embryonic development. A cell shape change that generates tissue layers is the apical constriction of epithelial cells, which promotes bending and invagination of cells in an epithelial sheet. During Drosophila gastrulation, a band of 18x70 prospective mesoderm cells on the ventral midline of the embryo apically constrict, forming a ventral furrow. While it is known that pulses of non-muscle myosin II (Myo-II) accumulations contract an actin meshwork to apically constrict these cells, how Myo-II is dynamically regulated to generate force is not understood. One regulatory candidate is phosphorylation of the Myo-II regulatory light chain (RLC) at two conserved sites, Thr20 and Ser21. Phosphorylation of these sites not only directs Myo-II mini-filament assembly, but also activates contractile motor activity. Using RLC phospho-mutants that lock Myo-II into different activity states, we find that mutants that disrupt modulation of RLC phosphorylation inhibit Myo-II contractile pulses. We observed that phospho-mimetic Myo-II mutants continuously constrict cells, causing the ventral tissue to tear. Thus, the pulsed Myo-II contractions are a possible mechanism to attenuate tissue tension while cells apically constrict. In contrast mutants that block Myo-II phosphorylation struggle to effectively generate cell contractions, and cells become round and appear to lose adhesion to each other. Analysis of Myo-II phospho-mutants will provide insight into the possible benefits of ratchet-like cell constriction versus continuous cell constriction.

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Regulation of epithelial morphogenesis by overlapping expression of Folded gastrulation (Fog), and its receptor, Mist. Alyssa J. Manning, Kimberly Peters, Stephen L. Rogers. Biology Department, UNC-Chapel Hill, Chapel Hill, NC.

Understanding morphogenesis, the set of processes by which cells are rearranged and change shape to form organs and

other higher-order structures, is crucial to our knowledge of biology. The Folded gastrulation (Fog)-Concertina (Cta) signaling pathway necessary for *Drosophila* epithelial folding is a fantastic system to study the principles morphogenesis. During gastrulation, a signal from the secreted protein Fog is received by cells of the presumptive mesoderm. Then, the Gα protein, Cta, is activated, which causes a signaling cascade to induce actin-based apical constriction and invagination of these cells. This same signaling pathway also controls other morphogenetic events, including invagination of the posterior midgut during embryogenesis and folding of imaginal discs during larval development. We have used RNAi screening in cell culture to discover a GPCR, Mist, which is a Fog receptor. *mist* RNA is specifically expressed in folds of imaginal discs, the presumptive mesoderm, and the posterior midgut. Specification and invagination of the mesoderm are induced by two transcription factors, Twist and Snail, which are specifically expressed in the mesoderm. Precisely patterned transcription of *fog* in this tissue is known to be activated by Twist. We now show that Snail is necessary for *mist* expression in the mesoderm. To test whether Mist is involved in morphogenesis in imaginal discs we altered levels of Mist, Fog, or Cta by RNAi and overexpression. Each of these molecules' normal expression levels and patterning is necessary for proper folding patterns. We have also made a deletion allele which disrupts *mist*expression by imprecise P-element excision. This allele shows that *mist* expression is also required for proper gastrulation movements. *mist* mutants phenocopy Fog and Cta mutants, exhibiting twisted gastrulation and improper invagination of mesodermal cells. Our data reveals that Fog and its receptor, Mist, are both patterned to robustly control the location and timing of epithelial morphogenesis in Drosophila.

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Misshapen regulates integrin levels to promote epithelial motility and planar polarity in Drosophila. Lindsay K. Lewellyn, Maureen Cetera, Sally Horne-Badovinac. Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Complex organ shapes arise from the coordinate actions of individual cells. The Drosophila egg chamber is an organ-like structure that lengthens along its anterior-posterior axis as it grows. This morphogenesis depends on an unusual form of planar polarity in the organ's outer epithelial layer, the follicle cells. Interestingly, this epithelium also undergoes a directed migration that causes the egg chamber to rotate around its anterior-posterior axis. However, the functional relationship between planar polarity and migration in this tissue is unknown. We have previously reported that mutations in the Misshapen kinase disrupt follicle cell planar polarity. Here we show that Misshapen's primary role in this system is to promote individual cell motility. Misshapen decreases integrin levels at the basal surface, which facilitates detachment of each cell's trailing edge. These data provide mechanistic insight into Misshapen's conserved role in cell migration. They also suggest that follicle cell planar polarity may be an emergent property of individual cell migratory behaviors within the epithelium.

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Role of Calcium and Rho family small GTPases in Single Cell Wound Repair. Maria Teresa Abreu-Blanco, Susan M Parkhurst. Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Cells and tissues are constantly exposed to mechanical and physical stresses, and their ability to respond to damage is critical for their survival. In particular, single cells must quickly repair their wounds to avoid cell death due to loss of cytoplasm and influx of ions. In *Drosophila*, single cell wound repair is mediated by specific spatial and temporal responses: plasma membrane is recruited as vesicles and from the wound border, an actomyosin ring is assembled serving as the contractile force driving closure, and the ring and plasma membrane are linked by E-Cadherin. We find that actin, Myosin II, Microtubules and E-Cadherin are all required for single cell wound repair. To the date, the only known signaling molecule that can trigger cell wound repair is calcium. In *Xenopus* oocytes the influx of calcium from the environment triggers membrane recruitment to the wound and its fusion with the plasma membrane. In the *Drosophila* embryo, we observed a wave of calcium around the wound area as soon as 10s post-wounding. We are currently investigating the role of this calcium wave in wound repair, and which downstream molecules are mediating this signal. Rho GTPases are well known cytoskeleton modulators and have been involved in coordinating multiple dynamic responses required by the cell. In our single cell model, Rho, Rac and Cdc42 rapidly accumulate around the wound, and segregate into dynamic zones. Importantly, genetic and pharmacological assays show that Rho, Rac and Cdc42 are required for wound repair, and each of them makes specific contributions to the assembly and organization of the actomyosin array. We also developed biosensor probes for each GTPase, using the Rho binding domains of different downstream effectors, to determine the spatial and temporal dynamics of active GTPases during the repair process. We find that Rho GTPases utilize specific effectors to mediate their signals. Significantly, we also observed crosstalk between the different GTPases and their signaling modules and the cytoskeleton.

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Branching Out: Genetic analysis of branch outgrowth in terminal cells. Tiffani A. Jones, Mark M. Metzstein. Human Gen, Univ Utah, Salt Lake City, UT.

Cellular morphology is critical for cell function. However, little is known about how individual cells generate their specific shapes. Larval terminal cells, a component of the respiratory system, are an excellent model for investigating questions of cell shape due to their elaborate branched morphology. Terminal cells initiate branching from a central branch, containing the cell body and nucleus. Subsequent side branches bifurcate from this central branch, with a general reduction in the diameter of successive branches. In previous work, we showed that PAR-polarity proteins (Par-6/Baz/aPKC/Cdc42) are required for terminal cell branching, but not outgrowth, and are downstream of the branchless/breathless FGF signaling pathway required for terminal cell outgrowth and branching. However, how branch outgrowth occurs mechanistically and how the PAR complex

may regulate this process is unknown. Our recent work has turned to testing vesicle trafficking pathways to elucidating the molecular mechanisms required for terminal cell outgrowth. In particular, we are focusing on a conserved complex, the exocyst. The exocyst complex is composed of the proteins Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 and is best known for its roles in facilitating polarized addition of post-Golgi derived vesicles to the plasma membrane. We find all 8 members of the exocyst complex, as well as the small GTPases Rab11 and Rab8, which are known to function in exocyst complex assembly, are required for terminal cell branching and outgrowth. The PAR protein Cdc42 has been shown to be required for exocyst protein localization in yeast and mammalian cell culture. We have found Cdc42 is required for exocyst protein localization in terminal cells and that PAR proteins and the exocyst act in a single genetic pathway to control terminal cell development. We suggest a model in which terminal cell development occurs through a process of branch specification via PAR complex activity, which directs exocyst complex mediated polarized exocytosis to facilitate terminal branch outgrowth.

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Live imaging of Drosophila neuroblast delamination reveals two stages with differential cytoskeletal dynamics. Yan Yan[1,2], Chris Doe[2]. 1) Division of Life Science, HKUST, Hong Kong, Kowloon, Hong Kong; 2) Institute of Neuroscience/HHMI, University of Oregon, Eugene, OR.

Ingression is a conserved morphogenetic process across species. During embryogenesis, individual cells frequently emigrate from an epithelial sheet and give rise to various cell types. In adult epithelial tissues, individual cell extrusion is utilized to maintain epithelial homeostasis. Here we document the neural stem cell (neuroblast) delamination process with high spatiotemporal resolution during Drosophila embryogenesis. We find that neuroblast delamination is a stereotyped process with two distinctive stages. In a first fast stage, delaminating neuroblasts decrease their apical domain incrementally correlated with medial myosin activity. In a second slow stage, the adherens junctions undergo prolonged remodeling associated with junctional myosin activity in the delaminating neuroblasts. Through analyzing Notch signaling mutants in which all the neuroectoderm cells attempt to delaminate, we find that the first fast stage can occur cell autonomously while the second stage is sensitive to aberrant cell-cell communication. Our analysis provided the foundation for further investigation of the molecular machineries that orchestrate the critical steps of cell ingression.

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Alp/Enigma family proteins cooperate in Z-disc formation and myofibril assembly. Frieder Schoeck, Stefan Czerniecki, Kuo An Liao, Anja Katzemich. Dept Biol, McGill Univ, Montreal, PQ, Canada.

The Drosophila Alp/Enigma family protein Zasp52 localizes to myotendinous junctions and Z-discs. It is required for terminal muscle differentiation and muscle attachment. Its vertebrate ortholog ZASP/Cypher also localizes to Z-discs, interacts with α-actinin through its PDZ domain, and is involved in Z-disc maintenance. Human mutations in ZASP cause myopathies and cardiomyopathies. Here we show that Drosophila Zasp52 is one of the earliest markers of Z-disc assembly, and we use a Zasp52-GFP fusion to document myofibril assembly by live imaging. We demonstrate that Zasp52 is required for adult Z-disc stability and pupal myofibril assembly. In addition, we show that two closely related proteins, Zasp66 and the newly identified Zasp67, are also required for adult Z-disc stability and are acting together with Zasp52 in Z-disc assembly resulting in more severe, synergistic myofibril defects in double mutants. Zasp52 and Zasp66 can cooperate because they both bind directly to α-actinin, and they can also form a ternary complex. Our results indicate that Alp/Enigma family members cooperate in Z-disc assembly and myofibril formation and based on sequence analysis we propose a novel class of PDZ domain involved in α-actinin binding.

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UpSET modulates open chromatin features at active transcribed genes. Hector Rincon-Arano, Jessica Halow, Jeffrey Delrow, Susan Parkhurst, Mark Groudine. Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Chromatin accessibility is one of the main structural features that distinguish transcribing genes from non-expressing genes. Interestingly, active promoter regions exhibit a more open chromatin structure than gene bodies, which is suggested to be consequence of the complex machinery and catalytic activities targeted to the transcriptional start site. The SET domain-containing protein UpSET is part of a Rpd3/Sin3 histone deacetylase complex associated with transcribing genes and lack of this protein results in female sterility. The recruitment of UpSET-containing complexes to active regions modulates histone acetylation of active promoter regions. To evaluate whether UpSET also modulates chromatin accessibility we developed an in situ M.SssI-based chromatin accessibility assay. Our results show that *upSET* germariums possess higher chromatin accessibility than wildtype. MNase I-based chromatin accessibility assays in RNAi-based UpSET knock down in Kc cells confirms higher chromatin accessibility around UpSET target genes. These results correlate with the ability of *upSET* mutants to increase Polycomb phenotypes, which are chromatin structure dependent. Unexpectedly, transcription of UpSET target genes is not disturbed; nonetheless off-target genes and repetitive sequences are up-regulated in *upSET* mutants and knock down cells. Accordingly, position effect variegation of transgene array, but not centromeric or telomeric, silencing is suppressed in *upSET* mutants suggesting a main functional role in euchromatic regions. In consequence, *upSET* mutant ovaries exhibit up-regulation of the Notch pathway, which affects cell lineage specification of polar cells. Altogether, our results suggest that UpSET is a key transcriptional modulator of open chromatin features to fine tune gene expression thereby avoiding spurious gene expression.

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Sorted cell ChIP-seq shows the molecular organization of Polycomb-repressed chromatin in the bithorax complex. Sarah K. Bowman[1], Aimee M. Deaton[1], Heber Domingues[2], Welcome Bender[2], Robert E. Kingston[1]. 1) Dept. of Molecular Biology, Massachusetts General Hospital, and Dept. of Genetics, Harvard Medical School, Boston, MA; 2) Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA.

The 300 kb of the Drosophila bithorax complex (BX-C) is the original model for studying gene repression by Polycomb group proteins. Decades of genetic experiments have led to the hypothesis that Polycomb organizes BX-C chromatin differently in each of the abdominal parasegments. However, studying the molecular organization of chromatin in individual parasegments has been technically difficult. We solved this problem by developing a sorted nuclei ChIP-seq pipeline. In this system, transgenic embryos produce tagged nuclei in single parasegments, in either PS4, 5, 6, or 7. Using FACS, we sort these tagged nuclei and perform small-scale ChIP-seq. Initial results show that the mark of Polycomb repression, H3K27me3, is lost from the active regions of the BX-C. The proximal H3K27me3-free region becomes progressively larger as we move from PS5 to PS7. The boundaries of the H3K27 methylation correlate precisely with previously identified CTCF binding sites. Correspondingly, the H3K4me3 active mark appears over the transcription start sites of BX-C genes as they emerge from the repressive environment. These results suggest that BX-C chromatin "opens up" at a molecular level in a proximal to distal direction, as previously hypothesized. By extending this study to other histone modifications and regulators of chromatin structure, we hope to gain insight into the molecular organization of repressed and activated chromatin in this uniquely well-annotated gene region.

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Genome-wide Analysis of the Binding Sites of the JIL-1 H3S10 Kinase and its Contribution to Modulation of Gene Expression. Kristen M. Johansen[1], Weili Cai[1], Chao Wang[1], Lu Shen[1], Yeran Li[1], Sanzhen Liu[2], Changfu Yao[1], Xiaomin Bao[1], Patrick S. Schnable[2,3], Jack Girton[1], Jørgen Johansen[1]. 1) Biochem, Biophys & Molec Biol, Iowa State Univ, Ames, IA; 2) Agronomy, Iowa State Univ, Ames, IA; 3) Data2Bio LLC, Ames, IA.

JIL-1 kinase localizes to euchromatic regions and is responsible for H3S10 phosphorylation at interphase. Genetic interaction assays show that JIL-1 can counterbalance the gene-silencing effect of the three major heterochromatin components Su(var)3-9, Su(var)3-7, and HP1a. In this study we have determined the genome-wide relationship of JIL-1 kinase mediated H3S10 phosphorylation with gene expression and the distribution of the epigenetic H3K9me2 mark. We show in wild-type salivary gland cells that the H3S10ph mark is predominantly enriched at active genes whereas the H3K9me2 mark largely is associated with inactive genes. Comparison of global transcription profiles in salivary glands from wild-type and *JIL-1* null mutant larvae revealed that the expression levels of 1,737 genes changed at least two-fold in the mutant and that a substantial number (39%) of these genes were upregulated whereas 61% were downregulated. Interestingly, salivary gland specific pathways were particularly affected by downregulation in the *JIL-1* mutant background suggesting that H3S10 phosphorylation may serve to keep genes transcriptionally active in a tissue and/or developmentally stage specific context. Furthermore, the results showed that downregulation of genes in the mutant was correlated with higher levels or acquisition of the H3K9me2 mark whereas upregulation of a gene was correlated with loss of or diminished H3K9 dimethylation. These results are compatible with a model where gene expression levels are modulated by the levels of the H3K9me2 mark independent of the state of the H3S10ph mark, which is not required for either transcription or gene activation to occur. Rather, H3S10 phosphorylation functions to indirectly maintain active transcription by counteracting H3K9 dimethylation and gene silencing. Supported by NIH grant GM62916.

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Piwi is linked to heterochromatin formation in the embryo of Drosophila melanogaster. Tingting Gu, Sarah Elgin. Department of Biology, Washington University in St Louis, St Louis, MO.

A persistent question in epigenetics is how heterochromatin is targeted for assembly at specific domains, and how that chromatin state is faithfully transmitted. Stable heterochromatin is necessary to silence transposable elements (TEs) and maintain genome integrity. Both the RNAi system and heterochromatin components HP1 and H3K9me2/3 are required for initial establishment of heterochromatin structures in fungi and plants. We utilized the newly developed Drosophila melanogaster transgenic shRNA lines to deplete proteins of interest at specific developmental stages to dissect their roles in heterochromatin assembly in early zygotes, and in maintenance of the silent chromatin state during development. Using reporters subject to Position Effect Variegation (PEV), we find that depletion of key proteins in the early embryo can lead to a loss of silencing (suppression of PEV) assayed at adult stages. The piRNA component Piwi is required in the early embryo for reporter silencing, but knock-down during larval stages has no impact. This implies that Piwi is involved in targeting HP1a when heterochromatin is established (late blastoderm), but that the silent chromatin state created is transmitted through cell division independent of the piRNA system. In contrast, HP1a is required for both initial assembly and the mitotic inheritance of heterochromatin. HP1a profiles in piwi mutant animals confirm that Piwi depletion leads to decreased HP1a levels in pericentric heterochromatin, particularly at TEs. Piwi is known to physically interact with HP1a, and is important for recruiting HP1a to some TEs in the female germ line. To establish whether Piwi's role is direct or indirect, experiments to tether Piwi adjacent to reporters are underway. The present results indicate that the major role of the piRNA system in targeting heterochromatin formation occurs in the early zygote during initial heterochromatin assembly, and further demonstrate that a failure of heterochromatin formation in the early embryo impacts the phenotype of the adult.

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Epigenetic regulation of olfactory receptor gene choice. Sarah Perry[1], Choon Kiat Sim[2], Sana Tharadra[1], Anand Ray[1]. 1) Entomology, UC Riverside, Riverside, CA; 2) Department of Genetics, Stanford University, Stanford, CA.

An olfactory neuron will express a single receptor or receptor pair from amongst a large gene family. It is not fully understood how the olfactory system is able to create and maintain such a complex map. Here we show that epigenetic mechanisms and chromatin structure play a role in receptor selection. We identify a chromatin modifying complex, MMB/dREAM , which is necessary for proper expression of the carbon dioxide receptor genes Gr63a and Gr21a in the antenna. The presence of Myb in the complex is required for normal expression of Gr63a/21a. Other members of the complex, Mip120 and E2F2, prevent aberrant expression of Gr63a in tissues other than the antennae. Loss of either of these members is associated with an increase in activating H3K4me3 histone modifications at the receptor gene locus throughout head tissue. Repressive chromatin is considered to be important for maintaining singular receptor expression in mammals. We find heterochromatic H3K9me2 modifications at olfactory receptor gene loci in the antennae including Gr63a. The histone methyltransferase responsible for these modifications, Su(var)3-9, acts in genetic opposition to myb and influences Gr63a expression. Finally, we show that another set of chromatin modifiers, histone deacetylases (HDACs), also participate in control of receptor choice. Treatment with HDAC inhibitors increases Gr63a expression in adults, potentially through alteration of chromatin structure. Our findings demonstrate a role for the MMB/dREAM complex in receptor gene choice and suggests that chromatin structure and its modifiers play an important role in creating and maintaining singular receptor expression in the olfactory system.

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The chromatin configurations of Polycomb Response Elements (PREs) define epigenetic states. Kami Ahmad[1], Guillermo Orsi[1], Steven Henikoff[2], Jorja Henikoff[2]. 1) Dept BCMP, Harvard Medical Sch, Boston, MA; 2) FHCRC, Seattle, WA.

PREs are regulatory elements that are essential to establish and maintain repression of large chromatin domains. A number of transcription factors bind at PREs and facilitate K27-trimethylation of histone H3, Polycomb recruitment, and gene repression. However, it is thought that both activating trithorax-Group (trxG) and repressing Polycomb-Group (PcG) factors bind simultaneously at PREs, and antagonistic interactions between these factors determine PRE activity. How these factors interact and are developmentally regulated is unknown. We have used micrococcal nuclease digestion of chromatin and paired-end sequencing (MNase-Seq) to define the occupancy of nucleosomes and transcription factors in two Drosophila cell lines, at base-pair resolution. We find that PREs are clusters of protected factor particles in both activating and repressing states. However, the specific configuration of factor binding differs in the two states. Analysis of underlying sequence motifs suggests that the trxG protein Trl binds and destabilizes nucleosomes at both activating and repressing PREs. Strikingly, at repressing PREs a novel occupied motif implicates an additional factor in reorganizing PRE-bound proteins into a more elaborate and stable bound configuration. To determine the composition of these factor complexes, we have developed a method for native immunoprecipitation of transcription factor and non-histone protein chromatin particles (MNase-IP-Seq), which we use to define the sites and modes of chromatin-complex interactions. We propose a model where trxG factors potentiate the chromatin of regulatory elements by increasing nucleosome dynamics, and cooperative interactions between PcG-engaged PREs and target promoters stabilize repressive complexes.

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Stuxnet Regulates PRC1-mediated Epigenetic Silencing by Promoting Ubiquitinated Polycomb Protein for Degradation. Juan Du[1], Junzheng Zhang[1], Feng Tie[2], Ying Su[1], Peter Harte[2], Alan Jian Zhu[1]. 1) Department of Cellular & Molecular Medicine, Lerner Research Institute, Cleveland Clinic, Cleveland, OH; 2) Department of Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland, OH.

By utilizing an *in vivo* RNAi screen strategy, we identify a new gene, *stuxnet*, that functions as a key component of Notch signaling, a process at the core of cell fate decisions in development, adult tissue homeostasis and cancer. To further study the biological function of stuxnet, we generate a *stuxnet* null allele and confirm that the transcript of the Notch receptor gene is reduced. Surprisingly, this stuxnet lethal mutation can be rescued by reducing the activity of Polycomb (Pc), an essential component of the Polycomb Repressive Group complex 1 (PRC1) that is known to epigenetically silence target genes critical for animal development. In accordance with a genetic interaction between *stuxnet* and *Pc*, Stuxnet protein physically interacts with and subsequently destabilizes ubiquitinated Pc protein through a proteasome-mediated degradation pathway. Our detailed structure/function analyses suggest that Stuxnet utilizes its ubiquitin-like domain (Ubl) to interact with the proteasome to facilitate Pc degradation. Consistently, overexpressed stuxnet leads to stereotypical homeotic transformation phenotypes associated with loss of PRC activity. Further chromatin immunoprecipitation experiments indicate that Stuxnet protein functions through Pc to epigenetically regulate transcription of a panel of PRC target genes, including *Notch, Ubx* and *Antp*. Thus, our work uncovers a novel mechanism for the control of the activity and stability of the PRC1 transcriptional silencing machinery in development.

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Telomere protection in Drosophila: functional analysis of the terminin complex. Grazia D. Raffa, Emanuela Micheli, Fiammetta Verni, Domenico Raimondo, Alessandro Cicconi, Laura Ciapponi, Giovanni Cenci, Stefano Cacchione, Maurizio Gatti.

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Drosophila telomeres are epigenetically determined, sequence-independent structures that are not maintained by telomerase, but by transposition to chromosome ends of specialized retroelements. Genetic and biochemical analyses have recently shown that fly telomeres are capped by terminin, a complex that includes at least four proteins: HOAP, HipHop, Modigliani (Moi) and Verrocchio (Ver). With the exception of Ver, which exhibits a structural homology with Stn1, the terminin proteins are not conserved outside the Drosophilidae and are all encoded by fast-evolving genes. Terminin localizes and appears to function only at telomeres just like shelterin, suggesting that terminin is a functional analogue of shelterin. We have now analyzed the structure of terminin using suitable protein truncations and DNA binding assays. HOAP binds double stranded (ds) and Ver single stranded (ss) DNA; Moi does not bind DNA but interacts directly with HOAP and Ver forming a bridge between the two proteins. Thus, the architecture of terminin is similar to that found in other telomere capping complexes including shelterin, where the ss DNA-binding protein Pot1 is connected to the TRF1/TRF2 ds DNA-associated proteins by the non-DNA-binding factor TPP1. Our data further suggest that Ver and Moi mask ss DNA at Drosophila telomeres, just like TPP1-Pot1 at human telomeres: When chromosome ends lack either Moi or Ver, telomeres form DNA repair foci that contain the phosphorylated form of the H2Av histone. Collectively, our results reinforce the idea that the basic mechanisms of telomere capping are conserved from yeast to flies and humans, and support a unifying model for telomere protection.

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Mannitol - a BBB disrupter is also a potent α-synuclein aggregation inhibitro for treating Parkinson's disease. Daniel Segal[1,2], Ronit Shaltiel-Karyo[1], Moran Frenkel-Pinter[1], Edward Rockenstein[3], Christina Patrick[3], Michal Levy-Sakin[1], Nirit Egoz-Matia[1], Eliezer Masliah[3], Ehud Gazit[1]. 1) Department of Molecular Microbiol & Biotech, Tel Aviv University, Tel Aviv 69978, Israel; 2) Sagol School of Neurosciences, Tel Aviv University, Tel Aviv 69978, Israel; 3) Department of Neurosciences, School of Medicine, University of California at San Diego, La Jolla, CA 92093, USA.

Misfolding and aggregation of α-synuclein (α-syn) is the hallmark of Parkinson's Disease. Osmolytes, e.g. polyols, are small molecules which accumulate under stress conditions and stabilize protein structure, acting as 'chemical chaperones'. They may reduce protein misfolding and aggregation in neurodegenerative diseases. The polyol Mannitol is a non-metablolized FDA-approved osmotic diuretic agent that also has BBB disrupting properties. We examined its ability to interfere with aggregation of α-syn in vitro and in vivo. Low concentrations of Mannitol (450 and 225 mM) were found to inhibit the in vitro formation of α-syn fibrils. High concentrations (900 mM) significantly decreased formation of tetramers and high molecular weight oligomers, and shifted the secondary structure from α-helical to a different structure, suggesting alternative potential pathways for aggregation. Feeding α-syn expressing Drosophila, with 75mM Mannitol dramatically corrected their behavioral defects and reduced the amount of α-syn aggregates in their brains. Daily injection (IP) of 1 g/kg Mannitol to mThy1-human α-syn transgenic mice caused a significant decrease of α-syn accumulation in several brain regions, suggesting that Mannitol promotes α-syn clearance from the cell bodies. Mannitol appears to have a general neuroprotective effect in the transgenic treated mice, which includes the dopaminergic system. No adverse effects were observed in control Mannitol-treated flies or mice. In conclusion, we suggest that Mannitol has a dual therapeutic mechanism for the treatment of Parkinson's Disease - a BBB disruptor that also serves by itself as a chemical chaperone correcting the pathogenic misfolding of α-syn.

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Bioinformatics-driven approaches to building new fly models of human disease. Stephanie E. Mohr[1], Yanhui Hu[1], Ian Flockhart[1], Juliane Schneider[2], Charles Roesel[1,3], Lizabeth Perkins[1], Norbert Perrimon[1,4]. 1) Dept Gen, Harvard Med Sch, Boston, MA; 2) Countway Medical Library, Harvard Med Sch, Boston, MA; 3) Grad Program in Bioinformatics, Northeastern University, Boston, MA; 4) Howard Hughes Medical Institute, Boston, MA.

Drosophila is used to model human diseases at cell, pathway, organ and organism levels, and to learn about the normal functions of disease-associated genes. Development of new fly disease models depends on 1) accurate associations between human disease terms, human genes and their fly orthologs, and 2) availability or production of relevant reagents. To improve the quality and ease of identifying fly orthologs of human disease genes, we developed the ortholog tool DIOPT (www.flyrnai.org/diopt) and DIOPT-DIST (www.flyrnai.org/diopt-dist), which incorporates disease information from Online Mendelian Inheritance in Man (OMIM) and genome-wide association studies (GWAS). Recent improvements include fuzzy search; inclusion of a tenth algorithm (OrthoDB) in the DIOPT 'voting system' output; and a combined automated and curated approach to map terms between OMIM and Medical Subject Headings. We used DIOPT-DIST to identify genes represented in the Drosophila RNAi Screening Center and Transgenic RNAi Project reagent collections, as well as nominate genes for production of new TRiP lines. A large number of our reagents could be put to immediate use to study disease gene orthologs. The diseases covered include ciliopathies, cohesinopathies, disorders related to lysosomes, peroxisomes or mitochondria, and enzyme deficiencies. In total, we identify 860 fly genes that are high-confidence orthologs (DIOPT score ≥8) matching 853 human genes and >1200 diseases. Many of these are rare, poorly understood, and/or not previously modeled in the fly. Altogether, our team, whose expertise spans bioinformatics, library science, and molecular genetics, is using a variety of approaches to make disease-relevant software tools and reagents better and easier to access, with the ultimate goal of facilitating meaningful disease-related studies at the lab bench.

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Early mitochondrial dysfunction leads to oxidative stress in a drosophila model of TPI deficiency. Stacy Hrizo[1,2], Isaac J Fisher[1], Bartholomew P Roland[2], Daniel R Long[1], Joshua A Hutton[1], Zhaohui Liu[2], Michael J Palladino[2]. 1) Biology, Slippery Rock University, Slippery Rock, PA; 2) Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Triose phosphate isomerase (TPI) is responsible for the interconversion of dihydroxyacetone phosphate to glyceraldehyde-3-phosphate in glycolysis. Point mutations in this gene are associated with a glycolytic enzymopathy called TPI deficiency. This study utilizes a Drosophila melanogaster model of TPI deficiency; TPIsugarkill is a mutant allele with a missense mutation (M80T) that causes phenotypes similar to human TPI deficiency. In this study, the redox status of TPIsugarkill flies was examined and manipulated to provide insight into the pathogenesis of this disease. Our data show that TPIsugarkill animals exhibit higher levels of the oxidized forms of NADH, NADPH; and glutathione in an age-dependent manner. Additionally, we demonstrate that mitochondrial redox state is significantly more oxidized in TPIsugarkill animals. We hypothesized that TPIsugarkill animals may be more sensitive to oxidative stress and that this may underlie the progressive nature of disease pathogenesis. The effect of oxidizing and reducing stressors on behavioral phenotypes of the TPIsugarkill animals was tested. As predicted, oxidative stress worsened these phenotypes. Importantly, we discovered that reducing stress improved the behavioral and longevity phenotypes of the mutant organism without having an effect on TPIsugarkill protein levels. Overall, these data suggest that reduced activity of TPI leads to an oxidized redox state in these mutants and that the alleviation of this stress using reducing compounds can improve the mutant phenotypes.

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Signaling pathways involved in 1-octen-3-ol mediated neurotoxicity in *Drosophila melanogaster*: Implication in Parkinson's Disease. Arati A. Inamdar, Joan W. Bennett. Department of Plant Biology and Pathology, Rutgers, The State University of New Jersey, New Brunswick, NJ.

The prevalence and growing incidence of PD point to the accountability of other environmental risk factors for the pathogenesis of PD. In addition to the existing neurotoxin, recently, natural toxins have been reported to be the causative agents for PD. Our lab reported the deregulation of dopamine homeostasis by newly reported natural toxin, 1-octen-3-ol. 1-octen-3-ol is a fungal VOC known to be emitted by all fungi. Fungal exposure leads to neurological and neuropsychiatric problems such as movement disorders, delirium, dementia, and disorders of balance and coordination in human populations. We have pioneered *Drosophila melanogaster*as a reductionist model to determine the mechanism of toxicity of 1-octen-3-ol which has shown to cause loss of dopaminergic neurons and PD like symptoms in flies. In this report, we have incorporated our inexpensive *Drosophila* model as an *in vivo* genetic model to identify the modulatory role of JNK and Akt signaling pathways in 1-octen-3-ol induced dopamine neurotoxicity. We found that AKt and JNK protect against 1-octen-3-ol mediated dopamine toxicity. Hence, our *Drosophila* model system allows unique opportunity to screen for the relevant signaling pathways involved in 1-octen-3-ol and other fungal VOCs mediated toxicity.

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4-aminoquinoline analogs rescue neurotoxicity in a Drosophila model of ALS based on TDP-43. Alyssa Coyne[1], Marilyn Roy[2], Ivy Lin[2], Joel Cassel[4], Mark McDonnell[4], Allen Reitz[4], Daniela Zarnescu[2,3]. 1) Department of Neuroscience, University of Arizona, Tucson, AZ 85721, USA; 2) Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721, USA; 3) Department of Neurology, University of Arizona, Tucson, AZ 85721, USA; 4) Biopharma, LLC, Pennsylvania Biotechnology Center, Doyleston, PA 18902, USA.

TAR DNA-binding protein (TDP-43) is an RNA and DNA binding protein that has been implicated in Amyotrophic Lateral Sclerosis (ALS). ALS is a progressive neurodegenerative disease for which there is currently no cure. Because the mechanisms behind TDP-43 action are poorly understood, it is difficult to pinpoint therapeutic targets. Increasing evidence however, supports a role in RNA metabolism and recently, TDP-43 was shown to directly bind $(TG)_n$ sequences. The high affinity binding of TDP-43 to TG oligonucleotides is inhibited by 4-aminoquinoline (AAQ) probes, which also lead to increased caspase cleavage of TDP-43 in vitro. To test their effect in vivo, we fed AAQ probes to larvae expressing wild-type and mutant TDP-43 in motor neurons (D42>TDP-43). These experiments show that AAQ probes but not a structurally related negative control rescue the lethality induced by TDP-43 overexpression in motor neurons. Furthermore, AAQs mitigate defects in larval locomotor activity due to TDP-43 neurotoxicity. Current experiments are aimed at determining the physiological effects of AAQs in vivo, using a battery of neuroanatomical and behavioral phenotypes caused by TDP-43 overexpression in motor neurons or glia. Our initial results provide novel insights into the physiological role of TDP-43's association with nucleic acids and suggest a novel therapeutic strategy for TDP-43 based ALS.

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A *Drosophila melanogaster* model identifies a critical role for zinc in initiating urinary stone formation. Thomas Chi[1], Man Su Kim[2], Nichole Bond[1], Sven Lang[3], Joe Miller[1], Gulinuer Muteliefu[3], Katja Bruckner[1], Arnie Kahn[3], Marshall Stoller[1], Pankaj Kapahi[3]. 1) UCSF, San Francisco, CA; 2) College of Pharmacy, Inje University, Republic of Korea; 3) Buck Institute for Research on Aging, Novato, CA.

Ectopic biomineralization is a driving force for kidney stones and other disorders where calcium hydroxyapatite is believed to serve as a nidus for mineralized deposits leading to calcification. Initiating factors for the calcification process are poorly understood. We developed a *Drosophila* model for urinary stone disease and screened for genetic inhibitors of stone

formation. Here we show that zinc (Zn^{2+}) is present in both *Drosophila melanogaster* Malpighian tubule stones and human renal biopsy material and plays a critical role in initiating urinary stones. We screened mineralization-associated human disease genes for their ability to induce stones in fly tubules. Upon xanthine dehydrogenase (*Xdh*) inhibition, flies formed 70% more stones compared to controls. Hydroxyapatite was confirmed in fly stones with a fluorescent bisphosphonate dye stain. Targeted screening of 50 genes of interest was then performed using concurrent inhibition with *Xdh* suppression. This identified 10 suppressors that mitigated fly stone formation. A member of the ZnT zinc transporter family conferred the greatest rescue, replicated with zinc chelation drug feeds. To better understand the mechanism by which zinc exerted its effects, synchrotron radiation-based analysis was performed on stone samples. This demonstrated the presence of Zn^{2+} in both *Drosophila* and human stones, implying that Zn^{2+} plays an important, previously unrecognized structural role in the initiation of human kidney stones. Our results implicate Zn^{2+} as a critical component for initiating stone formation whose manipulation could be leveraged as a therapeutic target. This work demonstrates for the first time translational utility of a genetically based *Drosophila* model for urinary stone disease with implications of applicability across multiple diseases involving ectopic biomineralization.

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Inhibition of JNK/dFOXO pathway and caspases rescues neurological impairments in *Drosophila* Alzheimer's disease model. Se Min Bang, Yoon Ki Hong, Soojin Lee, Kyoung Sang Cho. Biological sciences, Kunkok university, Seoul, Seoul, South Korea.

Amyloid-β-42 (Aβ42) has been implicated in the pathogenesis of Alzheimer's disease (AD). Neuronal *Aβ42* expression induces apoptosis and decreases survival and locomotive activity in Drosophila. However, the mechanism by which Aβ42 induces these neuronal impairments is unclear. In this study, we investigated the underlying pathway in theses impairments. JNK activity was increased in *Aβ42*-expressing brains, and the *Aβ42*-induced defects were rescued by reducing JNK or caspase activity through genetic modification or pharmacological treatment. In addition, these impairments were restored by*Drosophila forkhead box subgroup O* (*dFOXO*) deficiency. These results suggest that the JNK/dFOXO pathway confers a therapeutic potential for AD.

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Expression pattern analysis of 6,300 genomic fragments for *cis*-regulatory activity in the imaginal discs of *Drosophila Melanogater*. Aurélie Jory[1], Carlos Estella[1,3], Matt W. Giorgianni[1,4], Matthew Slattery[1,5], Todd R. Laverty[2], Gerald M. Rubin[2], Richard S. Mann[1]. 1) Department of Biochemistry and Molecular Biophysics, Columbia University Medical Center, 701 W. 168th Street, HHSC 1104, New York, NY 10032, USA; 2) Janelia Farms Research Campus, 19700 Helix Drive, Ashburn, VA 20147, USA; 3) Present address: Departamento de Biología Molecular, and Centro de Biología Molecular ''Severo Ochoa,'' Universidad Autónoma de Madrid, Madrid, Spain; 4) Present address: R.M. Bock Laboratories, University of Wisconsin-Madison, 1525 Linden Drive, Madison, WI 53706, USA; 5) Present address: Institute for Genomics and Systems Biology, University of Chicago, 900 E. 57th St. KCBD 10115, Chicago, IL 60637, USA.

Over 6,000 fragments from the genome of *Drosophila Melanogaster* were analyzed for their ability to drive expression of GAL4 reporter genes in the third-instar larval imaginal discs. About 1,200 reporter genes drove expression in the eye, antenna, leg, wing, haltere, or genital imaginal discs. The patterns ranged from large regions to individual cells. About 75% of the active fragments drove expression in multiple discs; 20% were expressed in ventral, but not dorsal, discs (legs, genital, and antenna), whereas around 23% were expressed in dorsal but not ventral discs (wing, haltere, and eye). Several patterns, for example, within the leg chordotonal organ, appeared a surprisingly large number of times. Unbiased searches for DNA sequence motifs suggest candidate transcription factors that may regulate enhancers with shared activities. Together, these expression patterns provide a valuable resource to the community and offer a broad overview of how transcriptional regulatory information is distributed in the*Drosophila* genome. Using this database and new computational and biochemistry results, we will present a deeper analysis of selected cis-regulatory modules (CRMs) involved in the proximo-distal patterning of the leg and antenna discs.

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DNA regulatory element usage is driven largely by developmental stage, even within distinct cell lineages. Daniel J. McKay[1], Jason D. Lieb[1,2]. 1) Department of Biology, The University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Carolina Center for Genome Sciences.

The defining feature of animal development is creation of a diversity of cell types and body parts from a single genome. Central to this process is differential regulation of gene expression. A prerequisite to understanding how genes are regulated differently in different cells is to identify all of the functional DNA elements in the genome. Recent advances in methods and technology have led to high-resolution maps of DNA regulatory elements across multiple stages of *Drosophila* development. However, these data were obtained from whole animals, and thus lack information on the cellular source of the signal. To determine how the genome is used in different cell lineages at different stages of development, we have generated genome-wide open chromatin and gene expression profiles from two distinct cell types and from two distinct axial positions at two stages of embryogenesis. While differences exist in the identity of DNA regulatory elements used in different cell types, we find that developmental stage has the greatest influence on which regions of the genome are utilized across all cells examined.

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Differential regulation of *sloppy-paired-1* transcription initiation and elongation by Runt and Even-skipped during Drosophila segmentation. Kimberly Bell[1,3], Saiyu Hang[2,3], J. Peter Gergen[3]. 1) Graduate Program in Genetics; 2) Graduate Program in Biochemistry and Structural Biology; 3) Department of Biochemistry and Cell Biology and the Center for Developmental Genetics Stony Brook University, Stony Brook, NY 11794-5215.

The initial metameric expression pattern of the *sloppy-paired-1 (slp1)* gene in the Drosophila blastoderm embryo is generated through two cis-regulatory elements, termed PESE and DESE for proximal and distal early stripe elements. These enhancers act in a non-additive manner to integrate inputs from distinct combinations of the pair-rule transcription factors Runt, Even-skipped (Eve), Fushi-tarazu (Ftz) and Odd-paired (Opa) resulting in fourteen two cell wide stripes in the segmented region of the embryo. This pattern consists of seven repetitive units, each comprised of four different cellular contexts (I-IV) of*slp1* transcription regulation. *Slp1* is actively transcribed in type II and IV cells, each with important contributions from Opa. In type I cells, *slp1* is not expressed due to repression by Eve. Chromatin immuno-precipitation (ChIP) experiments indicate this is due to an Eve-dependent block to transcription elongation mediated by PESE that involves antagonizing P-TEFb recruitment to the promoter. Similarly, Runt+Ftz act to repress expression in type III cells by blocking transcription elongation mediated by DESE. PESE activity is also repressed by Runt in type III cells. Interestingly, this repression involves blocking PESE dependent recruitment of PolII and the initiation of transcription, a different mechanism than DESE mediated repression in the same cellular context. This effect of Runt on PESE is consistent with a previous proposal that Runt prevents functional interactions between PESE and the *slp1* promoter. We will report results of experiments to determine if the distinct *slp1* expression states are reflected in changes in chromosome conformation involving different physical contacts between the promoter and other regions in response to regulatory inputs from Runt and other pair-rule transcription factors.

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Robust Hox-Mediated Transcriptional Regulation Utilizes a Combination of Flexible Binding Site Composition and Rigid Grammar. Juli Uhl, Lisa Gutzwiller, Arif Ghasletwala, Brian Gebelein. Developmental Biology, Cincinnati Children's Hospital, Cincinnati, OH.

'Designer enhancers' that selectively direct gene expression would be a useful research tool, but to create these we must first define the rules of enhancer organization. Many transcription factors bind common DNA motifs yet regulate gene expression in a tissue-specific manner. In Drosophila, the Abdominal-A Hox factor (AbdA) directs formation of specialized metabolic cells by activating *rhomboid* in a subset of sensory organ precursors, while in nearby cells AbdA restricts leg primordia to the thorax by repressing *Distal-less*. The enhancers for *rhomboid* (RhoA) and *Distal-less* (DMXR) both contain binding sites for AbdA, Extradenticle (Exd), and Homothorax (Hth), yet the order and spacing of the sites differs. In addition, RhoA requires a nearby dPax2 site while DMXR requires a nearby Sloppy-Paired (Slp) site for proper transcriptional outcomes. Here, we compare RhoA and DMXR using quantifiable reporter and DNA binding assays to understand the grammar of Hox-regulatory enhancers. Notably, we found that the Hox, Exd, and Hth motif in RhoA is capable of repression in place of that in DMXR. Moreover, we discovered that neither RhoA nor DMXR require Hth binding for *in vivo*activity despite the crucial role Hth plays in cooperative complex formation *in vitro*. Finally, we found that RhoA remains active when the dPax2 site is moved, but DMXR repression requires a particular orientation and spacing of Slp motifs relative to the nearby Hox motif. While the relationship between Hox factors and cofactors is complex, these data suggest that; 1-Functional gene regulation does not require cooperative binding of Hox and Exd/Hth. 2-There are two types of Hox:cofactor interactions; flexible dPax2:Hox configuration and inflexible Slp:Hox configuration. 3- Hox/Exd/Hth motifs are context-sensitive activators or repressors. Together, these data suggest that a combination of rigid grammar and flexible configuration of motifs regulates robust Hox-mediated gene expression.

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Autoregulation controls temporal progression of gene expression during development. Leslie A Dunipace, Angelike Stathopoulos. Biology, California Institute of Technology, Pasadena, CA.

It is widely accepted that multiple cis-regulatory modules (CRMs) may associate with individual genes to control temporal changes in expression throughout development. However, very little is understood regarding mechanisms of temporal control of gene expression and, in particular, of how the switch from one CRM to the next is accomplished. In order to further our understanding of these dynamics, we identified three CRMs that regulate the expression of *brinker* (*brk*) during the first 4 hours of embryonic development. Through the use of standard reporter assays as well as a series of deletions from a 32kb reporter construct, we found that two distal CRMs provide regulatory information for expression of *brk*; first in a narrow stripe pre-cellularization (5' CRM), and then in a broad lateral band post-cellularization (3' CRM). The third CRM is a promoter proximal element (PPE) which provides no expression when put into a standard reporter construct, but abolishes all early expression of *brk* when deleted from the large reporter construct. Using this PPE we showed that it is required for the activity of both the 5' and 3' CRMs when they are located at a distance and behind an insulating element. Although there is minimal overlap in the expression of the 5' and 3' CRMs at cellularization, there is a marked switch between the early and late acting CRMs. By expressing the large reporter constructs in a*brk* mutant background, we discovered that Brk itself is required for the proper timing of this exchange. Furthermore, if the early acting 5' CRM is placed near the promoter it delays the activity of the later acting 3' CRM, suggesting that a physical exchange at the promoter is required for proper timing of expression. Therefore,

through analysis of the *brk* cis-regulatory system we have uncovered (i) that two CRMs control spatially and temporally distinct patterns of *brk* expression; (ii) that expression of these distantly located CRMs requires the PPE; and (iii) that levels of Brk protein influence the switch from early-enhancer to late acting-enhancer in the early embryo.

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Tissue-specificity of Drosophila Developmental Gene Regulatory Networks. Matthew Slattery[1], Roumen Voutev[2], Rebecca Spokony[1], Lijia Ma[1], Richard Mann[2], Kevin White[1]. 1) Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL; 2) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

While a whole animal-based chromatin immunoprecipitation approach has been very successful at identifying cis-regulatory elements in Drosophila, an important question remains about what information will be gained by focusing on individual tissues. We have used genome-wide ChIP to map the binding patterns for selector genes, growth regulators and Polycomb group (PcG)-associated histone modifications in imaginal discs, progenitor tissues that give rise to limited subsets of the adult fly (eye, wing, etc.). Unlike ChIP experiments carried out using whole animals, which are composed of many different cell types, these experiments analyze the binding of these transcription factors in tissues with less cell-type complexity. Consistent with this limited amount of cell type diversity, and the fact that these tissues give rise to distinct parts of the adult fly, we found that the putative target genes regulated by these transcription factors and PcG display significant tissue-specificity. In many cases this specificity provides mechanistic or functional details that could not be gathered from whole animal-based approaches. However, all factors tested also display significant tissue 'common' binding -- binding shared across tissues -- and it appears that these common events can also be functionally relevant. Interestingly, regulators of developmental patterning show much more tissue-specific binding than general growth regulators, suggesting that focusing on individual tissues will be especially important for mapping out developmental gene regulatory networks.

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Regulation of *rhodopsins*: Single nucleotides are critical for photoreceptor subtype-specific expression. Jens Rister, Claude Desplan. New York University, Department of Biology, 100 Washington Square East, New York, NY.

Cis-regulatory elements (CREs) control where, when, and how strongly genes are expressed. We are interested in the regulatory mechanisms that generate the complex expression patterns of *rhodopsins* in specific photoreceptor subtypes involved in color vision. *Rhodopsins* are particularly suited for a *cis*-regulatory analysis, as their expression is mostly transcriptional, while compact CREs of less than 300 base pairs are sufficient to reproduce their endogenous expression patterns. Yet, it is still unclear how the combinatorial input of transcriptional activators and repressors (that is integrated in the CREs) controls spatiotemporal *rhodopsin* expression. We performed an extensive dissection of the *rhodopsin* promoters. They all contain an 11 base pair *rhodopsin* core sequence I (RCSI, consensus: TAATYNRATTN), which is necessary for *rhodopsin* expression. Multimerization of a generic RCSI (TAATYNRATTA) drives reporter expression in all photoreceptors. It was therefore suggested that the RCSI plays a role in the general activation of *rhodopsins*. However, each *rhodopsin* contains a preferred, highly conserved RCSI that differs from the consensus in 1-2 base pairs. Multimerization of each of these individual RCSI motifs did not drive reporter expression in all photoreceptors, as had been observed with the generic RCSI, but in subsets of photoreceptors. Moreover, point mutations affecting the single RCSI base pair differences in the wildtype promoter context led to an expansion of reporter expression into other photoreceptor subtypes. Depending on the respective RCSI, we found that the de-repression was either due to disruption of repressor sites or due to the generation of activator sites. Thus, these data suggest that subtle, but highly conserved differences in the RCSI are critical for subtype-specific *rhodopsin*expression. Single base pair changes therefore appear to be a major driving force in the evolution of mutually exclusive *rhodopsin* expression, a prerequisite for color vision.

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Chinmo prevents male-to-female sex transformation of somatic stem cells in the adult *Drosophila* testis. Qing Ma[1], Matthew Wawersik[2], Erika Matunis[1]. 1) Cell Biology Dept, The Johns Hopkins Sch Med, Baltimore, MD; 2) Biology Dept, The Col of William & Mary, Williamsburg, VA.

Drosophila sexual identity is controlled cell-autonomously via activation of the Sex-lethal sex determination cascade in embryos and by non-autonomous signals that regulate sexual dimorphism throughout development. Once gender of an organ has been assigned, it is widely viewed as permanent. Here, we show that a downstream target of the Jak-STAT pathway, *chronologically inappropriate morphogenesis (chinmo)*, is required for the active maintenance of male somatic cell identity in the adult testis. Partial reduction of Chinmo in the cyst stem cell (CySC) lineage of adult flies leads to a novel testis phenotype where germ cells over-proliferate and arrest as spermatogonia, and somatic cells form a layer of columnar epithelium that closely resembles the ovarian follicular epithelium. Lineage tracing shows that the columnar epithelium originates from squamous CySCs and cyst cells. This suggests that somatic cells acquire female identity in *chinmo* mutants while germ cells maintain male fate. Supporting this hypothesis, male form of Doublesex (DsxM), a male-specific somatic marker, is reduced in *chinmo* mutant CySCs and cyst cells. Additionally, the columnar epithelium in these mutants expresses ovarian follicle cell markers, while arrested germ cells express male specific markers, and RNAi of *chinmo* in the germline does not alter germ cell behavior. Also, loss of transformer, which is required for assignment of female fate in soma but not germline partially rescues the follicular epithelium phenotype in *chinmo* mutant testes. Thus, Chinmo plays a critical role in the maintenance of male fate in the adult *Drosophila*testis through the somatic sex determination pathway. Interestingly, the

mammalian Dsx homolog Doublesex and mab-3 related transcription factor 1 (DMRT1) is required to maintain male identity in the testis of adult mice, but the mechanisms are not understood. Thus, our work may elucidate mechanisms that regulate sexual maintenance in other model systems.

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Identification of genes modifying epigenetic plasticity during follicle cell differentiation. Ming-Chia Lee[1], Andrew Skora[2], Allan Spradling[1]. 1) Department of Embryology, Carnegie Institution of Washington, Baltimore, MD; 2) Ludwig Center for Cancer Genomics and Therapeutics, Johns Hopkins School of Medicine, Baltimore, MD.

Stem cells give rise to diverse cell types through cell differentiation. Differentiation programs are associated with a gradual reduction in cellular potency, accompanied by a corresponding stabilization in epigenetic plasticity potential. However, the chromatin-based mechanism underlying this differentiation-related reduction in epigenetic plasticity potential remains elusive. Here we report a new set of genes that play key roles in regulating epigenetic plasticity during ovarian follicle cell differentiation. With the capability of tracing epigenetic inheritance in vivo using the GAL4/UAS-GFP system, we performed systematic haploid deficiency screening at the second and the third chromosome to search for potential modifiers. As a result, we identified eight specific genes that dominantly modify the stabilization process of epigenetic plasticity, manifested as suppressed GFP variegation patterns as well as altered follicle cell differentiation. Interestingly, two of the most heavily represented classes of genes among the identified modifiers are cell cycle and epigenetic regulators. Together, our findings provide novel mechanistic insights on the molecular nature of epigenetic plasticity stabilization that underlies follicle cell differentiation.

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The Hox gene *Abd-B* controls stem cell niche function in the *Drosophila* testis. Fani Papagiannouli[1], Lisa Schardt[2], Nati Ha[1], Janina-Jacqueline Ander[1], Ingrid Lohmann[1]. 1) Developmental Biology, Centre for Organismal Studies (COS), Heidelberg, Germany; 2) Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany.

Stem cells reside in a specialized microenvironment, called the stem cell niche, which provides essential signals controlling stem cell behavior. Proper niche architecture is a key for normal stem cell function, yet only few upstream regulators are known. Here we report that the Hox transcription factor Abd-B controls niche positioning and integrity in the *Drosophila* testis by regulating integrin and actin localization in the neighboring somatic cyst cells. Loss of Abd-B results in centrosome misorientation in germline stem cells (GSCs) and reduced GSC divisions, leading to a dramatic reduction of pre-meiotic stages in adult testes, a hallmark of aging. Genetic dissection revealed that non-cell-autonomous organization of the stem cell niche downstream of *Abd-B* is mediated by diverse mechanisms, including the Boss-Sev pathway, linking integrin to the extracellular matrix and actin filaments. In order to systematically elucidate the network and hierarchy of events related to Abd-B function in the *Drosophila* testis, we aimed at identifying direct Abd-B target sites within the *Drosophila* genome using the in vivo binding-site profiling technique DamID (DNA adenine methyltransferase identification). Our data show for the first time that*Abd-B* provides positional cues upstream of integrin to maintain niche architecture and localization, ensure proper niche and GSC function, and prevent premature aging.

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Niche appropriation by *Drosophila* intestinal stem cell tumors. Parthive H. Patel[1,2], Devanjali Dutta[2], Bruce A. Edgar[1,2]. 1) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA USA; 2) German Cancer Research Center (DKFZ) and Center for Molecular Biology Heidelberg (ZMBH) Alliance, Heidelberg, Germany.

The importance of immune cells, fibroblasts, and vasculature recruited to the tumor microenvironment is widely appreciated, but how stem-derived tumor initiating cells interact with the stem cell niche prior to this, during tumor initiation, is poorly understood. Here we investigate intestinal stem cell (ISC) tumors generated in *Drosophila* by blocking Notch signaling. These differentiation-defective cells produce an autocrine, progenitor cell-specific EGFR ligand (Spitz), which supports early tumor growth. After achieving a critical mass the tumors induce JNK signaling and cytokines (Upd2,3) in neighboring enterocytes, and another EGFR ligand (Vein) in visceral muscle. These paracrine signals, normally used within the niche to support regenerative growth, accelerate tumor growth. Niche stress caused by the growing tumors enhances JNK activation and cytokine expression, driving a vicious cycle that would normally be kept in check by differentiation. We propose that niche appropriation by differentiation-defective stem cells may be a common mechanism of tumor initiation.

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Gastric Stem Cells Maintain the Adult Drosophila Stomach. Craig A. Micchelli, Marie Strand. Developmental Biology, Washington University School of Medicine, St. Louis, MO.

The adult Drosophila copper cell region or “stomach” is a highly acidic compartment of the midgut with pH < 3. In this region, a specialized group of acid-secreting cells similar to mammalian gastric parietal cells has been identified by a unique ultrastructure and by copper-metallothionein fluorescence. However, the homeostatic mechanism maintaining the acid-secreting “copper cells” of the adult midgut has not been examined. Here, we combine cell lineage tracing and genetic analysis to investigate the mechanism by which the gastric epithelium is maintained. Our investigation shows that a molecularly identifiable population of multipotent, self-renewing gastric stem cells (GSSCs) produces the acid-secreting copper cells, interstitial cells, and enteroendocrine cells of the stomach. Our assays demonstrate that GSSCs are largely quiescent but can be

induced to regenerate the gastric epithelium in response to environmental challenge. Finally, genetic analysis shows that EGFR signaling controls GSSC proliferation in the gastric epithelium. Characterization of the GSSC lineage in Drosophila, with striking similarities to mammals should advance our understanding of both homeostatic and pathogenic processes in the stomach.

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Neuron-produced Activinβ supports hematopoiesis in the *Drosophila* larva. Kalpana Makhijani[2], Brandy Alexander[2], Sophia Petraki[2], Michael O'Connor[4], Katja Brückner[1,2,3]. 1) Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research; 2) Department of Cell and Tissue Biology; 3) Department of Anatomy, University of California San Francisco, San Francisco, CA; 4) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

The Peripheral Nervous System (PNS) has been identified as a functional component of hematopoietic microenvironments and other stem cell niches, both in vertebrates and invertebrates. However, it remains largely unknown how sensory neurons and their inputs direct hematopoiesis or immune responses. To address these questions at the molecular and cellular level, we study the role of the PNS as a microenvironment in the hematopoietic pockets of the *Drosophila* larva. In this system, hemocytes reside in direct physical contact with segmentally repeated sensory PNS clusters and are induced to proliferate in these microenvironments (Makhijani et al. 2011). Using mutants, cell ablation, and other genetic manipulations that disrupt the PNS or generate ectopic neurons, we demonstrate that larval hemocytes functionally depend on the PNS regarding their localization and trophic survival. GRASP demonstrates direct neuron-hemocyte and glia-hemocyte contacts. Hypothesizing that *Drosophila* larval hematopoiesis is molecularly controlled by the PNS microenvironment, we screened key signaling pathways by in vivo RNAi and identified TGF-β related Activinβ (Actβ) as a PNS signal that supports larval hemocytes. Using cell type specific RNAi and dominant-negative transgene expression, we demonstrate complementary roles of PNS neuron-expressed Actβ, and hemocyte-autonomous Activin signaling through baboon (babo), punt (put) and dSmad2, all of which are required to control resident hemocyte adhesion/localization and number. *Drosophila* larval hematopoiesis shows parallels with vertebrate self-renewing tissue macrophages and hematopoiesis in the bone marrow niche. In the future, this system can be used to identify further, constitutive or neuronal activity-dependent mechanisms by which the PNS regulates hematopoiesis or immune responses.

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A genome-wide RNAi screen for Neuroblast cell cycle exit in *Drosophila*. Catarina Homem, Juergen Knoblich. IMBA, Vienna, Austria.

During development, *Drosophila* neural stem cells, the Neuroblasts (NBs), divide asymmetrically to self-renew and to generate a differentiated Ganglion Mother Cell (GMC). The GMC divides once more to generate two post-mitotic neurons or glia. *Drosophila* NBs undergo multiple rounds of divisions generating hundreds of neurons, which make up the nervous system of the fly. *Drosophila* NBs divide throughout development, but stop dividing and disappear just before entering adult stages. To maintain the correct number and type of neurons, it is essential to precisely regulate the time at which neurogenesis ceases. If these stem cells miss this proliferation stop this can lead to uncontrolled proliferation and tumor formation. Although several factors influencing neural proliferation have been identified, the underlying molecular mechanism scheduling the end of progenitor divisions remains enigmatic. NBs stop proliferating during pupal stages, suggesting that NB cell cycle exit happens as a response to either differential pupal nutritional status or to a stage specific humeral signal. By doing *ex-vivo* co-cultures of larval and pupal NBs we found that even in absence of extrinsic signaling pupal NBs exit cell cycle with similar timing to *in vivo*. This and other experiments show that NB proliferation termination is indeed cell intrinsically regulated. To identify novel genes regulating NB proliferation and division termination we performed a genome wide *in vivo* RNAi screen using central brain NBs as a model. In our assay we co-express Luciferase and RNA hairpins specifically in a subset of Nbs and their respective progeny. We then measure luminescence amounts in the adult head to assess Nb number and lineage sizes. From this screen we have identified approximately 80 genes that cause increases in NB size, NB number, NB life-span or their progeny number. Among these we find known NB tumor suppressors like Brat, Numb and Miranda. We also find previously uncharacterized tumor suppressors and 15 new regulators of NB growth and life-span.

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Drug screening on a new Drosophila cardiac model of Friedreich Ataxia. Veronique Monnier, Hervé Tricoire. BFA Unit, University Paris Diderot, 75205 Paris Cedex 13, France.

Friedreich Ataxia (FA), the most common hereditary ataxia, is characterised by progressive degeneration of the central and peripheral nervous system, hypertrophic cardiomyopathy and increased incidence of diabetes. FA is caused by reduced levels of frataxin, a highly conserved mitochondrial protein. Drosophila appears as an adequate animal model to study pathogenic mechanisms involved in FA and to test functionally pharmacological compounds. Several groups have previously developed Drosophila models of FA, in which dfh (the ortholog of Fxn) is downregulated by RNAi in various tissues, including the PNS and glial cells. We have developed a new Drosophila cardiac model of FA by targeting dfh-RNAi in cardiomyocytes with a specific RU486 inducible driver. In vivo real time imaging of Drosophila heart, using an innovative technology that we have recently developed, revealed profound impairments in heart function in these animals, including a strong increase in end-systolic and end-diastolic diameters and a decrease in Fractional Shortening. We used this new Drosophila model for drug screening and identified one compound highly efficient to prevent heart dysfunctions induced by dfh deficiency. This validates the use of this FA model to identify new potential therapeutic compounds that should be subsequently tested on other models

of the disease.

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Rescue of insulin signaling misregulation in a fly model of fragile x syndrome. Rachel E Monyak[1], Danielle Emerson[1], Yan Wang[1], Xiangzhong Zheng[2], Brian Schoenfeld[3], Sean McBride[1], Amita Sehgal[2], Thomas Jongens[1]. 1) Department of Genetics University of Pennsylvania Perelman School of Medicine Philadelphia, PA; 2) Howard Hughes Medical Institute and Department of Neuroscience University of Pennsylvania Perelman School of Medicine Philadelphia, PA; 3) Section of Molecular Cardiology Departments of Medicine and Molecular Pharmacology Albert Einstein College of Medicine Bronx, NY.

Fragile x syndrome (FXS) is the most common inherited cause of intellectual disability. Patients with FXS exhibit cognitive defects, autism, sleep disorders, ADHD and epilepsy. These symptoms occur as the result of loss-of-function of a single gene, *FMR1*. To understand how *FMR1* loss-of-function causes FXS, we study a *Drosophila* model of the disease in which the fly homolog of *FMR1, dfmr1*, does not function. The *dfmr1* mutant fly displays phenotypes reminiscent of those seen in FXS patients including defects in memory, social behavior (seen by abnormal naïve courtship) and circadian rhythmicity. We found that expressing *dfmr1* in the insulin-producing cells (IPCs) in the brain rescues the memory, naïve courtship and circadian defects of the *dfmr1* mutant fly, indicating that *dfmr1* expression in the IPCs is important for normal behavior. Since the IPCs regulate insulin signaling, we wondered whether this pathway could be misregulated in *dfmr1* mutant flies. We found that *dfmr1* mutant flies show increased levels of *Drosophila* insulin-like peptide 2 (dILP2) as well as increased PI3K and Akt activity, indicating that insulin signaling is increased in *dfmr1* mutant flies. We further found that we could rescue the memory, naïve courtship and circadian rhythmicity defects by genetically reducing insulin signaling in the *dfmr1* mutants. These results suggest that insulin signaling misregulation in *dfmr1* mutant flies contributes to the behavioral abnormalities of this fragile x model and reveals another pathway involved in the pathogenesis of FXS.

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Sphingosine 1-phosphate mediated suppression of dystrophic muscle wasting in Drosophila and mice. Mario Pantoja[1], Karin A. Fischer[1], Nicholas Ieronimakis[2], Timothy L. Dosey[1], Junlin Qi[1], Aislinn Hayes[2], Morayma Reyes[2,3], Hannele Ruohola-Baker[1]. 1) Dept Biochem, Univ Washington, Seattle, WA; 2) Dept Pathology; 3) Dept Labortory Medicine.

Presently, there is no effective treatment for the lethal muscle wasting disease Duchenne Muscular Dystrophy (DMD). Using Drosophila, we show that reduction of wunen, a lipid phosphate phosphatase 3, that inactivates the bioactive lipid Sphingosine-1-Phosphate (S1P), suppresses dystrophic muscle defects as assayed by myofibril integrity and movement. Furthermore, increasing S1P levels by reducing S1P lyase, Sply, or by upregulating lace, a serine palmitoyl-CoA transferase, also leads to suppression of dystrophic muscle degeneration. Importantly, suppression of dystrophic defects by S1P upregulation is evolutionarily conserved as we show that treatment of dystrophic mdx mice with the small molecule 2-acetyl-4(5)-tetrahydroxybutyl imidazole (THI), which elevates S1P levels systemically, significantly increases muscle fiber size and specific force while reducing DMD pathology of fibrosis and fat deposition. Moreover, delivery of THI to adult dystrophic flies phenocopies the genetic suppression observed with Sply reduction and shows that elevation of S1P in adult animals is sufficient to suppress muscle wasting. We further evaluate increased S1P signaling in dystrophic animals by treating flies with the S1P agonist, FTY720, and show that this drug significantly suppresses muscle degeneration. Furthermore, we will discuss dissecting the mode of action of S1P mediated suppression in both flies and mice as well as the use of Drosophila as a drug discovery tool for Duchenne Muscular Dystrophy.

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A kinome-wide RNAi screen in *Drosophila* glia reveals new kinases that mediate cell proliferation and survival in human glioblastoma. Renee Read[1,2], Tim Fenton[3], German Gomez[3], Jill Wykosky[3], Scott Vandenberg[4], Ivan Babic[3], Akio Iwanami[5], Huijun Yang[3], Webster Cavenee[3], Paul Mischel[3], Frank Furnari[3], John Thomas[2]. 1) Department of Pharmacology, Emory University School of Medicine, Atlanta, GA; 2) The Salk Institute for Biological Studies, Molecular Neurobiology Laboratory, La Jolla, CA; 3) Ludwig Institute for Cancer Research, University of California - San Diego, La Jolla, CA; 4) Department of Pathology, University of California - San Diego, La Jolla, CA; 5) Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan.

Glioblastoma (GBM), the most common primary malignant brain tumor, is incurable with current therapies. Genetic and molecular analyses show that GBMs frequently display mutations that activate receptor tyrosine kinase (RTK) and Pi-3 kinase (PI3K) signaling pathways. In *Drosophila melanogaster*, activation of RTK and PI3K pathways in glial progenitor cells creates malignant neoplastic tumors that display many features of human GBM. We used this *Drosophila* GBM model to perform a kinome-wide genetic screen for genes required for RTK-PI3K dependent neoplastic transformation. Human orthologs of novel kinases uncovered by these screens were functionally assessed in mammalian GBM models and human tumors. Our results revealed that a small number of these human kinases are subject to alterations in tumor cells. In particular, the atypical RIOK1 and RIOK2 kinases become overexpressed in GBM cells in response to Akt activity downstream of RTK and PI3K signaling. When overexpressed, RIOK2 upregulated Akt signaling and promoted tumorigenesis. Conversely, reduced expression of RIOK1 or RIOK2 disrupted Akt signaling and caused cell cycle exit, apoptosis, and chemosensitivity. These results imply that, in GBM cells, the RIO kinases create a feedforward loop that promotes and maintains oncogenic Akt activity. Further study of the RIO kinases as well as other kinases identified in our *Drosophila* screen may reveal new insights into signaling defects underlying GBM and related cancers.

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Functional characterisation of human synapse genes expressed in the *Drosophila* brain, applications in drug screening. Matt B. Mahoney, Lysimachos Zografos, R. Wayne Davies, J. Douglas Armstrong. Brainwave Discovery, LTD, Ardshiel, Main Street, Gartmore, FK8 3RJ, United Kingdom.

Brainwave Discovery, Ltd specialises in the rapid development of *in vivo* brain assays for chemicals acting on human central nervous system (CNS) targets. We use our expertise in "humanising" transgenic fruit flies (*D. melanogaster*) and expressing the human protein with spatial and temporal control in the insect brain. We have also developed and streamlined an array of phenotyping assays (climbing, courtship, neuronal activity) as well as histology methods in order to quantify and assess the effect of chemicals on fruit fly models of mental and neurodegenerative disease. Also, as part of the SynSys project (www.synsys.eu), in order to identify novel gene variants as disease associated candidates, we have selected and prioritised a list of human synaptic genes and variants (SNPs, mutants). Using the aforementioned fruit fly “humanising” and phenotyping pipeline we expressed these genes in the fruit fly CNS and tested for behavioural phenotypes, ranging from simple (i.e. climbing) through to more complex (i.e. courtship learning). In this work we will present the basic outline of our pipeline and how this has been applied to screen for synaptic genes involved in learning and memory or neurodegeneration processes. We will also show results underlining how these models can be used as excellent and rapid primary drug screening platforms enabling drug research and development divisions of pharmaceutical companies to make faster, cheaper and better informed decisions earlier in the drug discovery pathway.

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Renal proximal tubule receptors Cubilin and Amnionless mediate protein reabsorption in Drosophila nephrocytes. Fujian Zhang[1], Ying Zhao[1], Yufang Chao[1], Katherine Muir[1], Zhe Han[1,2]. 1) Department of Internal Medicine, University of Michigan, Ann Arbor, MI; 2) Department of Cell and Developmental Biology.

Filtration and reabsorption are two fundamental roles of the renal system. Remarkable similarities have been found between insect nephrocyte and the mammalian glomerular podocyte for filtration, but it remains unclear whether there is an organ or cell to perform protein reabsorption in flies. Here we show that the Drosophila nephrocyte has remarkable molecular, structural and functional similarities to the renal proximal tubule cell. From a genetic screen for genes required for nephrocyte function, we identified two novel Drosophila genes encoding orthologues of mammalian Cubilin and Amnionless (AMN), two major receptors for protein reabsorption in the renal proximal tubule. Mutations in Cubilin or AMN lead to Imerslund-Gräsbeck syndrome (IGS), a genetic disease associated with persisting proteinuria. We found that dCubilin and dAMN are specifically expressed in the Drosophila nephrocytes and function as co-receptors for protein uptake, suggesting that nephrocytes may carry out the similar function as renal proximal tubules. Targeted expression of human AMN in Drosophila nephrocytes is sufficient to rescue the protein uptake defect caused by dAMN RNAi knockdown, suggesting that functions of the Cubilin/AMN co-receptors are evolutionarily conserved from flies to humans. Electron microscopy analysis and toxin stress assay demonstrated that Cubilin/AMN-mediated protein reabsorption is not only required for maintaining nephrocyte ultrastructure, but also important for survivability of flies in toxic stress condition. Our data suggests that the insect nephrocyte combines filtration with protein reabsorption using evolutionarily conserved genes and subcellular structures, and can serve as a simplified model for both podocytes and renal proximal tubules.

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SMN is required for RNA splicing in sensory-motor circuits. Brian McCabe[1,2], Francesco Lotti[1], Erin Beck[1,2], Ben Choi[1,2], George Mentis[1], Christine Beattie[3], Livio Pellizzoni[1], Wendy Imlach[1,2]. 1) Pathology & Cell Biology, Columbia University, New York, NY; 2) Neuroscience, Columbia University, New York, NY; 3) Neuroscience, The Ohio State University, Columbus, OH.

Spinal muscular atrophy (SMA), the most common inherited cause of infant mortality, is a human disease characterized by motor neuron dysfunction and muscle deterioration due to depletion of the ubiquitous Survival Motor Neuron (SMN) protein. Drosophila SMN mutants have reduced muscle size and defective locomotion, motor rhythm and motor neuron neurotransmission. Unexpectedly, restoration of SMN in either muscles or motor neurons did not alter these phenotypes. Instead, SMN must be expressed in proprioceptive neurons and interneurons in the motor circuit to non-autonomously correct defects in motor neurons and muscles. SMN depletion disrupts the motor system subsequent to circuit development and can be mimicked by the inhibition of motor network function. Furthermore, increasing motor circuit excitability by genetic or pharmacological inhibition of K+ channels can correct SMN-dependent phenotypes. In addition, from a genome-wide screen, we have identified a novel protein Stasimon, that has both reduced expression in SMN mutants and can rescue motor circuit activity when restored to normal levels. We find that the regulation of stasimon splicing by SMN is conserved in the motor circuits of both zebrafish and mouse models of SMA. These results establish sensory-motor circuit dysfunction as the origin of motor system deficits in this SMA model and suggest that enhancement of motor neural network activity could ameliorate this RNA splicing disease.

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Widespread and distinct sequence signatures of combinatorial transcriptional regulation. M Kazemian[1], H Pham[2], M Brodsky[2], S Sinha[1]. 1) U of Illinois, Urbana, IL; 2) UMASS Med School, Worcester, MA.

There is a growing realization that transcriptional gene regulation is often combinatorial, with multiple transcription factors

(TFs) co-regulating the same genes, either independently or through direct or indirect interactions. Here, we explore the extent and diversity of combinatorial regulation in the Drosophila genome. We utilized the binding motifs of 322 TFs and chromatin accessibility data to produce computational TF-DNA interaction maps through different stages of embryonic development in fruit fly. We examined these binding maps to identify pairs of co-expressed TFs that either prefer to or avoid binding at common locations. We find that TF-TF aversion is as prevalent as co-binding, suggesting a less appreciated aspect of the combinatorial regulation. Several TFs had unusually many aversion partners including known chromatin remodeling TFs. We explored TF-TF co-binding and aversion partnerships in the context of nearly 100 gene expression domains and four stages of development, and found that the frequency of such partnerships varies greatly across expression domains. We then analyzed the common binding locations of TF-pairs for statistical patterns in terms of relative spacing and orientation between binding sites, using a newly designed statistical tool called “interacting TF signatures” (iTFs). We identified many instances of short distance biases between binding sites of TF-pairs including examples where such biases are stronger under certain relative orientations. To test if the genomic arrangement of these binding sites might reflect physical interactions between the corresponding TFs, we selected 28 TF-pairs whose binding sites exhibited short distance biases (<10bp) for further analysis. In vitro pull-down experiments revealed that ~65% of these pairs can directly interact with each other. For 5 of these pairs, we further demonstrate that they bind cooperatively to DNA if both sites are present with the preferred spacing. Overall, this study produces a comprehensive map of various types of sequence signatures of combinatorial TF action.

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Synergistic interactions between MSL complex and the CLAMP protein regulate Drosophila dosage compensation. Marcela Soruco[1], Jessica Chery[1], Eric Bishop[2,7], Trevor Siggers[3], Michael Tolstorukov[2,3], Alexander Leydon[1], Arthur Sugden[1], Karen Goebel[1], Jessica Feng[1], Peng Xia[1], Anastasia Vedenko[3], Martha Bulyk[3,4,5], Peter Park[2,3,6], Erica Larschan[1]. 1) Department of Molecular Biology, Cellular Biology and Biochemistry, Brown University, Providence, RI 02912; 2) Center for Biomedical Informatics, Harvard Medical School, Boston, MA 02115; 3) Division of Genetics, Department of Medicine, Brigham & Women’s Hospital and Harvard Medical School, Boston, MA 02115; 4) Department of Pathology, Brigham & Women’s Hospital and Harvard Medical School, Boston, MA, 02115; 5) Harvard-MIT Division of Health Sciences and Technology, Harvard Medical School, Boston, MA 02115; 6) Children’s Hospital Informatics Program, Boston, MA 02115; 7) Bioinformatics Graduate Program, Boston University, Boston, MA 02215.

In heterogametic species, the process of dosage compensation is required to equalize transcript levels between the sex chromosomes in males and females. The Drosophila Male-Specific Lethal (MSL) complex increases transcript levels on the single male X-chromosome to equal the transcript levels in XX females. However, the mechanism by which dosage compensation is targeted to the male X-chromosome is not understood because neither the MSL complex nor cis-acting DNA sequences are sufficient. Here, we demonstrate that a previously uncharacterized zinc-finger protein, CLAMP (Chromatin-Linked Adaptor for MSL Proteins), regulates X-chromosome specificity. CLAMP tethers MSL complex to the X-chromosome and exhibits a synergistic interaction with MSL complex that increases X-chromosome specificity. Also, CLAMP is highly enriched at likely "seed" sites prior to MSL complex recruitment. The discovery of CLAMP identifies a key factor that regulates the chromosome-specific targeting of dosage compensation and provides new insights into how sub- nuclear domains of coordinate gene regulation are formed within complex genomes.

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Akirin: a novel link between Twist transcription factor activity and Brahma chromatin remodeling complexes during embryogenesis. Scott J. Nowak[1], Hitoshi Aihara[3], Katie Gonzalez[2], Yutaka Nibu[3], Mary K. Baylies[2]. 1) Dept. of Biology and Physics, Kennesaw State University, Kennesaw, GA 30144; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY 10065; 3) Cell and Developmental Biology, Weill Cornell Medical College, New York, NY 10065.

The activities of developmentally critical transcription factors are regulated via interactions with accessory proteins that confer both tissue and target specificity for transcription factor activity. We identified Akirin, a highly conserved nuclear protein, as a novel cofactor of the key *Drosophila* mesoderm and muscle transcriptional regulator, Twist.
Like *twist* hypomorphic mutants, *akirin* mutant embryos have misattached or missing muscles and severely altered muscle morphology. Akirin interacts with Twist to facilitate expression of some, but not all, Twist-regulated genes during embryonic myogenesis. To regulate transcription, Akirin colocalizes and genetically interacts with subunits of the Brahma SWI/SNF-class chromatin remodeling complex at Twist-target genes. This suggests that Akirin mediates a novel link between Twist and chromatin remodeling complexes to facilitate Twist-regulated transcription during *Drosophila* myogenesis. These results also provide a common mechanism by which Akirin, through further interactions with chromatin remodeling factors, regulates the activities of multiple transcription factors during development, the immune response and homeostasis.

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Zelda's role in *Drosophila* zygotic genome activation. Yujia Sun[1], Sun Melody Foo[1], Chung-Yi Nien[1], Hsiao-Yun Liu[1], Kai Chen[2], Kevin O'Brien[1], Amruta Tamhane[1], Julia Zeitlinger[2], Christine Rushlow[1]. 1) Department of Biology, New York University, New York, NY 10003; 2) Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110.

During embryogenesis, developmental control is transferred from maternal gene products to the zygotic genome in a process called the maternal-to-zygotic transition. Previously, Zelda (Zld) was identified by our lab to be a key activator of the early zygotic genome in *Drosophila*, without which the transcription of many genes is affected, and the embryo ceases development

before gastrulation. However, the underlying mechanism of how Zld acts to ensure robust and timely activation of its target genes remains a mystery. The prevalence of Zld binding sites near transcription start sites, the great overlap between Zld-bound regions and "hotspots" where many other transcription factors co-occupy, and the early presence of Zld in nuclei prompted us to investigate the role of Zld in recruitment of transcription factors including RNA polymerase II. Here we present evidence that supports the hypothesis that Zld binding increases the accessibility of transcription factors to chromatin, and reveals the mechanistic role of Zld during zygotic genome activation. We propose a model of how Zld functions alone or together with transcription factors such as the Dorsal morphogen to activate target genes in a timely and robust manner during the maternal-to-zygotic transition.

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Analysis of evolution within the bHLH transcription factor family based on a complete set of DNA binding interaction specificities. Hannah Pham, Jianhong Ou, Scot Wolfe, Michael Brodsky. Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, MA.

We describe a comprehensive analysis of DNA binding specificities and protein-protein interactions within the bHLH family of transcription factors in D. melanogaster. bHLH proteins typically bind DNA as homo- or hetero-dimeric complexes to “E-box” related sequences, with some variant of CANNTG. We identify homo- or heterodimeric binding partners for all but one of the 56 bHLHs. The lone exception is Her, which binds to a unique, non-E-box sequence as a monomer, the first identified example of a monomeric, DNA binding bHLH protein. DNA binding specificities were also determined for all bHLHs, revealing highly diverse E-box related motifs that can vary at any position within the E-box and have different prefences flanking the E-box. This set includes the Tai/dSRC, a known coactivator protein; we find that Tai can directly binds DNA as a homodimer or a heterodimer with the JH receptors Met and gce. Analysis of bHLH sequences in 15 insect species reveals a core set of 52 bHLH proteins. D mel has 2 losses and 6 gains relative to the core set. The losses are in bHLHs conserved in mammals; our motif data combined with mammalian expression data reveal that other bHLHs present in D.mel have DNA binding and development expression related to the missing bHLH, suggesting a mechanism to compensate for loss of a core bHLH. 5 of 6 bHLH gains represent duplications that generate multiple proteins with similar binding specificities. However, the remaining gain is Her, a very recent derivative of the E(spl) family that acquired a new DNA binding specificity reflecting a novel DNA binding mechanism. Using the D.mel dataset to predict bHLH specificities in other insects, we find that most bHLH gains and losses are unlikely to result in the loss or gain of a particular DNA binding specificity. However, in rare cases, bHLH proteins evolve with substantial alterations in specificity determining residues, providing opportunities to regulate novel gene sets.

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Transcription Start Site Turnover in Drosophila using CAGE. Bradley J. Main, Hyosik Jang, Andrew Smith, Sergey Nuzhdin. MCB, Univ Southern California, Los Angeles, CA.

Random mutations can give rise to new promoter sequences that are functionally redundant. These promoters and their associated transcription start sites (TSS) then experience relaxed selection at one or both copies, resulting in death or functional divergence of one TSS. Thus, functionally equivalent TSS may move, or turnover, via this birth and death process. TSS locations can be identified using sequencing methods like cap analysis for gene expression (CAGE) that anchor sequencing reads to the 5’ end of mRNA. For this study, we developed a highly improved CAGE technique (Taq-ex CAGE) and employed it to assess TSS turnover among four Drosophila species: D. melanogaster (mel), D. simulans (sim), D. sechellia (sec), and D. pseudoobscura (Dpse). We identified 2849 high-confidence TSS in mel and found orthologs for the majority of them in each species (83%, 86%, and 55% for sim, sec, and Dpse, respectively). An appreciable number of mel TSS were unpaired due to extensive sequence divergence or lack of an ortholog within 500bp. These likely include distant turnover events, but may also include cases where the ortholog is expressed below detection. Overall, TSS were conserved, but ribosomal protein genes were highly enriched among genes with diverged TSS and turnover events were associated with divergence in expression. This suggests that TSS turnover is more common among certain types of genes and TSS changes contribute to cis-regulatory variation between Drosophila species.

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Role of regulatory small peptides in the control of gene expression. Francois Payre[1,2], Emilie Benrabah[1,2], Jennifer Zanet[1,2], Serge Plaza[1,2]. 1) Center for Developmental Biology, University of Toulouse,Toulouse, France; 2) CNRS, UMR5547, Toulouse France.

Recent high throughput studies have established that animal genomes express thousands of long non-coding RNAs (lncRNAs). However, small ORFs (i.e., <100 codons, called smORFs) are pervasive among lncRNAs and there is growing evidence that at least some smORFs are actually translated in small peptides. While the abundance of smORF-encoded peptides is likely underestimated, their functions and mechanistic roles are largely unknown. To address this question, we are focusing on the functional characterization of a long RNA that exerts its activity through the production of four small peptides. Previous works indicated that these smORF peptides are required at distinct steps of Drosophila development to control specific transcriptional programs. We report on new findings providing insights into the underlying molecular mechanisms.

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Bonus is maternally required for Dorsal nuclear translocation and zygotically for Dpp responsiveness in dorsal-

ventral axis formation. Janine Quijano, Michael Stinchfield, Stuart Newfeld. School of Life Sciences, Arizona State Univ, Tempe, AZ.

bonus is the fly counterpart to vertebrate Tif1/TRIM family members and is best known from studies of its post-embryonic mutant phenotypes. Analyses of larval and pupal functions revealed that it is a chromatin remodeling protein with roles such as nuclear receptor co-factor and transcription regulator. Here we provide the first report of bonus zygotic and maternal functions. In zygotic bonus mutant embryos we observed roughly 40% lethality with cuticles that were ventralized like dpp mutants. Further experiments revealed a loss of Dpp responsiveness, as shown by reduction in expression of the amnioserosa marker Hindsight. Our investigation also revealed that maternally supplied Bonus translocates to the nucleus synchronously with Dorsal and that its nuclear translocation depends upon Toll. Experiments utilizing bonus null germ line clones revealed that maternal bonus is required for Dorsal nuclear translocation. Overall, the data suggest that bonus has two distinct roles during embryonic dorsal-ventral patterning: first a maternal cytoplasmic requirement that facilitates Dorsal nuclear translocation that is unprecedented for any Tif1 protein and second a zygotic nuclear requirement for proper Dpp responsiveness. To our knowledge bon is the first identified gene required on both sides of the maternal to zygotic transition with different roles on each side. Further our data suggest a more intimate connection between signal transduction and chromatin remodeling, one whose disregulation may have a role in tumorigenesis.

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Nanobodies as novel tools to study morphogen gradient formation in vivo. Stefan Harmansa, Markus Affolter, Emmanuel Caussinus. Biozentrum, Universität Basel, Basel, Switzerland.

Monomeric antibody domains, so called nanobodies, have emerged as powerful tools to interfere with proteins of interest in vivo (Caussinus et al., NSMB 19, 117-121(2012)). We have generated membrane-bound versions of an anti-GFP nanobody in order to interfere with gradient formation of a GFP-tagged version of the Decapentaplegic morphogen (Dpp::GFP) in the Drosophila wing imaginal disc. We find that a nanobody fused to CD8, a protein which localizes all around polarized epithelial cells, is able to sequester extracellular Dpp::GFP and thereby prevents gradient formation. We are currently localizing nanobodies specifically to either the apical or the basolateral cell surface, aiming at dissecting the function of Dpp along the apical and basal compartments and identifying the respective contributions of apical and basolateral Dpp on wing growth and patterning.

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The dynamics of patterning in the Drosophila wing imaginal discs change under different environmental and internal cues. Marisa Oliveira[1], Alexander Shingleton[2], Christen Mirth[1]. 1) Instituto Gulbenkian de Ciência, Oeiras, Oeiras, Portugal; 2) Dept. of Zoology, Michigan State University.

Organisms require precisely coordinated developmental patterning programs to ensure the correct formation of structures and to produce a mature adult. To achieve this, the changes in gene expression that occur in tissues as they pattern need to be integrated with the systemic hormone levels that trigger developmental transitions. This integration must be robust across environmental conditions and between genetic backgrounds. This study addresses developmental coordination by examining how patterning dynamics in the wing imaginal disc of the fruit fly, Drosophila melanogaster, adjusts to environmental and systemic perturbations to ultimately produce functional adult tissues. We first described how developmental patterning programs progress over time. This was done by describing the development of the Drosophila third larval instar based on the gene expression pattern profile of the wing imaginal disc. These careful descriptions of the patterning events were used to compose a staging map for this period of development. We then used this map as a way to infer the intrinsic developmental clock of the tissue and organism. Secondly, we altered developmental timing, using temperature and genetic manipulations, to generate both fast and slow developing larvae. We then compared wing disc patterning between treatments. Surprisingly, not all gene expression profiles coordinate with developmental time in fast developing larvae. For slow developers, the developmental dynamics of patterning extend linearly with the developmental time. Finally, the beginning of wandering does not correlate with the patterning profiles, indicating that the hormonal cues regulating this developmental event do not coordinate patterning in the disc at this time. However, pupariation works as a checkpoint to which patterning profiles are aligned.

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Collapse of compartment boundaries and induced identity changes after massive damage in the imaginal discs of Drosophila. Salvador C. Herrera, Ginés Morata. Centro de Biología Molecular (CSIC-UAM), Madrid, Madrid, Spain.

One of the major questions in regenerative biology is how determined or differentiated cells can acquire pluripotency to repair a missing part of the organism. The wing disc compartments are established very early in development as lineage blocks and act as independent developmental units, as its cells never mix with other compartments. In our experiments we have inflicted massive cell death to either the posterior or the dorsal wing disc compartments and then have studied the regenerative response to the damage and the stability of the A/P and the D/V compartment boundaries. Strikingly, we have discovered that after damage both the A/P and the D/V compartment boundaries very frequently collapse and are later re-established. We detect cells crossing them in both directions and changing the activity of the identity genes (*engrailed*, *cubitus interruptus* or *apterous*) according to their new compartmental determination. We have found that this process is associated with loss of tension at the boundary and with alterations in the activities of factors involved in epigenetic regulation such as

the *Polycomb* and *trithorax* genes. We believe that after the damage some cells close to the A/P or the D/V borders descend to a naïve determination state and are later reprogrammed. We are trying to identify the mechanisms behind the acquisition of the new identities. By genetic manipulation, we are generating situations in which isolated wild type cells are surrounded by cells overexpressing an ectopic identity gene, e.g. the posterior identity gene *engrailed* in the anterior compartment. We observe that the isolated anterior cells acquire activity of their endogenous *engrailed* gene, indicating that it is induced by some signal/interaction with the surrounding cells. This result may open new questions on how developmental genes are regulated.

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Interchromosomal communication coordinates an intrinsically stochastic expression decision between alleles. Robert J Johnston, Claude Desplan. Biology, New York University, New York, NY.

Stochastic cell fate specification is critical for several patterning events in nervous system development. Though morphologically uniform, the *Drosophila* retina is composed of two randomly distributed types of ommatidia (unit eyes) defined by expression of light-detecting Rhodopsins. Stochastic expression of the PAS-bHLH transcription factor Spineless determines the random mosaic pattern. We have found that expression of Spineless is controlled by a two step process. First, each allele of the *spineless* gene randomly makes an intrinsic, On/Off expression decision governed by global activation coupled with stochastic repression. When the expression decisions disagree (one allele On and one allele Off), a second step involving interchromosal communication coordinates expression state between the two alleles. This effect does not depend on chromosomal pairing or endogenous *ss* chromosomal position but instead requires specific DNA elements to mediate regulatory interaction. Though individual *ss* alleles make independent stochastic choices, interchromosomal communication ensures that they are expressed in the same subset of cells. This mechanism coupling stochastic repression with interallelic expression coordination contrasts starkly with the noisy activation mechanisms seen in bacteria and the mono-allelic, stochastic activation mechanisms observed in the mouse olfactory and human color vision systems.

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Robustness of cell type identity in *Drosophila* embryos depleted for *bicoid*. Max V. Staller[1], Meghan D. Bragdon[1], Zeba B. Wunderlich[1], Norbert Perrimon[2], Angela H. DePace[1]. 1) Department of Systems Biology, Harvard Medical School, Boston, MA; 2) Department of Genetics and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA.

Developmental gene regulatory networks generate discrete cell types by buffering genetic and environmental variability to produce precise outcomes. It remains unclear how robustness to perturbation emerges from features of network architecture, such as node identities, connection strength, and network topology. To identify the relevant features, we quantify how the *Drosophila* embryonic segmentation network responds to a severe genetic perturbation. We removed *bicoid*, a key node in the network, and looked for new cell types as defined by cellular gene expression profiles. We developed a maternal Gal4 short hairpin RNA interference (shRNAi) strategy to deplete *bicoid* in blastoderm embryos. We quantitatively measured the expression patterns of 12 genes in the anterior-posterior patterning network at cellular resolution by *in situ* hybridization and two-photon microscopy. Using established methods, we combined data from many embryos into a computationally amenable gene expression atlas, which captures both the direct and indirect effects of *bicoid* knockdown. To our surprise, removing a key node from the network did not create any new cell types; instead, virtually all cell types in the *bicoid*-depleted embryo corresponded to cell types in the posterior half of the wild type embryo. Simple models of morphogen-based patterning fail to predict these data; we are currently developing alternative mathematical models of this highly interconnected network to contextualize our results. Analogous to the classical genetic experiments that uncovered the wiring of gene regulatory networks, our quantitative analysis will reveal how network architecture contributes to emergent properties such as canalization and robustness.

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BMP signaling likely had an ancestral role in providing global embryonic dorsal-ventral polarity in insects. Jeremy A. Lynch[1,2], Orhan Özüak[2], Thomas Buchta[2], Siegfried Roth[2]. 1) Molecular, Cell, and Developmental Biology, University of Illinois at Chicago, Chicago, IL; 2) Institute for Developmental Biology, University of Cologne, Cologne, Germany.

In Drosophila, the Toll signaling pathway plays the dominant role in patterning the embryo along the dorsal-ventral axis. The use of Toll signaling is not typical of most animals, and in fact a role for Toll in early axis formation has only been demonstrated in holometabolous insects. Most other animals depend heavily on BMP signaling for establishment of DV polarity and for patterning the germ layers. While this pathway plays an important role in Drosophila embryonic patterning, it is subordinate to Toll, and its function is restricted to the most dorsal regions of the embryo. Since the dependence on Toll appears to be a state derived within the insects, it is of interest to know when this trait arose in evolution. To address this, we examined the embryonic patterning system of the wasp Nasonia, which represents the most basally branching lineage of holometabolous insects, but undergoes an independently derived mode of embryogenesis that is highly similar to that of Drosophila. We have found that while Toll signaling plays a role in establishing ventral cell fates, BMP signaling (revealed by dpp RNAi) is crucial to provide global DV polarity to the embryo. We also have found evidence that a major function of BMP signaling is to repress an autoregulatory loop on the ventral side that is initiated by Toll signaling.

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A novel role of *Drosophila* P/Q type voltage gated calcium channel subunits in autophagy. Upasana Gala[1], Chao Tong[2,3], Manish Jaiswal[2], Hector Sandoval[2], Shinya Yamamoto[1], Vafa Bayat[1], Bo Xiong[1], Ke Zhang[4], Wu-Lin Charng[1], Lita Duraine[5], Kartik Venkatachalam[6], Hugo Bellen[1,2,5]. 1) Program in Developmental Biology, BCM; 2) Department of Human and Molecular Genetics, BCM; 3) Department of Molecular Biology, Zhejiang University; 4) Structural & Computational Biology & Molecular Biophysics Program, BCM; 5) Howard Hughes Medical Institute; 6) Department of Integrative Pharmacology, UTHSC.

Autophagy, characterized by the formation of a double membrane structure called autophagosome (AP) that sequesters intracellular cargo and delivers it to the lysosomes for proteolytic degradation, has been proposed to be especially important in long-lived post-mitotic neurons and defects in autophagy have been implicated in several neurodegenerative diseases (ND). We generated a collection of X chromosome mutants to identify essential genes in *Drosophila* involved in neurodegeneration. To find novel genes involved in autophagy that affect neuronal function, we re-screened this collection for defects in autophagy and identified ten alleles of *cacophony (cac)*, the pore-forming α1 subunit of voltage gated calcium channel (VGCC). Transmission electron microscopy of eye-specific neurons in *cac* flies shows an accumulation of late stage autophagic vacuoles (AV), suggesting defects in AP maturation. Cac, its accessory subunits and the SNARE complex are involved in neurotransmitter release through synaptic vesicle fusion in a Ca2+-dependent manner. Surprised that Cac affected autophagy, we tested flies mutant for the VGCC α2δ subunit, *straightjacket* and *vamp7*, a lysosomal SNARE protein and found that these phenocopy the cac mutants. Although Ca2+ and certain SNAREs have been shown to be required for autophagy, a VGCC has not previously been implicated in the autophagy pathway. We propose that Cac plays a role in autophagy by regulating the fusion of AVs with lysosomes. Mutations in VGCC subunits cause severe NDs such as spinocerebellar ataxia 6 and episodic ataxia type 2 but the molecular mechanisms are still undefined. We also propose that defects in Cac-mediated autophagy may be responsible for the aforementioned NDs.

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Zonda: A novel gene involved in autophagy and growth control. Mariana Melani[1], Maria Julieta Acevedo[1], Joel Perez Perri[1], Nuria Magdalena Romero[2], Pablo Wappner[1]. 1) Fundacion Instituto Leloir, Buenos Aires, Buenos Aires, Argentina; 2) Institute of Developmental Biology and Cancer, Nice, France.

Understanding the mechanisms by which multicellular organisms control the growth of cells, organs and the whole body is a central question in developmental biology. Studies in this field suggest that genetic mechanisms interlace with environmental clues to establish the final size of the organism. In this work, we describe the function of a novel gene that we have named *zonda* as a negative regulator of growth.*eyeless-flippase* induced *zonda* mutant clones generate larger heads when compared to controls. Conversely, overexpression of *zonda* in the head tissue leads to a pinhead phenotype, and mosaic overexpression of *zonda* in larval fat body cells provokes an autonomous reduction of cell size. Strikingly, Zonda subcellular localization is sensitive to the nutritional status of the larvae. In well-fed individuals Zonda presents a vesiculo-reticulated subcelullar distribution, without any clear colocalization with well-described organellar markers. When third instar larvae are starved for 4 hours, Zonda distribution changes dramatically to discrete foci. Zonda-containing foci partially colocalize with lysotracker and Lamp1 positive vesicles, and strongly colocalize with ATG-8, indicating that under these conditions Zonda is part of autophagy-induced structures. This remarkable colocalization led us to investigate a potential role of Zonda in autophagy. Indeed, we found that *zonda* mutant clones in the fat body of starved third instar larvae fail to incorporate lysotracker, characteristic of starvation-induced autophagy. Moreover, overexpression of *zonda* is sufficient to induce autophagy, as assessed by the nucleation of ATG8 in autophagosomes. Altogether, our results reveal that *zonda* is a novel autophagy gene likely to play a role at early steps of the autophagy cascade, thereby modulating growth control.

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***Ino80* is required for ecdysone-dependent regulation of PI3K/Akt signaling during *Drosophila* development.** Sarah Neuman, Robert Ihry, Arash Bashirullah. University of Wisconsin-Madison, Madison, WI.

During metamorphosis, many larval tissues, including the larval salivary glands, undergo programmed cell death in response to a pulse of the steroid hormone ecdysone. Ecdysone binding to its receptor initiates a cascade of gene expression that culminates in tissue-specific induction of the IAP antagonists *reaper* (*rpr*) and *hid involution defective* (*hid*). We have identified a mutant allele of the chromatin remodeling protein *Ino80* that displays defects in this salivary gland cell death response. *Ino80* mutant salivary glands do not initiate caspase activation despite induction of the death activators *rpr* and *hid*. Our results indicate that PI3K/Akt signaling is not properly downregulated in *Ino80* mutant salivary glands, resulting in dramatic increases in phospho-Akt levels. We demonstrate that constitutive activation of PI3K/Akt signaling is sufficient to resist ectopic expression of death activators, and that ecdysone signaling is normally required to inhibit PI3K/Akt signaling prior to the death response in salivary glands. Critical negative regulators of PI3K/Akt signaling, including *PTEN* and *S6K*, are induced in response to ecdysone, and the positive regulator *PDK1* is repressed in response to ecdysone. Our data suggests that *Ino80* is required for efficient induction of the *DHR3*-dependent mid-prepupal ecdysone response and that a defect in this cascade disrupts the timely inhibition of PI3K/Akt signaling. These results highlight the role of the ecdysone hierarchy in the regulation of PI3K/Akt signaling during development.

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Necrotic cell death is mediated by a specific chromatin-modifying pathway in fly and mammals. Kai Liu[1], Yuhong Li[1], Lianggong Ding[1], Hui Yang[1], Chunyue Zhao[1], Hermann Steller[2], Lei Liu[1]. 1) State Key Lab of Biomembrane and Membrane

Biotechnology, School of Life Sciences, Peking University, Beijing, China; 2) Strang Laboratory of Apoptosis and Cancer Biology, Howard Hughes Medical Institute, The Rockefeller University, NY.

Necrotic cell death (necrosis) plays important roles in many neurological diseases such as ischemic stroke, epilepsy and traumatic brain injury. Neuronal necrosis often results from acute calcium overload through glutamate receptors. However, the mechanism of necrosis execution is largely unknown. By genetic modeling calcium-overload-induced neuronal necrosis in *Drosophila*, we discovered specific chromatin changes in necrosis, including increased Histone H3 Serine 28 phosphorylation (H3S28ph), dissociation of polycomb repressive complex 1 (PRC1) from chromatin and increased H3 lysine 4 trimethylation (H3K4me3). Importantly, mutants of PRC1 enhance necrosis, whereas mutants of JIL-1 (the kinase generating H3S28ph to repel PRC1) and Trx (the histone methyltransferase generating H3K4me3 to antagonize PRC1) suppress necrosis. These results indicate neuronal necrosis is mediated by a chromatin-modifying pathway involving phosphorylation of H3S28 by JIL-1 to repel PRC1 and activate Trx. Moreover, we found this pathway mediates necrosis through mitochondrial fragmentation. Strikingly, this pathway is also activated in glutamate-induced necrosis in rat cortical neurons and ischemic mouse brains, and inhibition of the pathway suppresses neuronal necrosis *in vitro* and *in vivo*. These findings uncover a novel conserved mechanism of necrosis execution involving nucleus response and subsequent signal transduction to mitochondria, which provides promising drug targets and novel markers for necrosis-related diseases.

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The endocycle promotes aneuploidy at both ends of the spindle. Donald T. Fox[1,2], Ruth Montague[1], Kevin Schoenfelder[1], Benjamin Stormo[2], Sarah Paramore[1]. 1) Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC; 2) Department of Cell Biology, Duke University Medical Center, Durham, NC.

Polyploidy (extra chromosome sets) is a common property of both normal and cancerous animal cells. In cancer cells, polyploidy is linked to increased cell division errors, or chromosomal instability (CIN). Similarly, our study of Drosophila hindgut papillar formation found links between polyploid cell division and CIN. During hindgut metamorphosis, polyploid cells formed via the endocycle re-enter mitosis. As in mitosis of polyploid cancer cells, these papillar precursor divisions also exhibit CIN, leading to aneuploidy (cells with unbalanced chromosome content). Thus, CIN is a common property of polyploid cells, but mechanisms connecting polyploidy to CIN remain unknown. Through further characterization of papillar formation in flies, we now report two distinct mechanisms linking polyploidy via endocycles to CIN. First, we find the endocycle impairs localization of the spindle checkpoint regulator Mad2 to chromosomes. Using time-lapse imaging, we find Mad2 mis-localization coincides with chromosome mis-alignment defects and aneuploidy during papillar mitosis, indicative of failure in the Mad2-dependent checkpoint in anaphase delay. Second, we find polyploid papillar cells accumulate extra spindle poles, which fail to cluster into a bipolar spindle during anaphase. As a result, papillar development proceeds with frequent multipolar division. Contrary to multipolar division in diploid cells, we find multipolar divisions in polyploid cells frequently yield viable aneuploid daughters that contribute to organogenesis. Taken together, our study of papillar mitosis has identified two primary mechanisms by which endocycles disrupt faithful cell division: one at the chromosome end of the spindle (Mad2 mislocalization) and one at the spindle pole (failed pole clustering). Given recent findings that the endocycle induces aneuploidy and tumorigenesis in mammalian cells, our findings have potential implications for how the endocycle contributes to aneuploidy in cancer.

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Cell cycle remodeling is sufficient to repress apoptosis. Suozhi Qi, Christiane Hassel, Brian R. Calvi. Indiana University, Bloomington, IN.

Severe DNA damage usually triggers eukaryotic cells to undergo a programmed cell death called apoptosis. However, we have found that when cells are in the endocycle, which is characterized by alternating G and S phase, they do not apoptose in response to DNA damage. We are investigating how cell cycle remodeling modulates the apoptotic response. To do this, we are altering the cell cycle through RNAi knockdown of crucial cell cycle regulators and testing the apoptotic responses to DNA damage caused by irradiation. Knocking down Cyclin A or overexpressing Fizzy-related, a subunit of anaphase promoting complex, switches normal mitotic cells into the endocycle and inhibits apoptosis after irradiation. We have also found that arresting the cell cycle by knocking down Cdk1 or Cyclin E blocks the apoptotic responses in Drosophila. Our data indicate that cell cycle remodeling is sufficient to block apoptosis independent of other developmental inputs. These unexpected findings have important implications for understanding how polyploid tumor cells escape apoptosis and contribute to disease progression.

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The Molecular Chaperone Hsp90 is Required for Cell Cycle Exit. Jennifer L. Bandura[1,2], Huaqi Jiang[1,3], Derek W. Nickerson[1], Bruce A. Edgar[1,2]. 1) Fred Hutchinson Cancer Research Center, 1100 Fairview Ave., Seattle, WA 98109, USA; 2) German Cancer Research Center (DKFZ) - Center for Molecular Biology Heidelberg (ZMBH) Alliance, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany; 3) Current address: UT Southwestern Medical Center at Dallas, 6000 Harry Hines Blvd., Dallas, TX 75235, USA.

The coordination of cell proliferation and differentiation is crucial for proper development. In particular, robust mechanisms exist to ensure that cells permanently exit the cell cycle upon terminal differentiation, and these include restraining the activities of both the E2F/DP transcription factor and Cyclin/Cdk kinases. However, the full complement of mechanisms

necessary to restrain E2F/DP and Cyclin/Cdk activities in differentiating cells are not known. Here, we have performed a genetic screen in Drosophila, designed to identify genes required for cell cycle exit. This screen utilized a PCNA-miniwhite+ reporter that is highly E2F-responsive and results in a darker red eye color when crossed into genetic backgrounds that delay cell cycle exit. Mutation of Hsp83, the Drosophila homolog of mammalian Hsp90, results in increased E2F-dependent transcription and ectopic cell proliferation in pupal tissues at a time when neighboring wild-type cells are postmitotic. Further, these Hsp83 mutant cells have increased Cyclin/Cdk activity and accumulate proteins normally targeted for proteolysis by the anaphase-promoting complex/cyclosome (APC/C), suggesting that APC/C function is inhibited in cells lacking Hsp83. Based on these data, we propose that Cdh1/Fzr, an activating subunit of the APC/C that is required for timely cell cycle exit, is a client protein of Hsp83. Our results reveal that Hsp83 plays a heretofore unappreciated role in promoting APC/C function during cell cycle exit and suggest a mechanism by which Hsp90 inhibition could promote genomic instability and carcinogenesis.

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Phosphorylation of Caprin by Chk1(Grapes) May Control the Cell Cycle at the Mid-Blastula Transition. Helen X. Chen[1,3], Ophelia Papoulas[2,3], Paul Macdonald[1,3]. 1) Section of Molecular Cell and Developmental Biology; 2) Center for Systems and Synthetic Biology; 3) The Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712.

The mid-blastula transition (MBT) is the first embryonic development event requiring zygotic gene activity. One feature of the MBT is a slowing of the cell cycle. Previously, we reported that two translational regulators, *Drosophila* Fragile X mental retardation protein (dFMRP; FMR1) and Cytoplasmic activation/proliferation-associated protein (Caprin; Capr) collaborate to control the cell cycle at the MBT by mediating the normal repression of maternal *Cyclin B* mRNA. It remains unclear how the proper timing of the MBT is determined, and whether regulation of Capr or FMR1 activity might contribute to timing. Using precisely staged *Drosophila* embryo extracts we show that Capr is phosphorylated in multiple domains, with changes in phosphorylation state coinciding with the MBT. Three lines of evidence demonstrate that one responsible kinase for MBT-specific phosphorylation is Chk1/Grapes (Grp). First, immunodepletion of Chk1 from embryo extracts greatly reduces phosphorylation of the central domain of Capr (Capr298-630). Second, purified Chk1 phosphorylates Capr298-630 *in vitro*. Third, mutation of the serine within Capr298-630 that is phosphorylated by purified Chk1 blocks phosphorylation of Capr298-630 in embryo extracts. A role for Chk1 in MBT timing is suggested by a genetic interaction. Reducing the levels of both Capr and FMR1 in embryos from mothers heterozygous for mutation of each gene has no effect on MBT timing. However, the combination of loss of Capr activity and reduction of FMR1 activity results in altered MBT timing with a precocious mitosis. A similar phenotype was obtained when, in the background of reduced Capr and FMR1, the level of Chk1 was reduced. This raises the possibility that Capr phosphorylation by Chk1 contributes to control of MBT timing. Since Capr and FMR1 control the levels of Cyclin B, the precocious mitosis might be due to altered Cyclin B regulation.

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Ecdysone Signaling Antagonizes EGF Signaling in Germline-Cyst Cell Interactions of *Drosophila melanogaster* Testes. Ricky W Zoller, Cordula Schulz. Cellular Biology, University of Georgia, Athens, GA.

The testes of *Drosophila melanogaster* contain two populations of stem cells: germline stem cells and somatic cyst stem cells. Both stem cell populations produce daughter cells, the gonialblasts and the cyst cells, that together form a cyst composing of one germline cell enclosed by two cyst cells. The enclosed germline cell, the gonialblast, undergoes four rounds of mitotic transit amplification divisions before entering terminal differentiation. The cyst cells continue to engulf the developing germline cells until differentiation, where they become specialized cap and tail cyst cells. This codifferentiation of germ and cyst cells is a highly coordinated process and the mechanisms regulating the development from stem cells to more mature germ cells are not well understood. The programming of amplification and exit into differentiation requires interactions of the germ cells with the two accompanying cyst cells. Signaling via the EGFR regulates germline-soma association and the differentiation of the enclosed germline cells. We recently discovered that reduction of the ecdysone receptor (EcR) in cyst cells via RNAi knockdown promoted the differentiation of cyst and germline cells in EGF mutant testes. Through western blotting and immunofluorescence techniques, we confirmed that EcR is indeed expressed in testes and, specifically, in cyst cell nuclei. We next addressed if mutations in enzymes responsible for the biosynthesis of ecdysone had the same effect on EGF mutants as EcR. We found that several of these so-called Halloween genes are expressed in testes, as evidenced by RT-PCR, and their reduction markedly decreased the severity of the EGF mutant testis phenotype. Currently, we are dissecting the EcR pathway in cyst cells in more detail.

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Localization and functional analysis of Nmd and CG4701 AAA proteins in mitochondrial and microtubule dynamics in *Drosophila* spermatogenesis. Bethany L. Wagner, Lindsay A. Regruto, Melissa Lorenzo, Jessica Gerard, Sarah C. Pyfrom, Karen G. Hales. Department of Biology, Davidson College, Davidson, NC.

CG4701 and *nmd* are paralogous genes in *Drosophila melanogaster* that are associated with mitochondrial shaping defects during the early stages of spermatogenesis, resulting in recessive male sterility. Mutations in testis-enriched *CG4701* cause polynucleated spermatids, suggestive of cytokinesis defects during meiosis, and vacuolated Nebenkerne (mitochondrial aggregates). The broadly-expressed and essential gene *nmd* has hypomorphic alleles with differing phenotypes. In males mutant for one allele, mitochondria fail to aggregate, preventing Nebenkern formation; in contrast, males with another allele display cytokinesis defects similar to *CG4701*. Preliminary results from *nmd* knockdown using RNAi show mutant phenotypes

similar to the *nmd* allele with mitochondrial aggregation defects. Nmd localizes to mitochondria and centrosomes/basal bodies, and recent localization of a tagged version of CG4701 suggest that it colocalizes with Nmd. CG4701-RFP with a point mutation in the predicted transmembrane domain shows protein mislocalization and does not fully rescue the mutant phenotype. Both Nmd and CG4701 belong to the AAA ATPase family of proteins and are closely related to known microtubule severing proteins spastin and katanin. Therefore, we hypothesized that both Nmd and CG4701 interact with microtubules; we investigated the localization and expression of β-tubulin in nmd and CG4701 mutants using GFP-tagged versions of the proteins. β-tubulin-GFP partially rescued the *nmd* and *CG4701* mutant phenotype providing support for a functional connection between Nmd and CG4701 and microtubules. Alternative detection techniques in testes from mutants will answer whether microtubule dynamics are altered in the mutants.

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NPR2/3 define a novel nutrient stress pathway in the Drosophila ovary. Youheng Wei, John Reich, Weili Cai, Tanveer Akbar, Kuikwon Kim, Mary Lilly. Cell Biology and Metabolism Program, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

Drosophila oogenesis is highly sensitive to nutritional inputs. Under protein poor conditions, mid-stage (stage 8/9) egg chambers degenerate. In contrast, young egg chambers (stage 2 to 7) remain intact so that egg production can resume when nutrient availability improves. The pathways that protect young egg chambers under nutrient stress are poorly defined. During starvation the activity of the nutrient sensitive kinase, Target of Rapamycin (TOR) is down regulated. The inhibition of TOR activity triggers autophagy, a catabolic process that provides nutrients for cell survival during starvation through a lysosomal-mediated process of cytoplasmic degradation. In yeast the NPR2 and NPR3 proteins physically interact and mediate a response to amino acid starvation upstream of the TOR pathway and have been implicated in the regulation of autophagy. Intriguingly, in yeast NPR2 and NPR3 regulate early meiotic progression and sporulation (gametogenesis). We have determined that the basic metabolic functions of NPR2 and NPR3 have been conserved in metazoans. We found that, as is observed in yeast, in Drosophila NPR2 physically interacts with NPR3. Additionally, upon starvation the NPR2 and NPR3 proteins target to autophagosomes. Moreover, our data indicate that knocking down NPR2 or NPR3 in the female germ line results in ovaries being acutely sensitive to nutrient limitation. Specifically, in the absence of NPR2 and NPR3 young egg chambers die at high rates under starvation conditions. Our data suggest that this starvation sensitivity is due to the inability to down regulate TOR activity in response to nutrient stress. Finally, we defined a unique role for NPR2 and NPR3 in the regulation of early oogenesis. In summary, our data indicate that the evolutionarily conserved NPR2/3 complex regulates the response to starvation and gametogenesis in the Drosophila ovary.

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Tramtrack69 regulates epithelial tube expansion in the Drosophila ovary through Paxillin, Dynamin, and the homeobox protein Mirror. Nathaniel Peters, Celeste Berg. Dept of Genome Sciences/MCB Program, University of Washington, Seattle, WA.

Epithelial tubes serve as the infrastructure for organs and tissues and are essential for most multicellular life; faithful tube morphogenesis requires precise orchestration of cell signaling, shape, polarity, migration, and adhesion. In the Drosophila ovary, the follicular epithelium that encases each developing egg chamber forms a pair of epithelial tubes, the lumens of which act as molds for the eggshell respiratory filaments, or dorsal appendages (DAs). This system is a robust and accessible model for epithelial tube patterning, formation, and expansion. The Tramtrack69 (TTK69) transcription factor controls DA lumen volume by regulating tube expansion; the twin peaks (twk) mutation reduces TTK69 levels specifically during late oogenesis, inhibiting tube expansion and producing stunted eggshell DAs. Microarray analysis of wild type and twk ovaries, followed by in situ hybridization and RNAi of candidate genes, identified the focal adhesion scaffold Paxillin, the endocytotic regulator Shibire (Dynamin), and the homeodomain transcription factor Mirror as TTK69-regulated effectors of DA-tube expansion. These genes display enriched expression in DA-tube cells, reduced expression in twk, and RNAi phenotypes that are enhanced in a twk heterozygous background, indicating genetic interactions. Although Mirror is known to pattern the follicular epithelium prior to DA tube morphogenesis, we demonstrate that Mirror regulates DA-tube expansion independently of patterning, revealing a novel tube morphogenic role for this transcription factor. We show that Mirror, as well as TTK69, positively influences the expression of Paxillin, suggesting that these TTK69 effectors are in the same pathway. Finally, our results implicate several other genes, including shibire, as tube expansion effectors downstream of TTK69. Thus, our characterization of twk-differentially expressed genes identifies novel tube morphogenesis regulators, begins to elucidate the network of TTK69 effectors required for epithelial tube expansion, and significantly advances our understanding of this vital developmental process.

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Ovulation requires female reproductive tract secretions controlled by NR5a-family nuclear hormone receptors. Jianjun Sun, Allan Spradling. HHMI Laboratory, Department of Embyology, Carnegie Institution for Science, Baltimore, MD.

Ovulation is an important, general phenomenon yet its molecular control remains poorly understood. We report that *Drosophila* can be used to study the molecular genetic mechanisms regulating ovulation, and that several cellular and molecular mechanisms previously thought to be unique to mammals appear to be conserved in the fly. In particular, we found

that the NR5a-family nuclear hormone receptor Hr39 is required for ovulation, like its homolog LRH-1 in mice. In *Drosophila*, Hr39 controls the development of the glands of the female reproductive system, known as spermathecae and parovaria. Hence *Hr39*mutants lack reproductive tract secretions. The development of these glands was characterized for the first time and shown to involve asymmetric divisions, the Notch signaling, and the zinc-finger transcription factor Hindsight. Using this knowledge we manipulated the number of secretory cells and hence the amount of secretion in the reproductive tract. We showed that secretions play critically important functions in ovulation, sperm storage, and other aspects of reproduction. We also identified specific genes encoding secreted products in the spermathecae, including many that are conserved in mammals, and identified their likely functions.

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Using transcriptome and phosphoproteome profiling to identify genes that regulate the egg-to-embryo transition in D. melanogaster. Caroline V. Sartain, Amber R. Krauchunas, Jun Cui, Vanessa L. Horner, Jeffrey A. Pleiss, Mariana F. Wolfner. Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY.

After oogenesis, Drosophila oocytes transition from arrest to the ability to initiate embryogenesis if fertilized. This "egg activation" involves resumption and completion of meiosis, translation of proteins from stored maternal mRNAs, degradation of other maternal mRNAs, and changes to the vitelline envelope. Genetic screens identified maternal-effect genes needed for egg activation or to initiate embryogenesis, but more genes are certainly involved. Since there is little or no transcription during this transition, egg activation must be regulated by post-transcriptional and post-translational modification of pre-existing maternal mRNAs and proteins. Thus, we used transcriptomic and proteomic approaches to identify new molecules needed for egg activation: (1)We identified mRNAs that become translationally-competent. We'd shown that the GLD2 cytoplasmic poly-A polymerase WISPY is essential for egg activation. GLD2s extend poly-A tails of stored mRNAs to allow recruitment of cellular translation machinery. Using microarrays we identified RNAs whose poly-A tails depend on WISPY; these are likely to be newly-translated upon egg activation. We find that WISPY regulates poly-A tail length of RNAs from thousands of genes during egg activation. WISPY-regulated RNAs encode proteins in GO classes with likely roles in egg activation and early embryogenesis. (2)We examined phospho-modification of the proteome during egg activation. Changing phosphorylation state can cause an array of regulatory effects, and kinase and phosphatase activities are modulated during egg activation. Thus, we hypothesized that simultaneously changes in phosphorylation states of many proteins could underlie the cellular changes from oocyte to activated egg. Using 2-D gels and IMAC we identified 311 proteins that are phospho-modulated during egg activation; 83%; are conserved to mammals. RNAi knockdown of these molecules is identifying new genes needed for the oocyte-to-embryo transition.

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Intercellular Protein Equilibration through Somatic Ring Canals. Peter McLean. Genetics, Yale School of Medicine, New Haven, CT.

Ring canals are the stabilized remnants of arrested cleavage furrows, and provide direct cytoplasmic connections between sibling cells. Ring canals connecting germline cells are known for their participation in *Drosophila* oogenesis, but little is known about their role in the several somatic tissues in which they are also found. In this study we use the ovarian follicle cells to investigate the impact of somatic ring canals on protein movement between cells and across an epithelium. Here, we expand upon our previously reported results of photoactivatable GFP (PAGFP) and computational modeling that show intercellular protein movement to be robust, limited to syncytial groups that vary in size, and driven by passive diffusion. Using cells that express mosaic GFP, we provide evidence by Fluorescence Loss in Photobleaching (FLIP) and Fluorescent In Situ Hybridization (FISH) that ring canals permit equilibration of protein between cells with highly disparate levels of transcription. We also use a novel combination of markers to evaluate the extent and impact of protein movement relative to mitotic clones in follicle cells and wing imaginal discs. We provide evidence of robust intercellular exchange of GFP between the two lineages of the mitotic clone. We conclude that, depending on the experimental setup and proteins of interest, intercellular protein movement may alter the interpretation of clonal data in follicle cells. In sum, our results provide the first evidence for a role of intercellular bridges outside of the germline, a major function of which is to mediate equilibration of protein across an epithelium of transcriptionally mosaic cells.

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A non-canonical role for Yorkie and the Salvador/Warts/Hippo pathway in tracheal tube-size regulation. Renée M. Robbins, Samantha C. Gbur, Greg J. Beitel. Molecular Biosciences, Northwestern Univ, Evanston, IL.

The size of epithelial tubes is critical for organ function, yet the mechanisms of size control are poorly understood. In the Drosophila trachea, our group and others have demonstrated that cell junctions, apical extracellular matrix (aECM) and cell polarity proteins regulate tube size. Here, we show that the Salvador/Warts/Hippo (SWH) pathway also regulates tracheal tube size, but oppositely of what was expected from the previously characterized role of the SWH pathway. The SWH pathway regulates cell growth and proliferation by negatively regulating the transcription factor Yorkie (Yki), the fly homolog of YAP. Yki activity upregulates expression of genes that promote cell cycle progression and cell growth, so *yki* mutations typically reduce growth and were expected to decrease tracheal length. Surprisingly, *yki*mutant embryos have dramatically over-elongated trachea despite normal cell junctions and aECM. Similarly, mutations in *hippo*, *salvador* and *warts* cause shorter rather than longer trachea. Tracheal cell number is not affected in *yki* mutants, and quantification shows that cell volume and

length changes are not correlated in *yki* mutants. Thus, Yki does not appear to control tracheal tube-size via cell growth or cell division. Consistent with these results, during WT development, tracheal cell volume can decrease by 28% despite a 8% increase in length and a 294% increase in lumenal diameter between stages 14 and 16. Thus, embryonic tracheal tube size increases are not driven by cell size increases. Mutations in the Yki target *bantam* (a miRNA) did not change tracheal size, but mutations in *th/DIAP* (*Drosophilainhibitor of apoptosis protein*) strongly increased tracheal length. Since *yki* mutations do not alter tracheal cell number or growth, we conclude that Yki controls tracheal tube size through a novel, non-apoptotic function of DIAP that regulates the amount of apical membrane surface. We are currently using a DIAP-lacZ reporter to quantify Yki activity in tracheal cells and determine whether other tracheal pathways act through Yki to control tracheal tube-size.

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Mutual inhibition among postmitotic neurons regulates robustness of brain wiring. Marion G Langen[1,2,3], Marta Koch[1,2], Jiekun Yan[1,2], Natalie de Geest[1,2], Marie-Luise Erfurt[4,5], Barret D. Pfeiffer[6], Dietmar Schmucker[4,5], Yves Moreau[7], Bassem A. Hassan[1,2,3,4]. 1) VIB Center for Biology of Disease, VIB, 3000 Leuven, Belgium; 2) Center for Human Genetics, University of Leuven School of Medicine, 3000 Leuven, Belgium; 3) Doctoral Program in Molecular and Cognitive Neuroscience, Doctoral School of Biomedical Sciences, University of Leuven, 3000 Leuven, Belgium; 4) Vesalius Research Center, VIB, 3000, Leuven, Belgium; 5) Department of Oncology, University of Leuven School of Medicine, 3000 Leuven, Belgium; 6) Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA; 7) Bioinformatics Group, Department of Electrical Engineering, University of Leuven, 3000 Leuven, Belgium.

Brain connectivity maps display a delicate balance between individual variation and stereotypy, suggesting the existence of dedicated mechanisms that simultaneously permit and limit individual variation. We show that mutual inhibition among groups of neighboring postmitotic neurons during development regulates the robustness of axon target choice in a non-deterministic neuronal circuit. Specifically, neighboring postmitotic neurons communicate through Notch signaling during axonal targeting, to ensure balanced alternative axon target choices without a corresponding change in cell fate. Loss of Notch in postmitotic neurons modulates an axon's target choice. However, because neighboring axons respond by choosing the complementary target, the stereotyped connectivity pattern is preserved. In contrast, loss of Notch in clones of neighboring postmitotic neurons results in erroneous co-innervation by multiple axons. Our observations establish mutual inhibition of axonal target choice as a robustness mechanism for brain wiring and unveil a novel cell fate independent function for canonical Notch signaling.

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***Drosophila* epidermal cells function as phagocytes to clear degenerated dendrites during dendrite pruning.** Chun Han, Yuanquan Song, Denan Wang, Lily Jan, Yuh-Nung Jan. Howard Hughes Medical Institute, Departments of Physiology, Biochemistry, and Biophysics, Univ California, San Francisco, San Francisco, CA.

During the development of the nervous system, many neurons remodel their dendritic arbors to reshape neural circuitry. The excessive dendrites are pruned and go through degeneration programs during the dendrite remodeling. Prompt clearance of the degenerated dendrites from surrounding tissues is critical for maintenance of homeostasis and prevention of inflammatory responses. How the degenerated dendrites are cleared by phagocytosis and degraded in phagocytes is poorly understood. To address this question, we studied the clearance of degenerated dendrites during dendrite pruning of *Drosophila*dendritic arborization (da) neurons. By using the GEEM (gene expression with an independent enhancer-driven cellular marker) strategy to manipulate individual extraneural tissues that interact with da dendrites, we found that *Drosophila* epidermal cells, instead of hemocytes, are the main phagocytes in the engulfment and degradation of degenerated dendrites. To further analyze how dendrite debris is degraded in epidermal cells, we created a series of dendritic markers to trace the maturation of dendrite-derived phagosomes and established the first *in vivo* model system in *Drosophila* for analyzing phagosome maturation. We show that engulfment of degenerated dendrites by epidermal cells is mediated by scavenger receptor Drpr, and two members of CD36 family encoded by *croquemort* (*crq*) and*debris buster* (dsb) act at distinct stages of phagosome maturation. Lastly, we found that the phagocytic activity of epidermal cells facilitates dendrites fragmentation, demonstrating the coordination between neurons and phagocytes during dendrite degeneration.

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Control of cell proliferation in the embryonic CNS by Temporal, Hox and Notch cues. Stefan Thor. Dept Clinical and Exp Medicine, Linkoping Univ, Linkoping, OG, Sweden.

Substantial progress has been made with respect to cell fate specification in the nervous system. In contrast, less is known regarding the control of proliferation, such that proper numbers of each neural cell type is generated. In the embryonic Drosophila nerve cord, neuroblasts (NBs) generate the CNS by dividing asymmetrically, renewing themselves while budding off daughter cells, the ganglion mother cells (GMC). Each GMC in turn divides asymmetrically to produce two different neurons and/or glia. This is denoted a Type I division mode, because daughters divide once. The transcription factor Prospero plays a key role in controlling daughter cell proliferation in Type I daughters (GMCs). Recent studies have identified an alternate division mode, where NBs bud off daughters that directly differentiate. We propose that this division mode should be denoted Type 0, since daughter cells do not divide. However, the extent of Type I and Type 0 proliferation in the CNS, and the extent to which NBs display switches in the proliferation modes were hitherto unknown. By mapping several specific NB lineages, and conducting a global analysis of division mode, we find that half of all NB lineages in the nerve cord undergo a Type I to Type 0

switch. While Pros controls Type I daughter proliferation, Pros does not control Type 0 daughter proliferation. Instead, the switch from Type I to Type 0 mode is combinatorially controlled by the temporal genes castor and grainyhead, the Hox gene Antennapedia and Notch signaling. These regulatory cues all emerge in the latter part of many lineages, thus ensuring proper temporal control of the Type I to Type 0 switch. Analysis of 22 key cell cycle genes reveals that the dacapo gene (p21CIP/p27KIP) is the key player triggering the Type I to Type 0 switch. Dacapo expression is triggered late in switching lineages by the temporal, Hox and Notch cues, and ectopic expression of the regulatory cues or Dacapo is sufficient to trigger the proliferation switch. These findings reveal a novel global principle for proliferation control in the Drosophila CNS.

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Hippo-dependent cell fate specification is antagonized by multiple regulatory modules. Baotong Xie[1], David Terrell[1,2,3], Mark Charlton-Perkins[1,2], Brian Gebelein[2,4], Tiffany Cook[1,2,4]. 1) Division of Pediatric Ophthalmology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA; 2) Molecular and Developmental Biology Graduate Program, University of Cincinnati, Cincinnati, OH 45229, USA; 3) Physician Scientist Training Program, University of Cincinnati, Cincinnati, OH 45229, USA; 4) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA.

The Warts/Lats kinase plays central roles in the Hippo signaling pathway for tissue growth and neurogenesis. In Drosophila, Warts forms a bistable negative feedback loop with the Melted pleckstrin homology-domain protein to govern blue vs green photoreceptor fate. How this loop is generated and leads to changes in cell fate remains unclear. Here, we describe a hierarchical transcriptional regulatory network that functions upstream, within, and downstream of the melted-warts bistable loop to promote blue- and repress green- photoreceptor fate. This network includes a conserved feedforward loop between OTX and MAF transcription factors, and a multi-level feedback loop between Warts, the TEA factor Scalloped and the Yorkie/YAP transcriptional co-activator. Integration and re-implementation of the same regulatory modules guarantees unambiguous fate decisions, thus regulating both cell fate determinants and terminal differentiation genes. Our study defines cell-autonomous transcriptional regulators that integrate with the Hippo pathway to ensure robust and stable neuronal fate decisions.

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Adult neurogenesis in Drosophila. Eduardo Moreno, Ismael Hernandez-Fernandez, Christa Rhiner. University of Bern, Bern, Switzerland.

Using a new method to study cell proliferation in the adult we have observed de novo generation of neurons in the Drosophila brain during adulthood. This adult neurogenesis is restricted to some brain structures and is enhanced by damage. Implications and the genetic pathways involved will be discussed.

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3D mapping of the adult Drosophila brain: Towards a comprehensive digital atlas of secondary lineages. Darren C.C. Wong, Jennifer K. Lovick, Kathy Ngo, Jaison Omoto, Joseph Nguyen, Volker Hartenstein. MCDB, UCLA, Los Angeles, CA.

The central brain of Drosophila melanogaster is formed by approximately 100 bilaterally symmetrical lineages and these arise from the corresponding 100 neural stem cells (neuroblast) that derive from the early procephalic (head) neurectoderm. Neurons born in the embryo control larval behaviors and these may be reorganized during metamorphosis and subsequently contribute to adult neural circuit. Up until now, there has not been an exhaustive study that endeavors to map every lineage in the adult Drosophila brain. Extensive work has been put into understanding the lineages of the mushroom body and antennal lobe. A map of the secondary lineages has been generated in previous studies from our lab. It was found that the secondary lineages generate a tract (SAT) whose point of entry and trajectory in the neuropile is both invariant and characteristic in nature. Using the global marker BP106 (anti-neurotactin), in concert with green fluorescent protein -labeled clones, we could visualize and follow the SATs of all lineages to generate a comprehensive digital atlas of the secondary lineages at the adult stage. Using the SAT map we were able to identify and classify MARCM clones of secondary lineages in the adult. We show in this presentation the dynamically evolving map of SATs, the way in which SAT entrypoints and trajectories allow us to identify and classify MARCM clones of secondary lineages in the adult. We also present a 3D model of the adult brain, incorporating cell bodies and axonal tracts of secondary lineages.

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Development of astrocyte-like and ensheathing glia of the early larva ventral nerve cord. Emilie Peco[1], Sejal Davla[1], Stephanie Stacey[1], Matthias Landgraf[2], Don van Meyel[1]. 1) Centre for Research in Neuroscience, McGill University, Montreal, Qc, Canada; 2) Department of Zoology, University of Cambridge, UK.

CNS glia in mammals and invertebrates are of heterogeneous subtypes serving diverse and specialized functions. However, knowledge of the extent of glial diversity and how it arises during development is quite limited. The aim of our study was to explore diversity among neuropil-associated glia of the *Drosophila* ventral nerve cord, and more precisely among Longitudinal Glia (LG) composed of 9 identifiable cells derived from a unique glioblast. In late embryos, LG can be divided into subtypes based on gene expression profiles, but little is known about their mature properties (morphology, physiology) and functions in larvae. We used the Blown-out recombination system to selectively label and identify each LG cell in L1 larvae, and precisely examined their morphology and organization. Interestingly, we found that LG comprise 3 distinct and stereotypic glial subtypes: astrocyte-like, ensheathing and nerve-associated glia. Time-lapse analysis of LG development from their origin to

their mature state confirmed that these 3 subtypes derive from the same progenitor. What molecular mechanisms control the generation of distinct identities from a single progenitor? We found that Notch signaling, acting early in the lineage, controls alternative astrocyte-like and ensheathing glia fates. We also found that one effector of Notch in this process is the transcription factor Prospero. For each astrocyte-like glial cell, we then used landmarks positive for fasciclin 2 to map the positions of the cell bodies and the domains of the synaptic neuropil covered by their dense membranous processes. We found stereotypy with which they selectively associate with particular regions of the neuropil. Together, these results document a previously undiscovered pattern of differentiation, migration, and morphogenesis among CNS glia, setting the stage for future work to discover additional cellular and molecular mechanisms leading to diversification of form and function among CNS glia.

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Activity dependent active zone remodeling in the *Drosophila* visual system. Atsushi Sugie[1,2], Takashi Suzuki[2], Gaia Tavosanis[1]. 1) DZNE, Bonn, Germany; 2) Titech, Yokohama, Japan.

Neural activity contributes to the regulation of the precise localization and the number of synapses formed in a sensory system, allowing for adjustment to a changing environment. It is a fundamental question how synaptic molecular components are regulated to achieve synaptic plasticity. In this study, we visualized presynaptic active zones in photoreceptors of adult flies using *UAS-Brp$^{short\ mCherry}$* expressed in photoreceptor 8 (R8) with Rh6-Gal4. *Brp$^{short\ mCherry}$* accumulates in discrete puncta, presumably representing individual active zones as their number and distribution corresponds to previous EM data. Surprisingly, the discrete puncta of *UAS-Brp$^{short\ mCherry}$* observed in adult flies maintained in a 12h light/12h dark (LD) cycle were largely lost and the distribution of this marker became diffuse if the flies were kept in continuous light (LL) over a period of a day. This phenotype was reversible. We developed software-based detection of puncta distribution for quantitative analysis of *UAS-Brp$^{short\ mCherry}$*localization. The redistribution of *UAS-Brp$^{short\ mCherry}$* depended on activity as this phenotype was suppressed in *norpA* mutant, which abolishes the light-evoked photoreceptor potential. Conversely, the expression of *UAS-TrpA1* that leads to sustained activation of the photoreceptors caused diffused distribution of *UAS-Brp$^{short\ mCherry}$* even in flies maintained in continuous darkness (DD). The activity requirement, though, is not cell-autonomous. Indeed, in *hisCl1^{134},ort^{1}* mutant flies or blocking photoreceptor transmission with *UAS-Shibire^{ts}* the diffuse phenotype was suppressed even in LL. Thus, postsynaptic neurons regulate the activity-dependent synaptic modification in photoreceptors. These data demonstrate that activity can modulate the molecular composition of active zones and suggest a model of feed-back regulation within the circuit.

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MicroRNA-190 downregulates Bag of marbles to allow the switch from proliferation to differentiation in the *Drosophila* male germline stem cell lineage. Gonzalo H Olivares, Margaret T Fuller. Developmental Biology, Stanford University School of Medicine, Palo Alto, CA.

In many adult stem cell lineages, stem cell daughters commonly undergo a limited number of transit amplifying (TA) mitotic divisions before initiating terminal differentiation, allowing production of many differentiated progeny per stem cell division. The number of TA divisions must be tightly regulated: too few may lead to defective tissue regeneration, too many to abnormal growth and cancer. In the*Drosophila* male germline stem cell lineage, Bag of marbles (Bam) is required for cessation of TA cell divisions and onset of spermatocyte differentiation. Loss of function of *bam* causes male TA germ cells to continue proliferation without initiating differentiation. We have demonstrated that Mei-P26 facilitates accumulation of Bam protein in TA cells to allow the switch from proliferation to differentiation. Mei-P26 physically interacts with Ago1 and represses expression of micro-RNAs (miRNAs), suggesting that Mei-P26 regulates Bam protein expression post-transcriptionally via its 3'UTR. To test this hypothesis, I screened for miRNAs that when over-expressed generate a phenotype that resembles a *bam* mutant. I found that ectopic expression of miR-190 in germ cells caused overproliferation of spermatogonia resembling loss of function of *bam*, with germ cells enclosed in cysts and having branched fusomes consistent with TA identity. As in Mei-P26 mutants, miR-190 over-expression (OE) shows low levels of Bam protein, suggesting that forced miR-190 OE prevented Bam protein levels from reaching the threshold required to stop proliferation. A *bam* 3'UTR reporter with mutated miR-190 binding site show no repression at late stages. I am currently testing the model that the tumor suppressor Mei-P26 regulates expression or action of miR-190 to allow accumulation of Bam in early germ cells.

158B

Microtubule (MT)-dependent regulation of muscle length. Victoria K. Schulman[1,2], Eric S. Folker[2], Mary K. Baylies[1,2]. 1) Weill Cornell Graduate School of Medical Sciences, New York, NY; 2) Sloan-Kettering Institute, New York, NY.

Many muscle diseases are characterized by smaller, weaker myofibers, highlighting the fact that muscle size is critical for muscle function. To study the regulation of muscle size, we used the model organism,*Drosophila melanogaster*, a system that permits *in vivo* cell biological studies with an additional means to assess muscle function at later stages of development. Many aspects of morphogenesis determine overall muscle size, including nuclear number, cell volume, and myofiber length. We have focused on how muscle length is regulated because we have shown that larvae with shorter muscles exhibit significantly impaired muscle function. Through a combination of mutational analysis and RNAi-based screens, we have identified a number of factors that affect muscle length. Mutations in, or depletion of, Lis1, NudC, Rapsynoid (Raps/Pins), the minus-end directed MT motor Dynein heavy chain (Dhc), and its regulatory light chain (Dlc), all result in muscles that are shorter than controls. Conversely, mutations in, or depletion of, the plus-end directed motor Kinesin heavy chain (Khc), its regulatory light

chain (Klc), and Ensconsin (Ens), exhibit significantly longer muscles compared to controls. Finally, we have identified Sunday Driver (Syd) as a factor that coordinates Kinesin and Dynein activities as they pertain to muscle length determination. Although homozygous *syd* mutants produce muscles of the proper length, double heterozygotes of *syd* and *khc* have longer muscles than controls, and double heterozygotes of *syd* and *dhc* have shorter muscles than controls. This suggests that the adapter protein, Syd, is simultaneously regulating Dhc and Khc to influence muscle length. Collectively, these data suggest that muscle length is regulated by a MT-dependent process mediated by motor protein complexes that are coordinated by Syd to facilitate proper extension of the muscle pole.

159C

Frazzled/DCC facilitates cardiac cell outgrowth and attachment during dorsal vessel formation. Frank D. Macabenta[1,2], Amber G. Jensen[1,2], Yi-Shan Cheng[1], Joseph J. Kramer[1], Sunita G. Kramer[1,2]. 1) Pathology Department, UMDNJ/RWJMS, Piscataway, NJ; 2) Cell and Developmental Biology, Rutgers University, Piscataway, NJ.

Embryonic dorsal vessel formation is a highly stereotyped process that involves the migration and morphogenesis of 52 pairs of cardioblasts (CBs) in order to form a linear tube. This process requires spatiotemporally-regulated localization of signaling and adhesive proteins in order to coordinate the formation of a central lumen while maintaining simultaneous adhesion between CBs. Previous studies have shown that the Slit/Roundabout and Netrin/Unc5 signaling pathways facilitate site-specific de-adhesion between contralateral CBs in order to form a luminal space. However, the concomitant mechanism by which dorsoventrally-polarized attraction initiates cell shape changes and discrete localization of adhesive proteins remains poorly understood. Our findings support the idea that the axon guidance receptor Frazzled/DCC (Fra) plays an attractive role in lumen formation. *fra* mRNA is expressed in the dorsal vessel prior to and during lumen formation. Loss-of-*fra*-function results in cell shape change delays and alignment defects between contralateral CB rows; additionally, diminished or absent junctional domains are observed between CB pairs. Deletion mutants of both Netrin genes (*NetA* and *NetB*) exhibit phenotypes similar to that observed in *fra* mutants. Furthermore, overexpression of *fra* at high levels in the dorsal vessel results in delayed CB outgrowth. To localize Fra, we expressed a Myc-tagged *fra*transgene in CBs. In a wild type background, Fra-Myc accumulates at dorsal and ventral leading edges of paired CBs, corresponding to future sites of attachment. However, we observe mislocalization of Fra-Myc in a Δ*NetAB* background, suggesting a role for Netrin in mediating discrete Fra localization. Taken together, our data supports the idea that while Slit/Roundabout and Netrin/Unc5 signaling contribute to proper lumen formation by facilitating de-adhesion, Netrin/Frazzled signaling conversely allows for attraction and subsequent membrane outgrowth between CBs.

160A

The core complex of cuticle dynamics in *Drosophila* exoskeleton. Matthias Behr, Kapil R Patil, Yanina Y Pesch, Dominik Hölper. Life & Medical Sciences (LIMES) Institute, Carl-Troll-Str. 31, 53115 Bonn, Germany.

The arthropods cuticle plays important roles in growth control, wound healing and protects against dehydration, pathogens and toxins. It lines the apical surface of epidermis and many internal organs. Organization of the cuticle extracellular matrix (ECM) involves the polysaccharide chitin and associated proteins and enzymes. A newly synthesized cuticle requires further maturation and protection but underlying molecular mechanisms are poorly understood. We identified a core complex that is required for cuticle dynamics. Obstructor (Obst)-A (member of the *obstructor* multigene family) binds chitin and interacts with the cuticle modifier Knickkopf and the chitin deacetylase Serpentine. The core complex enables chitin ECM maturation and protects it from chitinase-dependent degradation. Loss of the core complex organization in *obst-A* mutants results in early larval lethality and cuticle integrity and molting defects. We then systematically knocked down enzymes that degrade chitin ECM in the epidermis and found severe cuticle defects similar to obst-A mutants. The presented mechanisms are required for cuticle dynamics throughout *Drosophila* development.

161B

MIPP1 functions at the basolateral domain to facilitate the generation of filopodia and the extension of lamellopodia of tracheal leading cells. Yim Ling Cheng, Deborah Andrew. Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD.

Multiple Inositol Polyphosphate Phosphatase 1, MIPP1, is a histidine phosphatase that dephosphorylates higher order inositol polyphosphates (InsP8 to InsP4). MIPP is highly conserved, but its biological function is unknown. *mipp1* was identified in our lab as a target of Trachealess, the major transcription factor regulating tracheal development. *mipp1* is highly expressed in all tracheal cells at early stages and the expression is maintained in only the intercalated branches (e.g. the dorsal branches), with enhanced expression in the branch tips at later stages. We generated a knockout allele of mipp1 and observed that 40% of dorsal branches have delayed sister cell intercalation (SCI), which is the process whereby tracheal tubes elongate by rearranging the cells from a side-by-side to an end-to-end configuration. C-terminal GFP-tagged fly MIPP1 localizes to the ER, consistent with localization of the mammalian protein; however, immunostaining with our recently generated MIPP1 antibodies shows that MIPP1 localizes to the plasma membrane. Topology/structure predictions and biochemical assays, including glycosidase treatment and trypsin protease digestion, reveal that most of the MIPP1 protein, including the active phosphatase domain, faces outside of the cell, with either a C-terminal transmembrane domain or a GPI-link. At early stages, MIPP1 localizes to both apical and basolateral surfaces. During branching morphogenesis, apical levels of MIPP1 decline and the basolateral levels associated with filopodia/lamellopodia increase. Overexpression of MIPP1 increases the number of filopodia and extension of lamellapodia in the leading cells. The pulling force that stimulates SCI is induced by FGF signaling in

the tracheal branch leading cells. We find that disruption of FGF signaling results in MIPP1 localizing to the apical surface even in the late stages. We propose that FGF signaling causes MIPP1 to preferentially localize to the basolateral domain where it facilitates the filopodial formation and lamellopodial extension, thus contributing to the pulling forces that underlie SCI.

162C

The PDZ domain protein Arc is required for proper invagination of the embryonic salivary glands. Rika Maruyama[1,2], Sarah Hughes[1], Deborah Andrew[2]. 1) Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada; 2) Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD.

Tubulogenesis is an important process during organogenesis since many organs, including the lungs, kidneys and vasculature, as well as many secretory organs, are composed of sophisticated tubular networks. Invagination is a key early step in the formation of tubes from polarized epithelial sheets; however, the molecular mechanisms coordinating invagination remain unclear. Fork head (Fkh), the founding member of the FoxA family of winged-helix transcription factors, is required for invagination of the salivary gland primordia to form salivary gland tubes. In a screen for Fkh targets that mediate salivary gland morphogenesis (1), we identified the *arc* gene. Arc is an adjerens junction-associated PDZ domain protein previously shown to be required for wing and eye development (2). *arc* mRNA is detected in multiple embryonic tubular organs, including the trachea, Malpighian tubules and the salivary glands, where *arc* expression depends on *fkh*. To examine the embryonic function of *arc* in more detail, we created*arc* null (KO) mutants by homologous recombination. *arc* maternal-zygotic KO mutants exhibit a range of morphological phenotypes linked to defects in invagination, including broader salivary gland invagination pits. Overexpression of *arc* in salivary glands using *fkh-Gal4* blocked salivary gland invagination and caused mislocalization of the apical membrane protein Crumb (Crb). We are continuing to explore the link between Arc and Crb, and the mechanisms through which Arc contributes to salivary gland invagination.

1. Maruyama et. al., PLoS One. 2011; 6(6):e20901
2. Liu and Lengyel, Dev. Bio. 2000; May 15; 221(2): 419-34.

163A

The large Maf factor Traffic jam functions to repress hub cell fate in the developing germline stem cell niche. Lindsey Wingert, Steve DiNardo. Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

The Drosophila testis is an excellent system for studying stem cell-niche interactions. The hub (niche) cells residing at the apical tip of the testis provide germline stem cells (GSCs) and cyst stem cells (CySCs) with signals promoting self-renewal and attachment. The somatic component of the testis is derived from a pool of somatic gonadal precursors (SGPs). SGPs facilitate the coalescence and compaction of the gonad through Ecadherin-mediated adhesion (Jenkins et al., 2003). Hub cell specification among SGPs is dependent on interaction with two additional cell populations. Notch activation by endodermal gut cells acts to positively specify hub cell fate while EGFR activation by germ cells acts to repress hub cell fate (Kitadate et al., 2007; Kitadate and Kobayash, 2010; Okegbe and DiNardo, 2011). Positively-specified hub cells undergo a mesenchymal to epithelial transition (MET) as they sort away from germ cells toward the periphery at the anterior pole of the gonad. During this process, they maximize attachments to each other via adherens and septate junctions and anchor via integrins (Le Bras and Van Doren, 2006; Tanentzapf et al., 2007). The remaining non-hub SGPs maintain their mesenchymal identity and encystment of germ cells. We are investigating Traffic jam (Tj), a transcription factor expressed in SGPs then downregulated in hub cells, for its role in MET. Tj mutant gonads display ectopic, epithelialized cells by septate and adherens junctional markers. These ectopic cells also express unpaired which encodes one of the self-renewal factors secreted by hub cells. We suspect that Tj functions in non-hub SGPs to maintain their preferential adhesion for germ cells in order to prevent them from becoming hub (Li et al., 2007).

164B

Machine learning-based functional characterization of heart enhancers uncovers novel cardiogenic roles for the transcription factors Myb and Su(H). Shaad M. Ahmad[1,4], Brian W. Busser[1,4], Di Huang[2,4], Elizabeth J. Cozart[1], Anton Aboukhalil[3], Sebastien Michaud[3], Neal Jeffries[1], Martha L. Bulyk[3], Ivan Ovcharenko[2], Alan M. Michelson[1]. 1) NHLBI, NIH, Bethesda, MD; 2) NLM, NIH, Bethesda, MD; 3) Harvard Medical School, Boston, MA; 4) Equally contributing first authors.

The development of a complex organ such as the *Drosophila* heart requires a network of signaling molecules and transcription factors (TFs), the combined activities of which are integrated by transcriptional enhancers. The *Drosophila* heart is composed of two distinct cell types, the contractile cardial cells (CCs) and the non-muscle pericardial cells (PCs). Here we combined machine learning of heart enhancer sequence features with chromatin immunoprecipitation sequencing (ChIP-seq) data for key cardiac regulators to computationally classify cell type-specific cardiac enhancers, thereby identifying related enhancers, their shared and unique sequence motifs, and novel *trans* acting factors which direct cell type-specific genetic programs. We initially found that addition of ChIP-seq data improves the performance of the enhancer classification. In addition, predicted cell type-specific enhancers are over-represented near the appropriate cell type-specific cardiac gene sets and are active in the heart when tested in transgenic reporter assays. Furthermore, many of the motifs learned by the classifier are recognized by TFs known to be involved in cardiogenesis, but some of the identified transcription factor binding sites (TFBSs) were novel. Within the latter category is a TFBS recognized by Myb, which we demonstrate experimentally acts in concert with the forkhead domain TF Jumeau to control cardiac progenitor cell divisions. Interestingly, machine learning revealed Suppressor of Hairless (Su(H)) TFBSs as a sequence feature that may discriminate between PCs and CCs. In

agreement with this hypothesis, Su(H) was found to repress a known PC gene in CCs. We thus show that machine learning can be utilized to recognize novel TFBSs and facilitate the identification of cognate TFs and their functions during organogenesis.

165C

Investigating the potential non-cell autonomous Robo2 function during lumen formation of the *Drosophila melanogaster* dorsal vessel. Judith J Canabal Alvear[1,2], Sunita G Kramer[1,2]. 1) Pathology Department , UMDNJ/RWJMS, Piscataway , NJ; 2) Cell and Developmental Biology, Rutgers University, Piscataway, NJ.

Biological tubes are required for the development of complex organisms given that they distribute important molecules to different parts of the organism. In this study, we investigate a previously unknown function for the transmembrane receptor Roundabout2 (Robo2) during lumen formation of the *Drosophila* dorsal vessel, a simple linear tube required to pump hemolymph throughout the embryo. Two major steps are required for dorsal vessel formation. First, specified cardioblasts (CBs) migrate in rows toward the dorsal midline of the embryo and second, the CBs undergo a series of cell shape changes to form a linear tube with a central lumen. The two rows of CBs are flanked on either side by two rows of non-muscle pericardial cells (PCs). While the PCs have been shown to be important for dorsal CB migration, a potential role for the PCs in mediating lumen formation in the adjacent CBs is unclear. CBs express a single Roundabout receptor (Robo1), while PCs express both Robo1 and Robo2. Our lab has shown that loss of Robo1 results in defects in lumen formation. However the role for Robo2 in this process has not been explored. The present work investigates the role for Robo2 in lumen formation through loss-of-function (LOF) and gain-of-function (GOF) studies. In *robo2* LOF embryos, we observe defects in CB lumen formation. Because Robo2 is expressed by the PCs, these findings suggest a non-cell autonomous role for Robo2 in this process. Furthermore, ectopic expression of *robo2* at low levels in the CBs results in a *robo* LOF phenotype, while expression of Robo2 at high levels in the CBs produces a strong *robo* GOF phenotype. These results suggest that Robo2 has the ability to both antagonize as well as mimic Robo function in the dorsal vessel. We are currently investigating the significance of this biphasic nature of Robo2, as well as determining its intrinsic role during lumen formation using a combination of genetic, structure function and live imaging analysis.

166A

Identification of transcription factors and chromatin regulators with novel roles in muscle morphogenesis. Krista C. Dobi[1], Marc S. Halfon[2], Mary K. Baylies[1]. 1) Dept Dev Biol, Sloan-Kettering Inst, New York, NY; 2) Biochem Dept, SUNY Buffalo, Buffalo, NY.

Skeletal muscles come in a variety of shapes and sizes, from the rounded muscles that control eye blinking to the elongated muscles of your legs that allow you to run. These muscles have different functions, and they also have different susceptibilities to diseases such as muscular dystrophy. Potential myoblast transfer and stem cell therapies to repair muscle wasting due to aging or disease will require the ability to generate muscles with specific morphologies. The 30 body wall muscles in each hemisegment of the Drosophila embryo are distinguishable by properties such as size, shape, attachment and gene expression. To identify new regulators of Drosophila embryonic muscle morphogenesis, we isolated muscle subsets using FACS, purified RNA from these cells and analyzed their transcriptional profiles by microarray. We identified ~600 differentially regulated genes, representing diverse functions like gene expression and cytoskeletal organization. GO analysis revealed that a significant number of up-regulated genes encode transcription factors and chromatin regulators. We confirmed mesodermal expression of these genes by in situ hybridization and tested whether loss of these factors disrupted the muscle pattern. We characterized 12 genes with novel functions in the Drosophila embryonic somatic muscle: zinc finger proteins CG8145, Lola, Alhambra and Charlatan; bHLH protein Cropped; T-box family member Midline; elongation factor Elongin-B; Mediator complex member Med13/Skuld; and chromatin regulators Little imaginal discs (Lid), Lysine-specific demethylase 2 (Kdm2), Grunge (Gug) and Sin3A. Our studies revealed new roles for highly conserved general transcription factors (Med13, Elongin-B) and chromatin regulators (Sin3A, Gug, Lid and Kdm2) in the regulation of muscle morphogenesis. Current experiments are providing us with a clearer picture of how regulation of transcription and chromatin structure is crucial for muscles to achieve distinct sizes and shapes.

167B

Cellular mechanisms of heart morphogenesis and lumen formation in *Drosophila*. Georg Vogler[1], Jiandong Liu[2], Timothy W Iafe[3], Rolf Bodmer[1]. 1) Development and Aging, Sanford Burnham Medical Research Institute, La Jolla, CA; 2) University of North Carolina, School of Medicine, Chapel Hill, NC; 3) New York University, School of Medicine, New York, NY.

The Drosophila embryonic heart is a key model system for understanding heart specification. Our previous studies indicate that heart morphogenesis requires Slit/Robo signaling, a function conserved in vertebrates. The mechanisms by which these and other signals control heart formation are still unknown. Due to its role in membrane dynamics, we investigated the role of the small GTPase Cdc42 during Drosophila heart development and found it to be required for cardiac cell alignment and heart tube formation. Mutant or constitutively active Cdc42 in the developing heart causes improper cardioblast alignment and formation of multiple lumina, suggesting that Cdc42 is required during discrete steps of cardiogenesis. Cell polarity and filopodia dynamics are unaffected by loss of Cdc42, therefore Cdc42 might have a different role during heart morphogenesis. To understand the regulation of Cdc42 and to identify new genetic interactors, we performed a genetic screen for modifiers of Cdc42. We identified the tyrosine kinase Abelson (Abl), and the non-muscle myosin-II zipper to strongly interact with Cdc42. Abl itself shows a requirement for coordinated heart tube assembly, and Zipper exhibits a dynamic localization pattern during

cardiogenesis, which depends on Cdc42 function, but is independent of Slit/Robo. Activation of the formin-like protein Diaphanous (Dia) produced defects similar to activated Cdc42, indicating that control of cell shape changes is a key regulatory step during heart morphogenesis. Our data suggest a novel mechanism of cardiac morphogenesis involving Abl, Cdc42, Dia and Zip acting in a common pathway during cardiac cell shape changes and orchestrated heart lumen formation.

168C

Elucidating the role of the nuclear hormone receptor E78 in *Drosophila* oogenesis. Elizabeth T. Ables[1,2], Kelly E. Bois[2], Daniela Drummond-Barbosa[2]. 1) Dept. of Biology, East Carolina University, Greenville, NC; 2) Dept. of Biochemistry and Molecular Biology, Johns Hopkins University School of Public Health, Baltimore, MD.

Nuclear hormone receptors (NHRs) have emerged as important regulators of mammalian and *Drosophila* adult physiology, affecting such seemingly diverse processes as adipogenesis, carbohydrate metabolism, circadian rhythm, stem cell function, and gamete production. Indeed, the steroid hormone ecdysone, and its cognate NHRs EcR and Usp, have multiple roles in *Drosophila* development and regulate key processes during oogenesis, including germline stem cell (GSC) function and follicle development. Other NHRs, including Hr39 and E75, also have known roles in the *Drosophila* female reproductive system; however, the function of most NHRs in oogenesis remains largely undescribed. Because of its similarity to mammalian PPARs and Rev-Erb, which are central to the control of metabolism and circadian rhythm, the NHR E78 is a particularly attractive candidate that may link oogenesis with the physiological status of the organism. In support of a role during oogenesis, we find that E78 appears to be weakly expressed in germ cells, and enriched in somatic border cells and late-stage follicle cells. We generated a predicted molecular null allele, $E78^{\Delta 31}$, and find that despite previous reports that hypomorphic *E78* mutants have no obvious fertility defects, homozygous viable $E78^{\Delta 31}$ females are sub-fertile. Decreased egg production is likely due to a combination of factors, including decreased GSC number and a partial block to vitellogenesis. We are currently investigating the mechanisms by which E78 regulates oogenesis. Taken together with the known roles of EcR, Usp, E75, and Hr39, our results suggest that NHRs may be critical for the broad transcriptional control of a wide variety of cellular processes during oogenesis.

169A

RTC1, a conserved SEA complex component, is required for early oogenesis in Drosophila. Weili Cai, Mary Lilly. NICHD, National Institute of Health, Besthesda, MD.

Meiosis is a variant cell cycle program for sexual reproduction in eukaryotes. We are interested in how meiosis is regulated in the context of a multicellular organism. Drosophila oogenesis is a powerful model system to study the regulation of meiotic progression and gametogenesis, and has proven especially useful in studying gene functions that are conserved in metazoans. Previously, we identified two genes, missing oocyte(mio) and seh1, required for the maintenance of the meiotic cycle during Drosophila oogenesis. In the absence of mio and seh1, the oocytes fate cannot be maintained. mio and seh1 oocytes enter the endocycle and develop as pseudo-nurse cells. Egg chambers are arrested and rarely develop beyond stage 5 of oogenesis. In yeast, the MIO and SEH1 proteins associate with a newly identified complex (SEA-complex). This complex regulates nutritional sensing and metabolism upstream of the Target of the Rapamycin (TOR) signaling pathway. RTC1 is another conserved component of the SEA-complex in yeast and has been implicated in the regulation of the early meiotic cycle and sporulation. To further study the function of this complex in Drosophila, we identified CG7609 as the homolog of RTC1 in Drosophila. We found that CG7609 physically and genetically associates with MIO and SEH1. CG7609 has an exclusively high transcription level in ovaries, indicating a potential important function in ovaries. We also identified a P-element insertion line as a potential null allele. RT-PCR experiments showed that homozygous flies of this P-element insertion line do not produce CG7609 transcript. CG7609 null mutants are not lethal but females are sterile, suggesting an important oogeneic function. Intriguingly, ovarioles from CG7609 mutants had multiple defects during oogenesis. In summary, our data strongly suggest that Drosophila RTC1, CG7609, is a component of the SEA-complex and plays a critical role in the regulation of meiotic progression and/or gametogenesis.

170B

Aging Related Oogenesis Defects of Upd3 Mutants. Michelle Giedt[1], Liqun Wang[2], Travis Sexton[3], Claire Venard[1], Douglas Harrison[1]. 1) Biology Department, University of Kentucky, Lexington, KY; 2) Department of Pathology, Brigham & Woman's Hospital, Harvard Medical School, Harvard New Research Building, Room 652, 77 Avenue Louis Pasteur, Boston, MA; 3) University of Kentucky College of Medicine, Cardiovascular Research Center, 741 S. Limestone St., Lexington, KY.

In Drosophila, Jak/Stat signaling has many developmental roles including several in oogenesis. In one, a gradient of activity patterns the A/P axis of the follicular epithelium, with the highest levels specifying terminal cell fates. The Upd ligand is secreted from the anterior and posterior polar cells, and reduction of Upd reduces terminal fates. Another ligand, Upd3, is also expressed in the polar cells, raising the question of whether Upd and Upd3 act redundantly or have distinct functions in oogenesis. To address this, mutants of upd3 were generated. Young upd3 mutant females are fertile but exhibit increasing frequency of unfertilized eggs and defective egg chambers as they age. Defects include egg chamber fusions and degenerating chambers that are also observed in Jak/Stat pathway mutants. To determine if the role of Upd3 is additive in follicular fate specification, terminal cells were examined. Mutants had a slight reduction in border cell numbers, but the biological significance of this is unknown. Because border cells are important in micropyle formation, examination of unhatched eggs was performed to determine if the decrease in fertility was due to morphological defects. Some eggs from mutants had

micropyles lacking a clear channel, but these occurred at a frequency too low to account for the decrease in fertility. The possibility of more subtle micropyle defects responsible for the low rate of fertilization is currently being investigated. Defects in posterior terminal cells were also examined. These cells give rise to the aeropyle, a respiratory structure located at the posterior. Loss of upd3 resulted in loss, reduction, or changes in shape of the cells in the aeropyle. This evidence suggests Upd and Upd3 act in an additive manner to specify follicle cell fates, with Upd3 increasing in importance as the female ages.

171C

Selective replication of functional mtDNA during oogenesis restricts the transmission of a deleterious mutation. Jahda H. Hill, Hong Xu. National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD.

Mitochondrial DNA (mtDNA) is prone to mutation accumulation, and how organisms limit transmission of deleterious mtDNA mutations across generations remains unknown. Utilizing flies heteroplasmic for a temperature-sensitive lethal mutation in the mitochondrial gene *mt:CoI*, we provide evidence of selection in the female germ line, where the frequency of the *mt:CoI*^{T300I} mutation decreased at the restrictive temperature. Further, using 5-ethynyl-2'-deoxyuridine (EdU) to label replicating mtDNA, we demonstrate that mtDNA replication occurs in the germarium stage of oogenesis, and concentrates around the fusome, a cytoplasmic structure mediating transport of mitochondria from somatic nurse cells to the oocyte. At the restrictive temperature, *mt:CoI*^{T300I} mitochondria are less effectively recruited to the fusome and display reduced mtDNA replication. These findings establish a previously uncharacterized developmental mechanism for selective amplification of healthy mtDNA, which may be evolutionarily conserved to prevent transmission of deleterious mutations.

172A

FGF mutants exhibit pleiotropic ovariole phenotypes relating to loss of epithelial sheath. Jihyun Irizarry[1,2], Angelike Stathopoulos[1]. 1) California Institute of Technology, Division of Biology, Pasadena, CA; 2) CIRM Bridges to Stem Cell Research Program, California State Los Angeles, Los Angeles, CA.

Fibroblast growth factor (FGF) signaling is crucial for many developmental processes including cell migration, survival, and differentiation in many species. Despite the broad importance of FGF signaling, no study has reported a function within the *Drosophila* ovary. The main objective of this study has been to elucidate the roles of FGF signaling during *Drosophila* ovarian morphogenesis. In particular, our study has focused on characterization of the *thisbe* gene, which encodes a *Drosophila* FGF ligand. We found that *thisbe* mutant females are sterile, and examination of the ovaries revealed significant phenotypes. The mutant ovaries lack two types of muscle tissue: the peritoneal sheath, an outer tissue which ensheaths ~20 ovarioles, and the epithelial sheath, a tissue that surrounds each individual ovariole thereby keeping them separate. Despite loss of these tissues, surprisingly, the sub-ovariolar compartments (i.e. germarium, egg chamber, stalk cell) were generated. However, these compartments exhibited intriguing phenotypes. In the ovarioles of *thisbe* mutant females, ectopic localization of polar cells and over-proliferation of stalk cells, follicle cells associated with developing egg chambers, were observed. Furthermore, the mutant exhibited developmental defects in oocyte development. We are currently investigating whether FGF's role in oocyte development is direct or indirect. For example, FGF signaling may be required to directly support proper GSC division/differentiation or, alternatively, the absence of the epithelial sheath may indirectly affect GSCs. As an initial step toward answering this question, we are examining ovary phenotypes at the pupal stage, when the peritoneal and epithelial sheaths form. Based on our preliminary results, we hypothesize that FGF signaling controls apical cell migration at the pupal stage.

173B

The Mitochondrial Protein Cytochrome c heme lyase is Necessary for Cell Polarity. Sarah E. Kleinsorge, Caryn Navarro. Graduate Program in Genetics and Genomics, BUSM, Boston, MA.

In Drosophila, the oocyte is specified and maintained through the asymmetric localization of cell cycle and cell polarity RNAs, proteins, and organelles such as mitochondria to and within the oocyte. We performed an EMS mutagenesis screen in Drosophila to uncover new genes important for cell polarity establishment and oocyte development. We discovered that one of the mutant lines isolated in the screen had a mutation in the highly conserved catalytic domain of the nuclear encoded mitochondrial protein Cytochrome c heme lyase (Cchl). In organisms such as humans, mice and flies all embryonic mitochondria are maternally inherited from the oocyte. However, the role of mitochondrial function in oocyte development is currently unknown. We therefore went on to characterize Cchl function during drosophila oogenesis. In Cchl mutant oocytes, cell polarity is initially established but not maintained. Cchl is known to function in the electron transport chain (ETC) to maintain proper ATP levels. In support of Cchl functioning in the ETC to maintain oocyte polarity we find that mutations in other genes necessary for ETC function show a similar phenotype as Cchl mutant oocytes. Therefore, we hypothesize that in Cchl mutant oocytes the energy level in the cell may not be high enough to maintain the processes leading to proper oocyte specification, such as dynein-mediated microtubule motor transport. However, the ETC also produces second messengers such as reactive oxygen species, calcium and ATP and these signals may trigger downstream pathways that are necessary to maintain oocyte polarity. Our current work focuses on determining which of these hypotheses are correct. Since the Cchl protein is both structurally and functionally conserved between flies and mammals, these studies could further our understanding of premature ovarian failure and reproductive ageing.

174C

Translational regulation at the oocyte to embryo transition in Drosophila. Iva Kronja[1], Bingbing Yuan[1], Kristina Dzeyk[2], Joanna Kirkpatrick[2], Jeroen Krijgsveld[2], Terry Orr-Weaver[1]. 1) Whitehead Institute, MIT, Cambridge, MA; 2) EMBL, Heidelberg, Germany.

Drosophila oogenesis is an excellent system to study the contribution of translational regulation to cell cycle progression. It is thought that two bursts of renewed protein synthesis are correlated with progression through meiosis, one at oocyte maturation and the other at egg activation. Identifying proteins whose levels increase at maturation will reveal candidates required for meiotic progression, and proteins upregulated at egg activation may lead to the players needed for completion of meiosis and the onset of embryogenesis. To identify these candidates, we applied two complementary genome-wide approaches: polysome profiling followed by mRNA sequencing and in vitro dimethyl labeling combined with quantitative mass spectrometry. Our proteomic approach showed that levels of only a limited set of proteins increase at oocyte maturation or egg activation. Surprisingly, we observed that a more drastic aspect of proteome remodeling is a decrease in protein levels. To understand if the differences in protein levels stem from changes in translation or protein stability, we performed genome-wide polysome profiling. This approach provided information on the translational status at egg activation for all mRNAs. Although the polysome profile of mature oocytes suggests slower translational initiation than in activated eggs, these two samples overall have comparable translational activity. Despite this similarity of the polysome profiles, at egg activation several hundred mRNAs are loaded onto polysomes while over a thousand mRNAs are released from the polysomes. Importantly, we observed a significant overlap among candidates identified by the proteome- and the translation-based methods, confirming the importance of translational regulation in proteome remodeling at egg activation. The agreement of the two methods also provides validation that the identified candidates may indeed be potentially important regulators of meiosis and early embryogenesis.

175A

Heterologous Segregations are established prior to chromosome congression in female meiosis I in *Drosophila melanogaster*.. Fiona M Lane, Ashley A Snouffer, William D Gilliland. Biological Sciences Department, DePaul University, Chicago, IL.

Heterologous Segregation (HS) occurs in female meiosis when the genome is rearranged to carry multiple chromosomes that lack homologs. In those cases, the heterologous chromosomes will segregate away from each other at high frequency. While this phenomenon has been known since 1936, the mechanism to establish the co-segregation has remained unclear, as these chromosomes are not homologs, did not recombine, and do not pair with each other like normal homologous chromosomes. We propose that the recently-discovered process of chromosome congression in female meiosis establishes HS. We have examined different chromosome configurations that undergo HS, and show using chromosome-specific fluorescent *in situ* hybridization that the rates of coorientation at metaphase arrest match the rates of chromosome segregation observed in the progeny. Data will be presented that indicates when the coorientation must occur and indicates possible mechanisms for establishing these co-segregational configurations.

176B

***asteroid* is required for oocyte determination in *Drosophila*.** Julie A. Merkle, Trudi Schüpbach. Howard Hughes Medical Institute, Department of Molecular Biology, Princeton University, Princeton, NJ.

A fundamental question in biology is how functional gametes are formed from the gerrmline stem cells. In many species, oogenesis establishes the molecular and developmental events necessary to promote fertility and embryonic development. How the oocyte establishes an identity and how that identity is maintained are processes that are still not well understood. In order to reveal new players involved in the control of these processes, we performed a mosaic screen on Chromosome 2L in *Drosophila* and isolated a set of 20 lethal mutations that display defects in oocyte specification and/or differentiation. In the majority of these mutants, clones produced 16 nurse cells and no oocyte. We are currently in the process of mapping these mutations and further characterizing the phenotypes. We mapped a mutation in one of these lines to *asteroid* (*ast*), a gene previously shown to interact with *Star* and *Egfr* in the *Drosophila* eye. The protein encoded by ast is conserved throughout metazoans and contains an XPG domain, suggesting a role for Ast in DNA repair. Future goals include further characterization of the *ast* mutant phenotype, as well as investigating its predicted nuclease activity, as to elucidate the mechanism by which Asteroid promotes oocyte specification and differentiation in *Drosophila*.

177C

The DExH box helicase region of Spindle-E is necessary for retrotransposon silencing and germline development. Caryn Navarro, Kristen Ott, Tram Nyguyen. Boston University School of Medicine, Boston, MA.

A large portion of the genome in many organisms contains transposable selfish genetic elements (TEs). TEs can self-replicate and insert into new locations, thereby causing genome instability. The tight regulation of TE transposition is critical in the germline because mutations that occur in these cells are inherited by offspring and may cause disease. A class of small RNAs, the Piwi associated RNAs (piRNAs) are responsible for suppressing the expression of TEs in the germline. Experiments in mice and Drosophila have shown that mutations that disrupt piRNA biogenesis cause elevated retrotransposon levels, defects in germline development, and sterility. To date, little is known about the molecular function of many piRNA pathway proteins. We have chosen to focus our studies on the functional characterization of the piRNA pathway protein, Spindle-E (SpnE) because it is necessary for the generation of most germline piRNAs and therefore likely plays a central role in piRNA

biogenesis. SpnE contains a DExH box and a Tudor domain as well as a Zn finger motif. Through the analysis of 12 new mutant spnE alleles, we have found that the highly conserved DExH box helicase region is required for piRNA pathway function, whereas the Zn finger motif is dispensable. Similar to most piRNA mutants, in *spnE* DExH box mutant ovaries, retrotransposon RNA levels are elevated, Dynein aggregates form, and Aubergine levels are reduced and the protein does not localize to the nuage properly. Additionally, eggs laid by these mutant mothers have severe dorsal/ventral patterning defects. Our mutant analysis also uncovered a new role for SpnE in ovary development. In 3-5 day old adult ovaries single spectrosome containing cells overproliferate and accumulate within the germarium. These cells fail to differentiate and continue to divide leading to a germ cell tumor. This phenotype is germline autonomous and strengthens over time. Our results indicate that SpnE functions at several times during Drosophila ovary development and the DExH box helicase region is important for its function in each of these processes.

178A

Three-dimensional epithelial morphogenesis in developing eggshells. Miriam Osterfield[1], XinXin Du[1], Trudi Schüpbach[1,2], Eric Wieschaus[1,2], Stanislav Shvartsman[1]. 1) Princeton University, Princeton, NJ; 2) Howard Hughes Medical Institute, Princeton, NJ.

Morphogenesis of the respiratory appendages on eggshells of Drosophila species provides a powerful experimental system for studying how cell sheets give rise to complex three-dimensional structures. In Drosophila melanogaster, each of the two tubular eggshell appendages is derived from a primordium comprising a patch of "roof" cells bordered by a row of "floor" cells, which form the upper and lower surfaces of the appendage, respectively. We previously demonstrated that the transformation of this two-dimensional primordium into a tubular appendage involves out-of-plane bending followed by a sequence of spatially ordered cell intercalations. These morphological transformations correlate with the developmental of complementary distributions of myosin and Bazooka. The observed distributions suggest a temporally varying pattern of line tensions on the apical side of the appendage primordium. Computational modeling shows that these patterns of tension could explain the main features of both tissue deformation and cell rearrangements observed during three-dimensional morphogenesis. We are further testing this model by examining the patterns of myosin distribution and the accompanying morphogenetic movements in mutants and in different Drosophila species with morphologically distinct eggshell appendages.

179B

Genetic and cytological dissection of mechanisms controlling mitochondrial DNA inheritance in *Drosophila melanogaster*. Jennifer Leigh Page, Patrick O'Farrell. BIOCHEMISTRY AND BIOPHYSICS, UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, SAN FRANCISCO, CA.

The evolutionary success of one mitochondrial genome over others relies on its partitioning into the cytoplasm of germ cells to contribute to the next generation, while the success of this next generation depends on acquisition of a competent compliment of mitochondrial genomes. Despite the importance of these factors in evolution and genetic health, we know little about the phenomena influencing the outcomes. Inheritance of mitochondrial DNA (mtDNA) follows patterns distinct from nuclear DNA. In higher eukaryotes, mtDNA inheritance is uniparental, provided only by the mother. We want to know how oocyte development in the Drosophila melanogaster female germline influences mitochondrial inheritance to the next generation. In flies, the future germline is specified quite early during oogenesis. Previous reports have suggested that through this process, mitochondria are specifically selected in order to ensure propagation of the most functional mitochondria to the next generation. We want to understand which maternal factors are necessary for recruiting mitochondria to the germ plasm, and whether there are mechanisms which survey mtDNA integrity and promote propagation of the best mitochondria. We propose to use genetic techniques to explore the maternal factors, such as oskar and vasa, that govern mitochondrial recruitment to the germ plasm, and cytological techniques to follow specific mitochondria during oogenesis in order to understand how the mitochondria are chosen. Preliminary evidence suggests that oskar is required for recruitment of mitochondria to the posterior, and that vasa also plays a key role. These and further experiments will help elucidate the mechanisms of mitochondrial inheritance through the female germline in Drosophila.

180C

Mio: Connecting Meiotic Progression to Metabolism in Early Oogenesis. John C Reich, Mary Lilly. CBMP, NICHD, Bethesda, MD.

In the Drosophila ovary, oocyte specification occurs in the context of a 16-germ cell cyst, where one of the 16-germ cells is designated as the future oocyte, and the other germ cells become polyploidy nurse cells. Multiple events contribute to this process, including the localization of oocyte specific proteins and RNAs, and the maintenance of the meiotic cycle specifically in the oocyte. Previously, our lab used a forward genetics approach to identify genes involved in oocyte formation/maintenance. Through this genetic screen, our lab identified *missing oocyte* (*mio*), a gene required to maintain oocyte specification during oogenesis potentially by maintaining the oocyte-specific meiotic arrest. Here we use a combination of genetics and fluorescent microscopy to show that *mio* mutant egg chambers (the precursor to an embryo) grow more slowly and have an increase in the catabolic process of autophagy compared to wildtype egg chambers, implicating *mio* in metabolism. In addition, *mio* mutant egg chambers have enlarged autolysosomes that can be seen by both fluorescent microscopy and EM, suggesting that *mio* may be involved in stopping autophagy. Consistent with this, under fed conditions *mio*mutant fat bodies do not show an increase in autophagy, but show a failure to stop autophagy during fat body

recovery from starvation. Given the localization Mio-eGFP at the lysosome, a hub for nutritional signaling, we believe that Mio may be involved in reactivating cell growth after cells have been subjected to stress. We are currently testing this model. Coordination of growth and metabolism are essential for proper organismal development, and our data suggest that *mio* is important in this process.

181A

A Role for *Prolyl-4-Hydroxylase Alpha* in Cell Migration During Oogenesis. Jinal S. Sheth, Michelle Starz-Gaiano. Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD.

During animal development, some cells are required to migrate at a precise time to fulfill their destiny. One such example is guided migration of border cells during oogenesis. Border cells are a group of 6-10 follicular epithelial cells that delaminate at the anterior of the egg chamber and migrate as a compact cluster posteriorly towards the oocyte. Developmental specification of border cells and their subsequent migration is induced by activation of the Janus Kinase and Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway. We have identified a gene, *prolyl-4-hydroxylase alpha (PH4alphaEFB)*, expressed in follicle cells at the time of border cell movements, that may be an important mediator of signaling. To characterize the function of the *PH4alphaEFB* gene better, we studied several putative loss of function mutations with transposable elements inserted at this locus. We determined the strongest mutant allele of *PH4alphaEFB* through genetic and molecular analysis and comparison with a deficiency. Loss of function mutations all affected oocyte growth, and some alleles also disrupted border cell migration. It has been known that PH4alphaEFB family members can regulate Collagen IV, a component of extracellular matrix. In addition, collagen is also well known for a role in shaping the elongated egg chamber, but its role in border cell movement is less clear. Other experiments suggest PH4alpha may interact with the receptor that activates STAT family. We are currently investigating a link between PH4alphaEFB and regulation of Collagen IV and STAT activation. The success of this project will contribute to a better understanding of border cell migration and may provide insight into cell movement more generally.

182B

Genes that act to destroy mitochondrial DNA in spermatids and enforce maternal only inheritance. Steven Z. DeLuca, Patrick H. O'Farrell. Dept. of Biochemistry, UCSF, San Francisco, CA.

It is widely appreciated that mitochondrial mutations and the mitochondrial genome itself (mtDNA) show maternal inheritance in metazoans. It is, however, not known why inheritance is restricted in this way. By following the male mtDNA in crosses, we found that it disappeared from within mitochondria during spermatogenesis. We summarize this finding and present results showing that the endonuclease, EndoG, and the mitochondrial DNA polymerase have roles in the disappearance of mtDNA. We followed male mtDNA by a polymorphism that is distinguishable by and quantifiable by PCR. None was found in recently fertilized eggs or in the mature sperm resident in the sperm storage organ of mated females. Using both cytological staining and GFP-mtSSB, we visualized mtDNA as numerous bright nucleoids that abruptly vanished from the huge mitochondria of late elongating spermatids. We have identified the mitochondrial endonuclease, EndoG, as being partially required at the late elongation stage for mtDNA elimination. In EndoG mutants, nucleoids declined in number more slowly, and the individualization complex later swept the remaining nucleoids out of the sperm. Expression of two independent RNAis during spermatogenesis revealed that knockdown of Tamas, the large sub-unit of the mitochondrial DNA polymerase, greatly stabilized mtDNA so that some of it persisted to mature sperm. Tamas includes a nuclease domain involved in proofreading, which we hypothesize contributes importantly to elimination of mtDNA during spermatogenesis. Removal of both EndoG and Pol-γ function resulted in even greater persistence of mtDNA in the mitochondria of developing spermatids, but the combination rendered males sterile. These findings suggest that a sophisticated program eliminates mtDNA during spermatogenesis and that this may be required for fertility as well as ensuring female only inheritance of mtDNA.

183C

Identifying new regulators of secretory capacity. Rebecca M. Fox, Xueni Chen, Deborah J. Andrew. Dept Cell Biol, Johns Hopkins Univ, Baltimore, MD.

In the Drosophila embryo, the salivary gland is the largest secretory organ, making it an excellent system in which to study the acquisition of high-level secretory function. We have shown previously that the bZip transcription factor, CrebA, and its mammalian orthologues, Creb3L1 and Creb3L2, are required to boost high-level secretory capacity through the direct regulation of components of the secretory pathway machinery. To identify other regulators of secretory function we performed microarray analyses to find the full complement of CrebA target genes. Of the nearly 400 genes whose expression went down in *CrebA* mutants, we chose to focus on genes encoding potential transcriptional regulators with clear human orthologues. Our initial analysis included whole mount in situs to look at the full embryonic expression patterns of each gene, as well as assaying for potential secretory defects in P-element or deficiency lines. Secretory defects present as defects in the cuticle secreted by the epithelial cells or as irregularities in the salivary gland or salivary gland lumen. Of the sixteen genes we characterized, Tudor-SN emerged as a promising potential regulator of secretory function based on this initial analysis. The Tudor-SN open reading frame is highly conserved and contains a Tudor DNA/RNA binding domain and four staphylococcal nuclease domains. Moreover, loss of *Tudor-SN* resulted in cuticle and salivary gland defects similar to those seen with loss of *CrebA*. Tudor-SN orthologues in other organisms have been implicated in multiple seemingly unrelated processes including

transcriptional activation, mRNA stabilization, small RNA processing, stress granule formation, lipoprotein phospholipid secretion and spliceosome assembly. Our initial experiments in Drosophila have confirmed that Tudor-SN is regulated by CrebA and have shown that the protein localizes to the ER. We expect that the characterization of CrebA targets, such as Tudor-SN, will provide key insights into the regulation and implementation of secretory function in p.

184A

Terminal cells lacking V-ATPase appear to form auto-cellular rather than seamless tubes. Deanne M. Francis, Amin Ghabrial. Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA.

The tracheal system contains three tube architectures: multi-cellular tubes that have lumens surrounded by two or more cells; auto-cellular tubes that form when a single cell wraps around a lumenal space and seals itself into a tube by making self-junctions; and seamless tubes that form intra-cellularly generation of internal apical membrane surrounding a lumen. Tracheal terminal cells are found at the end of the branched tracheal network, where they ramify on muscle and internal tissues, making dozens of seamless tubes. Mutations in *oak gall* (*okg*) and *conjoined* (*cnj*) were identified in a forward genetic screen designed to uncover genes required for tracheal morphogenesis (Ghabrial,A.S. 2011). I have focused on the role of *okg* and *cnj* in tracheal terminal cells. Terminal cells mutant for *okg* or *cnj* share identical cell rounding, branch pruning, and local air-filling defects. Careful analysis has revealed that *okg* and *cnj* affect the distribution of E-Cadherin within the terminal cell, and in the most extreme cases alter the type of tube architecture present within the terminal cell such that tubes expected to be seamless instead appear to have auto-cellular junctions. Positional cloning revealed that *okg* encoded the E-subunit of the vacuolar (V)-ATPase and *cnj* encoded the G-subunit of the same multi-subunit complex. In fact, these two proteins hetero-dimerize (Ma et al., 2011). The V-ATPase holoenzyme acidifies intracellular organelles and is important for many cell processes (Nishi and Forgac. 2002). Interestingly, the V0 subunit of the V-ATPase has been shown to have an independent function in membrane fusion (Bayer et al, 2003). We find that depletion of V1 and V0 proteins cause similar phenotypes in tracheal terminal cells, suggesting that loss of the acidification function of the V-ATPase is responsible for the terminal cell phenotype. Current efforts towards distinguishing among possible models to explain the conversion of seamless to seamed tubes in *okg* and *cnj* mutant terminal cells will be presented.

185B

Role of *expansion* in *Drosophila* tracheal tube diameter regulation. Ekaterini Iordanou, Rachana R. Chandran, Mina Essak, Lan Jiang. Biological Sciences, Oakland University, Rochester, MI.

The regulation of optimal tubular sizes is a fundamental process that is critical for the function of human lungs, kidneys, and blood vessels. Aberrant alterations in tube sizes during development lead to devastating diseases such as polycystic kidney disease. The *Drosophila* tracheal system is one of the most powerful model systems used to study tubular epithelial morphogenesis. Despite the recent advances in understanding tubular organ formation, the mechanisms by which cells assemble into tubes, with highly regulated lengths and diameters, are still not well understood. The apical luminal matrix has been shown to be important in the prevention of tube over-expansion; however, mechanisms that mediate apical secretion of specific luminal components are poorly understood. We identified a novel, evolutionarily conserved, *Drosophila* protein, Expansion (Exp), which is required for tracheal tube-size regulation. In *expansion* mutants, uni-cellular tracheal branches develop bubble-like cysts. In addition, the secretion of certain luminal proteins is defective. We further demonstrate that the apical localization of Rab11, a member of the family of small GTPases, and Rip11, a Rab11-interacting protein, is significantly reduced in*expansion* mutants. In addition, Rab11-mediated apical secretion is required for the secretion of certain luminal proteins. Therefore, *expansion* is required for tube-size regulation partially by controlling Rab11/Rip11-mediated apical section of the luminal matrix. The *expansion* phenotype exemplifies a role for this novel protein in epithelial lumen formation and tube-size control.

186C

Myogenesis of the smooth muscles surrounding the testes of Drosophila melanogaster males. Jessica Kuckwa[1], Christina Hornbruch-Freitag[1], Loreen Susic-Jung[1], Uwe Lammel[2], Renate Renkawitz-Pohl[1]. 1) Developmental Biology, University of Marburg, 35043, Marburg, Germany; 2) Neurobiology, University of Muenster, 48149, Muenster, Germany.

Myoblast fusion and myotube differentiation has been extensively studied in the Drosophila embryo, but limitations occurred due to functional redundancies and maternally supplied components. We here focus on the male reproductive system as a model to study myogenesis of different types of muscles. The inner genitalia of males consist of the testes, which emerge from the gonads and the remaining genital organs, i.e. paragonia, ejaculatory duct, sperm pump and seminal vesicles, developing out of the genital imaginal disc. We identified the muscles of the testes, seminal vesicles and sperm pump to be multinucleated. Paragonia and ejaculatory duct are encircled by a mononucleated muscle layer. All muscles derive from myoblasts of the male genital disc. Upon all characterized Drosophila muscles, the testes musculature is very special: their unique filament arrangement is reminiscent of vertebrate smooth muscle fibers. Smooth muscles have not been described in Drosophila, so far. Analyzing the developing muscle fibers, we revealed that the myoblasts building up the muscles of the seminal vesicles and/or the testes begin to get multinucleated on the male genital disc. The nascent myotubes then migrate over the seminal vesicles onto the testes where they spread out and elongate. This process is accompanied by expression of the mesodermal transcription factor DMef2, needed for specification and differentiation of embryonic myoblasts. The immunoglobulin proteins Duf and Sns are similarly expressed. Duf and Sns function during embryonic myoblast fusion as well

as in ommatidia spacing during eye development. knocking down either sns or duf by RNAi in the developing testes muscles lead to some scattered filaments, arranged like the little rods in the Mikado game (Mikado-phenotype). All in all, we currently suppose that the multinucleated smooth muscles surrounding the testes develop by myoblast fusion during migration of the myoblasts/myotubes on the genital disc.

187A

Identification of somatic factors controlling ovarian development by *RNAi* screening. Chun-Ming Lai[1,2], Yueh Cho[1], Hwei-Jan Hsu[1]. 1) Inst Cellular & Organismic Biol, Academia Sinica, Taipei, Taipei, Taiwan; 2) Molecular and Biological Agricultural Sciences Program, Taiwan International Graduate Program, National Chung-Hsing University and Academia Sinica, Taipei, Taiwan.

Germ cells are the only cell type that transmits genetic information to the next generation. Germ cell development and germline stem cell (GSC) specification require interaction with their somas. The molecular mechanisms underlying these processes, however, are largely unknown. The *Drosophila* ovary is an excellent model in which to study germ cell-soma interactions, as it produces eggs daily, houses well-characterized GSCs, and is easily manipulated. To identify factors in the soma that affect germ cells, we conducted a small-scale *RNAi* targeting screen. We first selected 560 *UAS-RNAi* lines with targets involved in the control of female fertility from the NIG-Fly stock center. We individually overexpressed these *RNAi* lines in the soma during ovarian development using a *bab1-GAL4* driver, and examined egg production and ovary morphology at the adult stage. We identified 42 candidate genes that potentially function in the soma and contribute to ovarian development. Among these genes, Transformer, Sex lethal and Traffic jam have been reported to control soma specification and function, validating our approach. Eight of our candidate genes encode proteins belonging to the Notch, Bmp, Hh, Wnt and Ras signaling pathways, indicating that these pathways are critical for appropriate soma development. Other candidate genes encode proteins that regulate cell cycle progression, cell movement, chromatin remodeling, cytoskeleton arrangement, and transcriptional regulation. We are currently investigating how these genes regulate soma-germ cell interactions during ovarian development.

188B

Analysis of *Neprilysins 1-5* in *Drosophila melanogaster* reveals parallels between mammalian and invertebrate roles in reproductive fitness. J. Sitnik[3], C. Francis[1,2], R. Huybrechts[4], M. Wolfner[3], P. Callaerts[1,2]. 1) Laboratory of Behavioral and Developmental Genetics, KULeuven, Leuven, Belgium; 2) VIB Center for the Biology of Disease, Leuven, Belgium; 3) Dept. of Molecular Biology and Genetics, Cornell University, Ithaca NY, USA; 4) Zoological Institute, KULeuven, Leuven, Belgium.

Members of the M13 class of metalloproteases have been implicated in a variety of diseases including cardiovascular disorders and Alzheimer's. In addition to their importance in human disease, some members of this zinc-metalloprotease family are expressed in human reproductive tissues. Further, they have been shown play a role in reproductive fitness for both male and female mice. Beyond the role of one family member, Neprilysin, in degrading tachykinins in the uterus and the sperm of mammals, very little is known about how these proteases regulate reproduction. We sought to use *Drosophila* as a model to dissect this. The *Drosophila melanogaster* genome contains 24 M13 class protease homologs, some of which are orthologs of human proteases including Neprilysin and are expressed in the reproductive tracts of either sex. Using RNAi we individually targeted each of the 5 *Drosophila Nep* genes *(Nep1-5)* to determine their importance in reproduction. Reducing expression of *Nep1* or *Nep2* in the CNS or spermathecae of females causes a reduction in egg-laying; expression of *Nep2* in females is also important for the hatchability of laid eggs. Females homozygous for a null mutation in *Nep2* also show defects as hosts of sperm competition, suggestive of roles for female-derived *Nep2* in sperm storage or utilization. Reducing expression of *Nep1-5* in males did not cause dramatic fertility defects which suggests that these genes may not be essential for reproductive fitness in the male or that they may overlap in function. Our results are in support for a reproductive role for Neprilysin members of the M13 class of metalloproteases, particularly in the female. Further work is needed to elucidate the roles of the other 19 M13 class homologs in reproduction.

189C

The H4K16 histone acetyltransferase *chameau* is a putative target of Doublesex. Emily Clough[1], Cale Whitworth[2], Erin Jimenez[2], Hania Pavlou[3], Megan Neville[3], Stephen Goodwin[3], Mark Van Doren[2], Brian Oliver[1]. 1) Laboratory of Cellular and Developmental Biology, NIDDK/NIH, Bethesda , MD; 2) Department of Biology, Johns Hopkins University, Baltimore, MD; 3) Department of Physiology, Anatomy and Genetics,University of Oxford, Oxford, UK.

Doublesex (DSX) is a sex-specifically spliced DMRT family member transcription factor that regulates somatic sex determination in *Drosophila*. Although *dsx* is an essential regulator of sexual differentiation, few direct targets have been characterized. We have used multiple genome-wide approaches to assay DSX occupancy across diverse developmental and chromatin contexts including chromatin immunoprecipitation-sequencing (ChIP-seq) and DNA Adenine Methylation-Identification (DamID). These studies have yielded thousands of sites that are concentrated near transcriptional start sites. Furthermore, the DNA captured beneath the peaks is enriched for previously identified DSX binding sequences. In order to make connections between putative target genes associated with DSX occupancy and *dsx* phenotype, we are knocking down these genes specifically in *dsx*-expressing cells to evaluate their role in sexual differentiation. Our DSX occupancy experiments reveal that DSX protein is associated with the gene *chameau* (*chm*) at a site within one of it's large introns that also contains a DSX-binding sequence. *chm* is characterized as an H4K16 acetyltransferase with no known role in sexual differentiation.

Knockdown of *chm* in *dsx*-expressing cells produces several *dsx*-like phenotypes including defective rotation of male genitalia, sterility and male sex comb bristles that are reduced in number with thin, pointed ends. Further evidence of a role for *chm* in somatic sex determination is provided by the observation that *chm* genetically interacts with *dsx* to promote terminal filament formation, a feature of the female gonad. Collectively, these data suggest that *chm* is a direct target of DSX and may impact sexual differentiation of many tissues.

190A

A Genomic Analysis of Sex Determination. Erin Jimenez[1], Cale Whitworth[1], Emily Clough[2], Brian Oliver[2], Mark Van Doren[1]. 1) Biology, Johns Hopkins University, Baltimore, MD; 2) NIDDK, National Institutes of Health, Bethesda, MD.

Sex determination pathways are diverse throughout the animal kingdom, but converge upon conserved genes that encode products that regulate sexual dimorphism. One such downstream factor across many diverged sex determination pathways is the Drosophila doublesex (dsx) gene. The role of dsx is highly conserved in different insects and dsx homologs (dsx, mab-3 related transcription factors, DMRTs) play roles in sexual differentiation in a diverse array of metazoans. In Drosophila, nearly all manifestations of sexual dimorphism between males and females are regulated by dsx, yet there are only three known direct targets of DSX, which cannot account for the differences in regulation by DSX in sexually dimorphic tissues. To gain a comprehensive understanding of DSX targets, we have discovered genes whose transcription is more immediately regulated by DSX by performing transcriptome analysis on samples where the DSX isoform has been acutely changed from DSX-F to DSX-M and vice versa. To control DSX status between sex specific isoforms, we have temperature-controlled alleles of the splicing regulators Transformer-2 and Transformer, which renders expression of either DSX-F or DSX-M temperature dependent. Thus, by switching between DSX protein isoforms DSX-M or DSX-F for an acute period of time, followed by expression profiling, we identified which genes are up- or down-regulated, in response to a change in DSX isoform. By comparing these results to experiments that determine where the DSX protein is bound in the genome, and genetic analysis that identifies new dsx-interacting genes, we have identified a number of target genes. Since the Drosophila gonad represents an excellent model to dissect how DSX acts on a particular time and place to promote development of a sexually dimorphic tissue, we are examining these target genes for roles in gonad sexual development. This research will provide insight into conserved genes that regulate developmentally similar pathways whose outcome generates major differences observed between the sexes.

191B

Female-expressed genes that affect the post mating response in Drosophila melanogaster. Alexandra L. Mattei, Jessica L. Sitnik, Frank W. Avila, Amber R. Krauchunas, Mariana F. Wolfner. Cornell University, Ithaca, NY.

Seminal fluid proteins (SFPs) from male Drosophila cause behavioral and physiological changes in mated female flies. These changes, collectively called the female post mating response (PMR), include rejection of further mating, increased feeding, increased egg production, decreased lifespan, and changes in gene expression. It is not well understood how proteins produced by the female fly contribute to the PMR. We are studying the roles of three female-expressed proteins in the PMR; we are using systemic or localized knockdown in females followed by assessment of PMR in those flies. For two genes, encoding angiotensin converting enzyme (ANCE) and neuropeptide like precursor 3 (nplp3), the phenotypes of knockdown females suggest roles in determining the number of eggs laid for 10 days post-mating. A third gene, encoding the sex peptide receptor (SPR), is already known to be essential for elevating egg-laying post-mating. However, SPR's ligand (the sex peptide) is also essential for controlling the release of sperm stored in the mated female. We are testing whether SPR is necessary for this aspect of SP action. Our results will enhance the understanding of the genes, proteins and mechanisms involved in male-female interactions in reproduction. They have potential application to the control of insect pests, such as the mosquito Aedes aegypti that is the vector for dengue fever, Chikungunya, and yellow fever.

192C

Genetic basis for developmental homeostasis of germline stem cell niche number: a network of Tramtrack-group nuclear BTB factors. Mathieu Bartoletti[1,2,3*], Thomas Rubin[2,3], Fabienne Chalvet[2,3,4], Sophie Netter[1,2,3], Nicolas Dos Santos[2,3], Emilie Poisot[2,3], Melanie Paces-Fessy[2,3,5], Delphine Cumenal[5], Frederique Peronnet[5], Anne-Marie Pret[1,2], Laurent Theodore[1,2,4]. 1) Centre de Génétique Moléculaire - UPR 3404, GIF SUR YVETTE, France; 2) Departement de Biologie, Univ Versailles St-Quentin, Versailles, France; 3) Laboratoire de Génétique et Biologie Cellulaire, Equipe Associée 4589, Univ Versailles St-Quentin, Versailles , France; 4) Departement de Biologie, Univ Paris-Sud, Orsay, France; 5) Biologie du Développement UMR 7622, CNRS et UPMC, France.

In the insect ovary, each germline stem cell (GSC) niche is embedded in a functional unit called an ovariole. The number of ovarioles varies widely among species. It remains generally unknown how the number of stem cell niches is controlled in the ovary. In *Drosophila*, morphogenesis of ovarioles starts in larvae with the formation of terminal filaments (TFs), each made of 8-10 cells that pile up and sort in stacks. TFs constitute organizers of individual GSC niches during larval and early pupal development. In the *melanogaster* subgroup, the number of ovarioles varies interspecifically from 8 to 20. Here we show that *pipsqueak*, *Trithorax-like*, *batman* and the *bric-à-brac* (*bab*) locus, all encoding nuclear BTB/POZ factors of the Tramtrack Group, are involved in limiting the number of ovarioles in *D. melanogaster*. At least two different processes are differentially perturbed by reducing the function of these genes. We found that when the bab dose is reduced, sorting of TF cells into TFs was affected such that each TF contains fewer cells and more TFs are formed. In contrast, psq mutants exhibited a greater number of TF cells per ovary, with a normal number of cells per TF, thereby leading to formation of more TFs per ovary than

in the wild type. Our results indicate that two parallel genetic pathways under the control of a network of nuclear BTB factors are combined in order to negatively control the number of GSC niches.

193A

Apontic acts as a JAK/STAT pathway regulator in the Drosophila testis niche. Kathryn A. Bus, Archana Murali, Michelle Starz-Gaiano. University of Maryland Baltimore County, Batimore, MD.

Production and maintenance of adult stem cells depends upon a complex microenvironment, called a niche. Stem cells provide the basis for all subsequent differentiated tissues. Thus, the dynamics of the niche are complex and although some mechanisms of this environment are established, there are questions that need to be investigated. The Drosophila testis is an excellent model system to study the genetic and molecular interactions needed during stem cell self-renewal and differentiation. The testis niche is comprised of a group of cells, known as hub cells, which are surrounded by germ line stem cells (GSCs) and somatic cyst stem cells (CySCs). Several laboratories have shown that activation of the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway is necessary for maintaining both types of stem cells. We have shown that Apontic (APT), a transcription factor and STAT signaling feedback inhibitor, is highly expressed in the somatic stem cells of the testis, as well as in early daughter cells. When *apt* is overexpressed in the soma, there are fewer CySCs, while overexpression in the germ line has no effect. In *apt* loss of function mutants, we observe more Zfh-1-positive CySCs, and an expanded domain of GSCs. The *apt* mutant phenotype is distinct from those due to mutations in other STAT targets or regulators, such as *socs36e*. *apt* mutant cells display altered adhesion and morphological properties in ovarian follicle cells, and parallel changes may explain delayed CySC differentiation in the testis. Thus, additional CySCs in *apt* mutants may permit GSC self-renewal by acting as a secondary niche or altering the properties of the microenvironment. We are currently utilizing a mosaic clonal analysis to determine the effects of *apt* on the two cell populations and how it affects adhesion molecules in the niche environment. Thus, this work supports a new role for a Jak/Stat regulator, APT, in the testis niche and suggests APT is required for the maintenance of the stem cell populations.

194B

The Role of miR-310s in Regulation of Somatic Cell Differentiation in Drosophila Ovary. Omer Cicek, Halyna Shcherbata. MPRG of Gene Expression and Signaling, Max Planck Institute, Goettingen, Germany.

miRNAs are short noncoding RNA molecules, which have regulatory roles in gene expression and which have been shown to act on diverse cellular and physiological processes such as cell survival, death, and fate determination. Among many fine-tuned homeostases throughout the body, continuous generation of adult tissues from their respective adult stem cells is tightly regulated. Stem cell divides asymmetrically into another stem cell and a daughter that undergoes differentiation. An open question of great importance is how stem cell progeny determines appropriate cell fate. Problems in cell specification can result in permanent loss of regenerative tissue or excessive cancerous overproliferation. We use the Drosophila ovary as a model system to study the specificity of cell differentiation, since ovarian soma bears different types of cells; for example, terminal filament and cap cells are mitotically inactive, while follicular epithelium and stalk cells are constantly replenished by follicle stem cell division. Stalk cells are terminally differentiated, while follicle cells undergo mitotic and endomitotic cell division. Our data show the role of the recently evolved miR-310s complex that consists of miR-310, 311, 312, and 313 in the process of regulation of somatic cell fate in the Drosophila ovary. This miRNA complex is expressed in terminally differentiated stalk cells. The deletion of miR-310s results in multilayered epithelial phenotypes that are enhanced due to stress conditions. We found that Rab23, a negative regulator of highly evolutionary conserved Hedgehog (Hh) pathway that has been shown to determine cell fate in the ovary, is a miR-310s target in somatic cells. Taken together, our data show that cell specification can be adjusted by miRNAs in response to external conditions: miR-310s fine tune the strength of Hh signaling that in turn regulates cell fate determination.

195C

Putative sperm chromatin condensing proteins and their respective conserved domains in 12 sequenced species of *Drosophila*. Zain A. Alvi, Tin-Chun Chu, Angela V. Klaus. Department of Biological Sciences, Seton Hall University, South Orange, NJ.

Our current research is aimed at identifying and analyzing proteins that are involved in sperm chromatin condensation in the original 12 sequenced *Drosophila* fly species. The process of nuclear transformation occurs due to the interaction of three sperm basic proteins (SNBPs), transition protein (TPL94D in *Drosophila melanogaster*), and CTCF (chromatin insulating and DNA - zinc finger binding protein). The SNBPs can be divided into histone group (Histone H1 linker like and involved in chromatin condensation in *Drosophila melanogaster*); protamine-like proteins (DNA binding and present in*Drosophila melanogaster*); and true protamines (not found in *Drosophila*). Using the reference sequences for *Drosophila melanogaster* SNBPs (Mst35Ba, Mst35Bb, and Mst77F), we previously identified the putative sequences for the SNBP proteins in *D. simulans*, *D. sechellia*, *D. yakuba*, *D. erecta*, *D. anannassae*, *D. mojavensis*, *D. virilis*, *D. willistoni*, *D. grimshawi*, *D. pseudoobscura*, and*D. persimilis*. Our current work suggests that Mst77F and TPL94D are conserved in the melanogaster species subgroup, but not conserved in the rest of the subgenus Sophophora or in subgenus Drosophila, whereas Mst35Ba, Mst35Bb, CTCF, are conserved among the original 12 sequenced species of *Drosophila* flies. Mst35Ba, Mst35Bb, TPL94D, CTCF, and Mst77F all have a putative conserved DNA binding domain. Additionally, Mst77F appears to have a conserved protein-protein interaction domain. We are also analyzing chromatin condensation patterns during nuclear transformation

in *Drosophila*sperm nuclei. Our hypothesis is that the type of SNBPs present in the sperm nucleus will affect the pattern of chromatin condensation, which in turn will affect the species-specific shape of the sperm nucleus.

196A

Lipid signaling between soma and germline is required for *Drosophila* spermatogenesis. Geulah Ben-David, Josefa Steinhauer. Department of Biology, Yeshiva College, New York, NY.

Lysophospholipids are single fatty acid chain phospholipids that can promote proliferation, motility, and survival when added to the media of cultured cells. In mammals, lysophospholipid signaling has been linked to cancer progression and has been implicated in normal physiology and development. Mechanisms that regulate lysophospholipid levels in vivo are not well understood. One pathway by which lysophospholipids are generated is the Lands cycle, which converts membrane phospholipids to lysophospolipids by removal of a fatty acid chain (deacylation) via the activity of phospholipase A_2 (PLA_2). PLA_2 activity is counterbalanced by the activity of membrane-bound O-acyltransferase (MBOAT) family enzymes, which catalyze the reacylation of lysophospholipids into phospholipids. Oysgedart (Oys) and Nessy (Nes) are *Drosophila* MBOAT family lysophospholipid acyltransferases (LPLATs). Adult male *oys nes* mutants are sterile with a complete block in spermatid individualization. Here we show that*oys* and *nes* RNAs are expressed in the testis, as are four of the nine *Drosophila* PLA_2 genes. We are testing whether these PLA_2s are required for spermatogenesis using RNAi. The spermatogenesis defect of *oys nes* mutants can be rescued by expression of Oys cDNA in the somatic cyst cells, but not the germline. In *oys nes* mutants, molecular markers of cyst cell development are expressed normally, and cyst cell membranes also appear normal with a fluorescent membrane marker. Additionally, *oys nes* mutant embryos display defects in germ cell migration, a process that relies on lipid signaling, and *oys* and *nes*are required in the soma for this process. Together, our data suggest that Oys and Nes mediate cell communication between soma and germline in two stages of development, by regulating the availability of lysophospholipid signals. These studies may provide a foundation for investigating the roles of lysophospholipid signals in cell communication and fertility.

197B

Studying the effects of Hsp27 phosphorylation on viability and fertility. Emily Furbee[1], Joseph Ayoob[2], Jonathan Minden[1]. 1) Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Department of Computational and Systems Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

The small heat shock protein Hsp27 plays many roles within the cell. Some of Hsp27's chaperone roles are to regulate caspase activity and actin dynamics. Although the exact molecular details underlying each of these functions are poorly understood, phosphorylation appears to play a central role in Hsp27 regulation. To further study the role of Hsp27 and its phosopho-regulation *in vivo*, we used ΦC31 site-directed transgenesis to express either unphosphorylatable ($Hsp27^{SA}$) or phosphomimetic ($Hsp27^{SD}$) versions of Hsp27 under the control of Hsp27's endogenous promoter. We were surprised to find that ectopic expression of phospho-mutant proteins in an Hsp27 wild-type background produced distinct phenotypes. Embryos that are homozygous for wild-type Hsp27 and $Hsp27^{SA}$ die in late embryogenesis or as early larvae. These mutants exhibit fragmented or absent trachea, suggesting defects in tracheal morphogenesis, which is an actin-dependant process. In contrast, embryos that are homozygous for wild-type Hsp27 and $Hsp27^{SD}$ survive to adulthood, but the males are completely sterile. Interestingly, these males display defects in the caspase-dependant individualization stage of spermatogenesis, featuring disrupted actin-based individualization complexes. Here we present the initial molecular dissection of both of these phenotypes that includes live microscopy and histochemical analysis with a focus on how each isoform affects caspase activation and actin dynamics.

198C

The role of Tudor-SN in spermatogenesis and the Piwi-piRNA pathway. Hsueh-Yen Ku, Vamsi Gangaraju, Haifan Lin. Stem Cell Center and Department of Cell Biology, Yale University School of Medicine, New Haven, CT.

PIWI proteins associate with piRNAs and function in epigenetic programming, post-transcriptional regulation, and transposon silencing to protect germline development. Based on the size exclusion chromatography and mass-spectrometry (MS) analysis of Drosophila embryos, we identified Tudor-SN (Tudor staphylococcal nuclease, TSN), an evolutionarily conserved protein, as a PIWI-interacting protein. Tudor-SN contains five staphylococcal nuclease-like domains (SN1-SN5) and a methyl lysine/arginine recognizing Tudor (Tud) domain. Tudor-SN has been shown to participate in a variety of RNA regulations, such as RNA-induced silencing complex (RISC), cleavage of hyper-edited miRNAs, and mRNA splicing. Here we show that Tudor-SN interacts with PIWI in vivo, and they are colocalized in the primordial germ cells (PGCs) in early embryos. Tudor-SN is ubiquitously expressed and is enriched in the cytoplasm of both germline and somatic cells in ovaries and testes. In tudor-sn mutant testes, spermatocytes are overexpanded, creating an enlarged tumour-like phenotype. In addition, mature sperms are present in the apical region of the mutant testis. Further genetic analysis demonstrated piwi mutant rescues tudor-sn mutant phenotype in a dosage-dependent manner. Our results suggest that Piwi and Tudor-SN antagonize each other to ensure proper spermatogenesis in Drosophila. We are currently working on the deep sequencing of tudor-sn mutant testes to examine the role of Tudor-SN in the piRNA pathway and mRNA regulations.

199A

Characterizing the genetic basis for mitochondrial shaping defects in *emmenthal* mutants of *Drosophila*

***melanogaster*.** Will S. Mitchell, Karen G. Hales. Department of Biology, Davidson College, Davidson, NC.

The regulation of mitochondrial dynamics in many organisms and cell types is important to viability and involves the highly specific and choreographed interactions of many gene products. A recessive male sterile mutation in *Drosophila melanogaster*, *emmenthal*, is associated with meiotic cytokinesis failure, vacuolated Nebenkerne (mitochondrial aggregates) and non-motile sperm. The emmenthal mutation was generated in a P-element insertion screen (Castrillon et al., 1993, *Genetics* 135: 489). The P-element insertion site was located using plasmid rescue and found to be among a cluster of genes with similar temporal and spatial expression in the *Drosophila* testis, though the insertion was not within any one gene. Initial RT-PCR analysis suggested that this insertion causes altered gene expression of a subset of these genes, which have no characterized homologs in other organisms and which have no recognizable protein domains. At least two of these genes appear to be transcribed in a polycistronic mRNA. The genes associated with the *emmenthal* phenotype may thus represent novel functions vital for mitochondrial regulation.

200B

Roles for testis-enriched ATP synthase subunits in mitochondrial shaping during *Drosophila* spermatogenesis. Eric M. Sawyer, Olivia Brown, Yihharn Hwang, Lauren Ivey, Kelsey E. Sheaffer, Conroy Field, Taylor Gunnell, Karen G. Hales. Department of Biology, Davidson College, Davidson, NC.

After meiosis in wild type *Drosophila* spermatids, mitochondria aggregate near the nucleus, and their membranes rearrange to form two large mitochondrial derivatives folded into a structure called the Nebenkern. During sperm tail elongation, the two derivatives unfurl and lengthen, and one derivative ultimately remains to power the sperm flagellum. Males homozygous for the *ms(2)1400* mutation exhibit mitochondrial clumping during the elongation stage of spermatogenesis, leading to male sterility. A lack of mitochondrial fusion bypasses the mitochondrial elongation defect, demonstrating that *ms(2)1400* does not directly cause elongation failure but instead a defect in internal Nebenkern structure. *CG7813*, encoding a testis-enriched paralog of the d-subunit of ATP synthase with an additional protein domain, is the gene associated with the *ms(2)1400* phenotype. In addition to its paradigmatic catalytic role, ATP synthase also plays an important role in cristae shaping, a property that has been described in other models, particularly yeast. In prior research, *CG7813* was investigated using RNAi knockdown via the GAL4-UAS system. The resulting phenotypes resembled the clumping during elongation found in the *ms(2)1400*mutants. To further understand the function of *CG7813*, current efforts are focused on constructing a transgene for rescue experiments and GFP-tagged transgenes to visualize protein localization in developing spermatids. To explore the role of additional ATP synthase subunits in mitochondrial dynamics, we knocked down expression in the testis of subunits g, F6, b, and α in the testis using RNAi. Results demonstrated a variety of mutant phenotypes primarily involving Nebenkern structure, such as vacuolated Nebenkerne, abnormal mitochondrial shaping, and ultimately non-motile sperm tails. The results serve as a further indication of the important role of ATP synthase in mitochondrial morphology.

201C

Cooperation of Mad and Akt signaling in a *Drosophila* model of epithelial plasticity. Courtney Onodera[4,8], Björn Gärtner[5,8], Samantha Aguinaldo-Wetterholm[2,9], David Casso[2,9], J. Alex Rondon[2,6,9], Samuel Meier[5], Aiguo Tian[2,7], Brandy Alexander[2], Rik Derynck[1,2,3], Jun S. Song[1,4], Julia Zeitlinger[5], Katja Brückner[1,2,3]. 1) Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research; 2) Department of Cell and Tissue Biology; 3) Department of Anatomy; 4) Institute for Human Genetics, University of California San Francisco, San Francisco, CA; 5) Stowers Institute for Medical Research, Kansas City, MO; 6) present address: Genentech; 7) present address: UT Southwestern; 8) equal contribution; 9) equal contribution.

Transforming Growth Factor-β (TGF-β) and Bone Morphogenetic Protein (BMP) cooperate with Akt signaling in many systems of epithelial plasticity during development and in fibrosis and tumor metastasis. However, the molecular basis of this cooperation remains incompletely understood. *Drosophila* has been an excellent model to study epithelial architecture and epithelial plasticity in vivo, yet no cell-based system has been available to take advantage of *Drosophila* in the molecular dissection of epithelial plasticity. We now introduce KaBrü1D, a *Drosophila* epithelial cell line closely related to wing imaginal disc cells, that undergoes BMP/decapentaplegic (dpp) induced epithelial plasticity, similar to the elongation of wing imaginal cells during thorax closure of the developing fly. Based on an RNAi screen comprising all *Drosophila* kinases and phosphatases, expression profiling, and ChIP analyses, we identified Mad targets and genes functionally involved in Dpp/BMP-induced epithelial plasticity. Akt/Tor signaling is essential in this process, and activity of this pathway is enhanced over the course of several days of BMP stimulation, consistent with a secondary transcriptional wave leading to elevated receptor tyrosine kinase signaling. Focusing on the mechanism of cooperation between the BMP and Akt pathways, we identified differential binding of Mad to transcriptional targets, and we dissect this regulation in cell culture and during thorax closure in vivo.

202A

The *Drosophila* BMPRII, Wishful thinking, is required for eggshell patterning. Rob Marmion[1], Milica Jevtic[2], George Pyrowolakis[2], Nir Yakoby[1]. 1) Department of Biology and Center for Computational and Integrative Biology, Rutgers University, Camden, NJ; 2) Institute for Biology I, Albert-Ludwigs-University of Freiburg, Freiburg, Germany.

The *Drosophila* eggshell is an established model to study cell signaling, tissue patterning, and morphogenesis. The bone morphogenetic protein (BMP) signaling pathway is a crucial regulator of tissue growth during multiple stages of *Drosophila* development. During oogenesis, the role of the type I BMP receptor, *thickveins* (*tkv*), on spatial distribution of signaling and eggshell patterning, has been established. However, BMP signaling requires a heterocomplex of type I and type II

receptors. We found the type II receptor, *wishful thinking* (*wit*), to be dynamically and non-uniformly expressed in the follicle cells, which are a mono-layer of epithelial cells engulfing the developing oocyte. We found wit to be transcriptionally regulated by BMP signaling and necessary for BMP signaling in the follicle cells. Of importance, we demonstrate that WIT is essential for proper eggshell morphology. Interestingly, we discovered two independent enhancers that combinatorially recapitulate the endogenous pattern of WIT. The first enhancer is expressed uniformly throughout the follicle cells, and the second is restricted to the anterior domain. The dynamics of the two enhancers suggest that they are regulated by the epidermal growth factor receptor and BMP signaling, respectively. Since this locus contains no traditional P-MAD/MED binding site, and the genetic evidence supports that the second enhancer is regulated by BMP signaling, we are in the process of discovering this novel regulatory sequence.

203B

A Novel Role for UDP-GlcNAC in Dpp Signal Antagonism. Matthew J. Moulton, Gregory Humphreys, Anthea Letsou. Human Genetics, University of Utah, Salt Lake City, UT.

mummy (*mmy*), a member of the *raw* group of signaling antagonists, encodes the single Drosophila UDP-*N*-acetylglucosamine pyrophosphorylase. Mmy's effects on signal antagonism are most evident in the context of embryonic dorsal closure. In this developmental context, the JNK/AP-1 signaling cascade transcriptionally activates Dpp signaling in leading edge (LE) epidermal cells. Whereas *dpp* expression is confined to LE cells in wild-type embryos, it expands ectopically into the dorsolateral epidermis in *mmy* mutant embryos, establishing Mmy as a *dpp* antagonist. To identify downstream effectors of Dpp signal restriction, we screened the 25 Drosophila glycosyltransferases potentially utilizing UDP-GlcNAc downstream of Mmy. Embryos depleted of several of these transferases phenocopy *mmy* loss-of-function phenotypes. In at least one case, transferase depletion leads to *dpp* expresson beyond the LE in the epidermis. These data suggest a role for GlcNAc modifications that is critical to development and the control of signaling.

204C

GPI-mannosyltransferase 2 shapes the Hedgehog morphogen gradient. Yi-Nan Lee[1], Haiwei Pi[2], Cheng-Ting Chien[1]. 1) Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan; 2) Department of Biomedical Sciences, Chang-Gang University, Taoyuan 333, Taiwan.

Hedgehog (Hh) signaling controls a wide spectrum of developmental processes such as tissue patterning and axon guidance. Hh is concentrated at cell surface by the interaction of lipid moieties with heparin sulfate proteoglypicans (HSPGs) residing in the extracellular matrix. Glypicans are HSPGs with a glycosylphosphatidylinositol (GPI) modification at the C-terminus of core proteins for membrane anchorage. In*Drosophila*, two functionally interchangeable glypicans Dally and Dally-like (Dlp) are required for Hh gradient formation in wing discs. Signaling-coupled internalization of Hh in a complex with cognate receptor Patched (Ptc) also depends on the function of the membrane attachment of glypicans. However, non-cell autonomous activities of Dally and Dlp have been observed in wing development. In this study, we investigate the functions of GPI anchor on Hh responses and morphogen gradient formation by studying mutants for GPI mannosyltransferase II (GPI-MT2), an enzyme for GPI anchor synthesis. We show that Dlp modification, processing and localization are affected in *gpi-mt2* mutants. In genetic mosaic assay, *gpi-mt2* is required cell autonomously for the expressions of short and medium ranges of Hh target genes. However, low levels of Hh signaling are unaffected in mutant clones for null *gpi-mt2* . In contrast, the responses to low and intermediate levels of Hh signaling are enhanced in *gpi-mt2*hypomorphic mutants. In correlation with the long-range effect on Hh signaling activity, both secreted and anchorless forms of Dlp are increased in the larval hemolymph, and Hh levels are increased in the anterior compartment of wing discs. Finally, we show that knockdown of *gpi-mt2* in wind discs cause defect in wing development, which can be rescued by the coexpression of secreted and membrane-anchored forms of Dlp. We propose that secreted and anchored Dlp forms play distinct roles in Hh signaling with secreted Dlp in promoting Hh long-range diffusion.

205A

Balancing Hedgehog, a retention and release equilibrium given by Dally, Ihog, Boi and Shifted/dWif. David Sánchez Hernández, Aphrodite Bilioni, Ainhoa Callejo, Ana-Citlali Gradilla, Carmen Ibañez, Emanuela Mollica, M.Carmen Rodríguez-Navas, Eleanor Simon, Isabel Guerrero. CBMSO, Madrid, Madrid, Spain.

Hedgehog (Hh) can signal both at a short and long-range, and acts as a morphogen during development in various systems. We studied the mechanisms of Hh release and spread using the Drosophila wing imaginal disc as a model system for polarized epithelium. We analyzed the cooperative role of the glypican Dally, the extracellular factor Shifted (Shf, also known as dWif), and the Immunoglobulin-like (Ig-like) and Fibronectin III (FNNIII) domain-containing transmembrane proteins, Interference Hedgehog (Ihog) and its related protein Brother of Ihog (Boi), in the stability, release and spread of Hh. We show that Dally and Boi are required to prevent apical dispersion of Hh; they also aid Hh recycling for its release along the basolateral part of the epithelium to form a long-range gradient. Shf/dWif on the other hand facilitates Hh movement restrained by Ihog, Boi and Dally, establishing equilibrium between membrane-attachment and release of Hh. Furthermore, this protein complex is part of thin filopodia-like structures or cytonemes, suggesting that the interaction between Dally, Ihog, Boi and Shf/dWif is required for cytoneme-mediated Hh distribution during gradient formation.

206B

The interactions among upd-family ligands. Qian Chen, Douglas Harrison. Dept Biol, Univ Kentucky, Lexington, KY.

The JAK/STAT signaling pathway is the major signaling cascade in response to a variety of cytokines and growth factors in vertebrates and it is highly conserved. But unlike vertebrates, the Drosophila JAK/STAT signaling pathway has only three identified ligands: Unpaired(Upd), Upd2 and Upd3. The expression patterns of upd2 and upd3 overlap with that of upd during several developmental processes. upd2 and upd are expressed in identical stripes in embryos, while upd3 and upd are co-expressed in the polar cells of egg chambers and posterior region of eye discs. Given the overlapping expression pattern, we hypothesize that the three ligands cooperatively regulate the JAK signaling by forming different ligand complexes. We tested the physical interaction among the three ligands by Bimolecular Fluorescence Complementation (BiFC). All three ligands show the ability to form homodimers, and the interaction of Upd2 and Upd3 homodimers were stronger than Upd homodimers. Upd2 and Upd3 were able to form heterodimers with Upd individually as well. Homotypic interaction between Upd3 molecules was also detected in a yeast two-hybrid assay. To determine the putative functional domains of the upd-family ligands, we compared the sequence of the three ligands and identified six short conserved domains. We substituted each of the conserved domains on upd3 with five alanine residues individually and tested their function in a luciferase reporter assay. All six alanine substitutions dramatically reduced the Upd3 ability in stimulating JAK signaling. Interactions between the six alanine substituted upd3 molecules with intact ligands will be tested in BiFC assay to see if any of them are responsible for ligand interactions.

207C

A novel calcyphosine-like protein facilitates border cell migration during oogenesis. Lathiena A. Manning, Michelle Starz-Gaiano. Biological Sciences, University of Maryland Baltimore County, Baltimore, MD.

Collective cell migration is crucial to an organism's capacity to perform morphogenesis thereby creating body plans and organ systems. Cells that move as clusters must maintain their primary adhesions to their migratory counterparts while altering adhesions to stationary cells allowing for detachment and subsequent movement. We are employing the Drosophila melanogaster oocyte development to study cell detachment and migration amongst a small population of specialized cells referred to as border cells . Border cells differentiate and detach from the anterior epithelium and migrate posteriorly toward the oocyte while remaining in a cluster. Border cells display the characteristics of collective cell migration as they move. Both the single Drosophila steroid hormone, Ecdysone and JAK/STAT signaling pathways are essential in the expression of specific genes needed for coordinating border cell migration. Our work focuses on an uncharacterized calcyphosine-like protein (CAPSL) that potentially acts downstream of both signaling pathways. This calcyphosine-like protein contains EF hand domains, which are known for binding calcium. Though this protein is present in several tissue types, the specific function remains a mystery. We set out to determine the specific role that CAPSL plays in border cell migration. Gene expression analysis demonstrated the presence of the CAPSL in border cells prior to detachment and during early migration. Various alleles that reduce expression of the gene in the border cells disrupted their proper migration. We are testing the hypothesis that the calcyphosine-like protein disrupts actin dynamics preventing the cellular rearrangements needed for migration.

208A

An *in vivo* RNAi screen identifies components of the JAK/STAT signaling that regulate cell migration. Afsoon Saadin, Michelle Starz-Gaiano. Biological Sciences, UMBC, Baltimore, MD.

The Janus Kinase and Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway is involved in essential biological processes including cell fate determination, cell migration, cell proliferation, normal function of immune system, and stem cell maintenance. Out of different events that can be regulated by JAK/STAT signaling, cell migration is particularly interesting since it is not only required for normal embryonic development but can also lead to detrimental outcomes, such as tumor metastasis. Migration of a cluster of cells termed border cells in the *Drosophila* ovary is an excellent example of collective cell migration, which resembles metastasis of some carcinoma cells. Border cells arise within the follicular epithelium, and are required to migrate to the oocyte to contribute to a fertilizable egg. The requirement for the well-conserved components of STAT signaling pathway, including the activating cytokine, its receptor, JAK, STAT, SOCS and APONTIC, during border cell migration is well-studied, however, the functions of other potential regulators of the pathway during this process are not yet known. To find new components of the pathway that govern cell migration, we knocked down predicted STAT modulators using RNAi expression in follicle cells, and assayed for defective cell movement. We have identified a number of candidate genes that function during cell invasion, and these are currently being further characterized.

209B

JAK/STAT pathway plays two opposite roles in *Drosophila* spermatogenesis. Lingfeng Tang, Douglas Harrison. Department of Biology, University of Kentucky, Lexington, KY.

Germline cells in the testis are derived from germline stem cells (GSCs) at the tip and undergo a stereotyped pattern of divisions and differentiation to form mature sperm. The somatic hub cells at the tip express *upd*, a ligand for the JAK/STAT pathway that has roles in the maintenance of both Cyst stem cells (CySCs) and GSCs in the testis. We found that *upd3* is also expressed in the hub, and mutants of*upd3* have fewer CySCs and GSCs. Interestingly, JAK/STAT is also activated in elongated cyst cells which are away from the tip. The knockdown of JAK/STAT in the somatic cyst cells leads to impaired spermatid individualization, as shown by fewer cystic bulges, waste bags and individualization complexes and no sperm in the seminal vesicle. Activation of caspases in elongated spermatids is required for individualization. The knockdown of JAK/STAT in cyst

cells almost completely eliminated the activation of two effector caspases: Drice and DCP1. Forced expression of hid, the initiator caspase, significantly rescued the impaired individualization phenotype. JAK/STAT is activated in elongated cyst cells, while caspases are activated in spermatids enclosed by cyst cells. Candidate downstream signals from cyst cells that might regulate caspase activation in spermatids were examined. Hedgehog is expressed in the cyst cells, and over-expression impaired the activation of caspases. Knockdown of hedgehog and STAT simultaneously in cyst cells is able to partly rescue the phenotype of STAT knockdown. We concluded that JAK/STAT activity in somatic cyst cells promotes individualization in spermatids by stimulating caspase activity, perhaps partly by inhibiting Hedgehog activity. JAK/STAT pathway is not only required for the maintenance of stem cells in the tip, but also required for individualization away from the tip during late differentiation, thus plays two opposite roles in *Drosophila* spermatogenesis.

210C

***Drosophila* glypican Dally regulates Upd distribution and JAK/STAT signaling activity in eye development.** Jia You[1], Yan Zhang[2], Wenyan Ren[2], Xinhua Lin[1,2]. 1) Dev Biol, Cincinnati Chld Hosp Med Ctr, Cincinnati, Oh; 2) 1State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China.

Highly conserved janus kinase (JAK)-signal transducer and activator of transcription(STAT) pathway is a well-known signaling system that is involved in numerous biological processes. In *Drosophila*, this low-redundant signaling cascade is activated by ligands of Unpaired(Upd) family. Therefore the regulation of Upd distribution is one of the key issues in controlling JAK/STAT signal. Heparan sulfate proteoglycans (HSPGs) are macromolecules that regulate distribution of many ligand proteins including Wingless, Hedgehog and Decapentaplegic(Dpp). Here we show that during *Drosophila* eye development, HSPGs are also required in normal Upd distribution and JAK/STAT signaling activity. Loss of HSPG biosynthesis enzyme Brother of tout-velu(Botv), Sulfateless (Sfl), or glypican Division abnormally delayed(Dally) and Dally-like protein(Dlp) will lead to reduced levels of Upd and reduction of JAK/STAT signaling activity. Overexpression of Dally is sufficient to accumulate Upd and up-regulate the signaling activity. In vitro luciferase assay also shows that Dally promotes JAK/STAT signaling activity, and is dependent on its heparin sulfate chains. These data suggest that Dally is a major regulator of Upd distribution and JAK/STAT signaling activity.

211A

Spatial and temporal analysis of axonal transport in primary neuronal cultures from Drosophila larvae. Gary Iacobucci, Noura Abdel Rahman, Aida Andrades Valtueña, Shermali Gunawardena. Biological Sciences, State University of New York at Buffalo, Buffalo, NY.

Efficient intracellular transport is essential for healthy cellular function and structural integrity. Problems in this pathway have recently been implicated in neuronal cell death and disease. To spatially and temporally determine how transport defects are initiated, we have developed a primary neuronal cell culture system from Drosophila larval brains. Immunohistochemical characterization indicates that these primary neurons are similar to larval neurons in vivo. The staining patterns of numerous synaptic markers mimic the patterns seen in fixed whole mount third instar larvae. We also visualize, live, the movement dynamics of several cargos/organelles. In day 1 and day 2 old cultures we observe robust bi-directional movement of six cargos/organelles. Using a MATLAB based single-particle tracker program we have analyzed the movement behavior of these cargos/organelles at each time point. Temporal analysis shows transport dynamics of these cargos change with time. Significant increases or decreases in segmental velocities observed at day 2 relative to day 1 negatively correlate to increases or decreases in pause frequency and/or duration. In contrast to WT larval brain cultures, neuronal cultures from motor protein reduction larval brains show reduced movement of cargos with increased numbers of stalled cargo and axonal blocks. Strikingly, we find that axonal blockages are not fixed, permanent blocks that impede transport as previously thought, but are instead dynamic. Under some motor reduction conditions, blocks resolve while under other conditions they do not. Over time, these neuronal cultures from mutant larval brains show defects in neuronal growth. Taken together, our results propose that non-resolving blocks may initiate deleterious pathways leading to death and degeneration while resolving blocks are benign.

212B

***Drosophila* Tempura, a novel Rab geranylgeranyl transferase subunit, modulates Notch signaling via Rab1 and Rab11.** Wu-Lin Charng[1], Shinya Yamamoto[1,2], Manish Jaiswal[2,6], Vafa Bayat[1,3], Bo Xiong[1], Ke Zhang[4], Hector Sandoval[2], Gabriela David[1], Hsiang-Chih Lu[1], Kuchuan Chen[1], Hugo Bellen[1,2,4,5,6]. 1) Program in Developmental Biology; 2) Dept of Molecular and Human Genetics; 3) Medical Scientist Training Program; 4) Program in SCBMB; 5) Dept of Neuroscience; 6) Howard Hughes Medical Institute; The Neurological Research Institute; Baylor College of Medicine, Houston, TX.

To identify novel players in Notch signaling we performed an F3 forward genetic screen and isolated an uncharacterized *Drosophila* gene, which we named *tempura*. *temp* mutants exhibit notum bristle loss and wing notching. The balding is caused by loss of Notch signaling during cell fate determination, as shown by multiple neurons per sensory cluster. In addition, the density of mutant sensory clusters is increased, indicating a Notch signaling defect during lateral inhibition. We observed an accumulation of Delta in sensory organs, which may be the cause of the cell fate defect. On the other hand, a positive Notch signaling modulator, Scabrous, cannot be properly secreted which leads to the lateral inhibition defect. *temp* homologs are found in all vertebrate species but have not been characterized in any model organisms. It encodes a protein with a domain showing homology to α subunit of Rab geranylgeranyl transferase (RabGGT). This complex contains an α and a β subunit and adds geranylgeranyl groups to Rab proteins with the assistance of Rab escort protein (REP). Without

this modification, Rab distribution is altered and vesicle trafficking is impaired. We propose that Temp functions as a novel α subunit of RabGGT and modulates specific Rabs involved in trafficking of Notch signaling components. Indeed, Temp interacts with RabGGT β subunit, REP, and Rabs. Overexpression of dominant negative Rab1 and Rab11 can phenocopy Sca accumulation and notum balding, respectively. We also observed a severe misdistribution of Rab1 and Rab11 in *temp* mutants. Hence, Temp modulates Rab1/Rab11 and regulates Notch signaling through Sca and Delta. We are currently testing whether this modulation also occurs in vertebrates.

213C

Identification of novel maternal neurogenic genes that are potential components of Notch signaling in *Drosophila*. Takuma Gushiken[1,2], Kenjiroo Matsumoto[1,2], Takahiro Seto[2], Ryo Hatori[1,2], Shunsuke Shimaoka[1,2], Tomoko Yamakawa[1], Takeshi Sasamura[1], Kenji Matsuno[1]. 1) Department of Biological Science, Osaka university, Japan; 2) Department of Biological Science and Technology, Tokyo University of Science, Japan.

Notch signaling regulates many cell-fate specifications through local cell-cell interaction in *Drosophila* development. Notch signaling is involved in "lateral inhibition" that prevents proneural cells that neighbor a neuroblast from choosing the neuroblast-fate during neuroblast segregation. Thus, in the absence of Notch signaling, proneural cells differentiate into neuroblast at the expense of epidermoblasts. Therefore, the disruption of Notch signaling leads proneural cells to differentiate into neuroblast at the expense of epidermoblasts and results in the hyperplasia of neuronal cells in *Drosophila* embryos, which is referred to as the "neurogenic phenotype".

Although mutants that show neurogenic phenotype in their homozygotes have been studied extensively in *Drosophila*, we probably failed to identify many mutants that potentially lead to neurogenic phenotype, because maternal supply of their gene functions can suppress this phenotype. To overcome this problem, we screened mutants that showed neurogenic phenotype in embryos homozygous for them and lacking their maternal contribution. This phenotype is designated as "maternal neurogenic phenotype", and genes whose mutants show maternal neurogenic phenotype are called maternal neurogenic genes.

To identify novel maternal neurogenic genes, we screened mutants on the left arm of the second chromosome, which covers about 20% of the *Drosophila* genome. From this screen, we identified 2 mutants that showed maternal neurogenic phenotype. The summary of this screen and molecular genetics analyses of these maternal neurogenic genes will be presented. These studies will contribute to the understanding of the molecular mechanisms of Notch signaling.

214A

Direct regulation of *broad* expression by Notch signaling during the mitotic/endocycle switch in *Drosophila* follicle cells. Dongyu Jia[1], Yoichiro Tamori[1], George Pyrowolakis[2,3], Wu-Min Deng[1]. 1) Department of Biological Science, Florida State University, Tallahassee, FL 32306. USA; 2) Institute for Biology I, Faculty of Biology, Albert-Ludwigs-University of Freiburg, Hauptstrasse 1, 79104 Freiburg, Germany; 3) BIOSS Centre for Biological Signalling Studies, Albert-Ludwigs-University of Freiburg, 79104 Freiburg, Germany.

During *Drosophila* oogenesis, the follicle cells sequentially undergo three distinct cell-cycle programs: the mitotic cycle (stages1-6), the endoreplication cycle (also called the endocycle, stages 7-10a), and gene amplification (stages10b-13), through which chorion genes are selectively amplified. Activation of Notch signaling in the follicular epithelium (FE) at around stages 6/7 is essential for the proper entry of the endocycle. Notch induces the expression of zinc finger protein Hindsight and suppresses homeodomain protein Cut to control the mitotic/endocycle (M/E) switch. Here we report that *broad* (*br*), encoding a family of zinc-finger transcription factors, is a direct transcriptional target of Notch/Supressor of Hairless (Su(H) site binding of CSL complex (CBF-1, Su(H), Lag-1)) in the FE. We show that the early pattern of Br expression in follicle cells, uniformly expressed in the FE starting at stage 6, is established by Notch signaling. We identified a putative Su(H) binding site at the *br* early enhancer (*brE*) region, mutation of this site significantly reduced the expression of a reporter in the FE after Notch activation. The regulation of *brE* by Notch singlaing appears to be tissue-specific, as similar regulation does not exist at the dorsal/ventral boundary in the wing imaginal disc, where Notch is also active. We further demonstrate that *br* function is involved in the M/E switch and Br acts in parallel to Hnt during the endocycle.

215B

Rescue of Notch signaling in cells incapable of GDP-L-fucose synthesis by gap junction transfer of GDP-L-fucose in *Drosophila*. Kenjiroo Matsumoto[1], Tomonori Ayukawa[2], Ishikawa O. Hiroyuki[3], Akira Ishio[1], Tomoko Yamakawa[1], Takuya Suzuki[1], Kenji Matsuno[1]. 1) Biological Science, Osaka university, Toyonaka,Osaka, Japan; 2) Medical Science, Akita university, Toyonaka,Akita, Japan; 3) Science, Chiba university, Chiba,Chiba, Japan.

Notch and its ligand interactions are essential for ligand dependent Notch signaling. Notch contains epidermal growth factor (EGF)-like repeats, many of which have *O* -fucose glycan modification that regulate Notch-ligand binding. This modification requires GDP-L-fucose as a donor of fucose. A possibility that GDP-L-fucose is supplied intercellraly was considered, however the molecular basis of GDP-L-fucose transportation have not been explored in depth. Here, our *Drosophila* study showed that GDP-L-fucose is supplied intercellularly through gap junctions in vivo (PNAS, 2012). Moreover, the gap junction-mediated supply of GDP-L-fucose was sufficient to support the fucosylation of Notch EGF-like repeats (PNAS, 2012).

216C

The *Drosophila* CREB binding protein gene *nejire* is involved in multiple signaling and cell migration processes in

follicle cells. Zhiqiang Shu, Dongyu Jia, Wu-Min Deng. Biological Science, Florida State University, Tallahassee, FL.

Drosophila oogenesis encompasses some of the most fascinating biological changes at the cellular level, e.g. cell growth and migration. These cellular events are regulated spatially and temporally by multiple signaling pathways. Among these, the activation of Notch pathway induces a switch from the mitotic cycle to the endocycle in the somatic follicle cells. To understand how Notch activation is precisely regulated and interacts with other pathways, we employed an *in vivo* RNAi screen and identified *nejire* (*nej*), the *Drosophila* homolog of CREB binding protein gene. We found that *nej* knockdown (KD) follicle cells showed defects in the expression of Notch signaling markers Cut. Interestingly, *nej*-KD cells also showed defects in border cell migration and dorsal appendage formation. The role of *nej* in border cell migration is related to the JNK signaling because misexpression of *puckered*, a negative regulator of the JNK pathway, alleviated the *nej*-KD- induced border cell migration defect; whereas the involvement of *nej* in dorsal appendage formation is related to EGFR signaling, as revealed by downregulation of the EGFR target genes. We also observed an intriguing phenotype in follicle cells that cover the oocyte during stages 9-11. These *nej*-KD follicle cells appeared to have lost their epithelial morphology and undergone concerted migration to cover only the posterior end of the oocyte. This phenotype resembles that of epithelial-mesenchymal transition (EMT), a fundamental process that governs morphogenesis in multicellular organisms and is reactivated in a variety of diseases, especially in the progression of carcinoma and tumor metastasis. Our results indicated that *nej* regulates the expression of adhesion molecules, such as E-cadherin and Armadillo in this EMT-like process. In summary, our studies reveal the involvement of *nej* in multiple signaling pathways, and in cell migration in different follicle cell groups. Further studies on *nej* will determine how this gene integrates different signaling inputs and regulates complex cellular events such as migration and differentiation.

217A

Identification of me31B from an in vivo RNAi screen as a potential regulator of Notch Signaling. Muhammed Soylemez, Dongyu Jia, Wu-Min Deng. Department of Biological Science, Florida State University, Tallahassee, FL. Muhammed Soylemez, Dongyu Jia, Wu Min Deng. BIOLOGICAL SCIENCE , FLORIDA STATE UNIVERSITY, TALLAHASSEE , FL.

The Drosophila somatic follicle cells are excellent for the study of cell-cycle regulation and cell differentiation. During oogenesis, the follicle cells sequentially undergo three variations of cell cycle programs, the mitotic cycle, the endocycle and gene amplification. Notch signaling activation is required for the switch from the mitotic cycle to the endocycle (the M/E switch) and its downregulation is necessary for the switch from the endocycle to gene amplification (the E/A switch) in these cells. Recently, we have found that Broad, a zinc-finger transcription factor, is directly up-regulated by Notch signaling during the M/E switch in the follicle cells (Jia and Deng, unpublished data). During late oogenesis, Broad is also regulated by EGFR and Dpp pathways for chorionic appendage formation. To explore how these different signaling pathways regulate follicle cell differentiation and cell cycle switches, we performed an in vivo RNAi screen to examine the effect of induced knockdown of gene expression on Br expression during oogenesis. So far, 350 different RNAi lines have been screened and about 20 of them showed defects in either early or late Br expression in follicle cells. Knockdown of Me31B, a putative RNA helicase belonging to the DEAD-box family, resulted in disruption of the Br early expression pattern during the endocycle stages. In addition, we found that Hindsight and Cut, both of which are Notch targets in follicle cells, are also regulated by Me31B, suggesting a potential role of Me31B in Notch signaling. Further studies are being conducted to gain more insight into the relationship between Notch signaling and Me31B and their effects on cell cycle regulation, differentiation and growth.

218B

E(y)1, a component of the transcription initiation complex, is required for Notch signaling activation in Drosophila. Gengqiang Xie, Dongyu Jia, Wu-Min Deng. Department of Biological Science, Florida State University, Tallahassee, FL, 32306.

The Notch signaling pathway plays pivotal roles in a variety of developmental events, including cell differentiation, proliferation and apoptosis in multiple metazoan tissues. Dysregulation of this pathway has been linked to several inherited genetic diseases and cancer. In a large-scale RNAi screen for genes involved in follicle-cell differentiation and cell-cycle switches, we identified that e(y)1 is required for the Notch-dependent mitotic-to-endocycle transition in follicle cells at stage 6/7 of oogenesis. We further show, by monitoring the reporters of Notch activity, that E(y)1 positively regulates the Notch signaling pathway both in follicle cells and in the wing imaginal development. E(y)1 has been shown to be a component of transcription factor TFIID complex and/or SAGA histone acetyltransferase complex, suggesting an important function for gene transcriptional regulation. As expected, epistatic analysis indicates that E(y)1 acts in the level of the Notch transcription factor complex. We are currently investigating the mechanism by which E(y)1 regulates the Notch signaling pathway at the transcriptional level.

219C

***Drosophila pecanex* activates Notch signaling via unfolded protein response (UPR).** Tomoko Yamakawa[1], Yu Atsumi[1], Takeshi Sasamura[1], Naotaka Nakazawa[1], Emiko Suzuki[2], Mark E. Fortini[3], Kenji Matsuno[1]. 1) Osaka Univ, Osaka, Japan; 2) Gene Network Lab, NIG, Japan; 3) Thomas Jefferson Univ, Philadelphia, USA.

Notch (N) signaling is an evolutionarily conserved mechanism that regulates a broad spectrum of cell-specification through local cell-cell interaction. The homozygous mutant flies of *pecanex* (*pcx*) are viable, but *pcx* homozygous females mated with the *pcx* mutant males produce embryos that show an *N*-like neurogenic phenotype, suggesting that *pcx* encodes a component

of N signaling. Pcx is a multi-pass membrane protein. However, its biochemical functions are still unknown.

Here we established that Pcx is a component of the N-signaling pathway. Pcx was required upstream of activated form of N, probably in N-signal-receiving cells, suggesting that *pcx* is required prior to or during the activation of N. We found that Pcx was an endoplasmic reticulum (ER) residential protein. In addition, ER was enlarged in the embryos homozygous for *pcx* lacking its maternal contribution. However, such ER enlargement was not observed in embryos homozygous for *N* or *Presenilin*. These results suggest that the ER enlargement is not due to the disruption of N signaling.

Hyper-induction of the unfolded protein response (UPR), by the expression of activated *Xbp1* or dominant-negative *Heat-shock cognate 70-3*, suppressed the neurogenic phenotype and ER enlargement caused by the absence of *pcx*. A similar suppression of these phenotypes was increased by the overexpression of *O-fucosyltransferase 1*, an N-specific chaperon. Taking these results together, we speculate that the reduction of N signaling in embryos lacking *pcx* function might be attributable to defective ER functions, which are compensated for by up-regulation of the UPR and possibly by enhancing N folding.

220A

UIF, a large transmembrane protein with EGF-like repeats, can antagonize Notch signaling in *Drosophila*. Hongtao Zhang[1,2], Gengqiang Xie[1,4], Jun Ma[1,3], Renjie Jiao[1]. 1) State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, the Chinese Academy of Sciences, Beijing, China; 2) Graduate School of the Chinese Academy of Sciences, Beijing, China; 3) Divisions of Biomedical Informatics and Developmental Biology, Cincinnati Children's Research Foundation, Cincinnati, OH, USA; 4) Department of Biological Science, The Florida State University,Tallahassee, FL 32306, USA.

Notch signaling is a highly conserved pathway in multi-cellular organisms ranging from flies to humans. The diversity, specificity and sensitivity of the Notch signaling output are regulated at distinct levels, particularly at the level of ligand-receptor interactions. Here, we show that the *Drosophila* gene *uninflatable* (*uif*), which encodes a large transmembrane protein with eighteen EGF-like repeats in its extracellular domain, can antagonize the canonical Notch signaling pathway. Overexpression of Uif causes Notch signaling defects, which can be rescued by Notch target gene expression. Further experiments suggest that overexpression of Uif inhibits Notch signaling *in cis* and acts at a step that is dependent on the extracellular domain of Notch, which suggest that Uif can alter the accessibility of the Notch extracellular domain to its ligands during Notch activation. However, *uif* loss-of-function did not reveal any detectable phenotypes that are reminiscent of Notch activation. Nevertheless, a wing cell size reduction upon Uif depletion indicates that Uif may have a role in the control of cell growth. We further demonstrate that the intracellular domain of Uif is responsible, to a large extent, for its role in cell size control. Further investigations combining genetic and biochemical approaches are in progress to shed light on how Uif controls cell growth.

221B

Interaction between juvenile hormone and insulin/IGF-like signaling mediates lipid homeostasis during lactation in the tsetse fly, Glossina morsitans. Aaron A. Baumann[1], Joshua B. Benoit[2], Veronika Michalkova[2], Paul Mireji[3], Geoffrey M. Attardo[2], John K. Moulton[4], Thomas G. Wilson[5], Serap Aksoy[2]. 1) HHMI Janelia Farm Research Campus, Ashburn, VA; 2) School of Public Health, Yale University, New Haven, CT; 3) Department of Biochemistry and Molecular Biology, Egerton University, Njoro, Kenya; 4) Department of Entomology and Plant Pathology, University of Tennessee, Knoxville TN; 5) Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH.

Juvenile hormone (JH) mediates reproductive maturation in most insects, acting via the bHLH PAS transcription factor MET. The Drosophila genome contains both Met and its paralog Gce, genes with similar function in development but not in reproduction. Similarly, annotation of the tsetse (Glossina morsitans) genome revealed distinct Met and Gce orthologs. Tsetse flies employ viviparous reproduction, in which females nourish a developing intrauterine larva with a protein- and lipid-rich milk secreted from a modified accessory gland. We examined roles for Met, Gce, and insulin signaling (IIS) during the lactating and dry (non-lactating) stages of tsetse pregnancy, using a combination of hormone application and siRNA-mediated gene suppression. Perturbing either the JH or IIS pathway interfered with lipid homeostasis that is critical for tsetse lactation, suggesting JH/IIS interaction in this physiology. Specifically, siRNA reduction of Met but not Gce expression resulted in 1) elevated expression of the lipase bmm, a FOXO target gene, and 2) reduced expression of the class II histone deacetylase HDAC4, a FOXO modulator. Met reduction diminished fecundity and reduced stored lipids, similar to phenotypes obtained via knockdown of the JH biosynthetic enzyme JHAMT and inverse of phenotypes resulting from insulin or JH treatment. These phenotypes suggest that manipulation of JH/IIS pathways can prolong dry periods of the tsetse pregnancy cycle by promoting lipid storage in the fat body.

222C

The Male Accessory Gland: A novel model to evaluate new ER stress genes. Clement Y. Chow, Andrew G. Clark, Mariana F. Wolfner. Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY.

The endoplasmic reticulum (ER) is a large organelle that is responsible for synthesis, maturation, and delivery of a variety of proteins essential for cellular function. ER dysfunction occurs when misfolded proteins accumulate in the ER lumen, causing ER stress. The cell responds to ER stress with the "unfolded protein response" (UPR). The UPR can return the ER to homeostasis by attenuating protein synthesis, activating transcriptional signaling cascades, and refolding or degrading misfolded proteins in the ER. ER stress can be a primary cause or secondary effect of many human diseases. Drosophila is an

ideal, if underutilized genetic model with which to dissect the conserved ER stress response. In a previous screen of the DGRP, we found a large number of genes contributing to genetic variation in ER stress response in Drosophila. Over 50% of the genes we found had no previously known function in ER stress response. Additionally, ~50%; of these putative ER stress genes were essential for viability. To further characterize the function of these new genes in the ER stress response, we developed an *in vivo* system. We use the male accessory gland (AG) as our assay system. This AG synthesizes and secretes numerous proteins that are transferred to the female during mating. Because of its large secretory role, the AG requires optimal ER function. Indeed, the AG has the highest basal expression of genes that are upregulated under ER stress conditions. We subjected the AG to ER stress by locally expressing a misfolded rhodopsin or by ex vivo treatment with tunicamycin. Both ER-stress-inducing treatments impaired AG function: accessory gland protein production was reduced, as was male fertility. Our results identified a set of phenotypic, transcriptional, and translational markers indicative of ER stress in the AG. We show that these markers accurately predict ER stress when known ER stress genes such as BiP are perturbed in an AG-specific manner. Thus, the AG will be a useful and quantitative model for efficiently testing the novel ER stress genes identified in our variation studies.

223A

Phosphatidylinositol Synthase regulates the polarized deposition of basement membrane components. Olivier Devergne, Trudi Schüpbach. Department of Molecular Biology, HHMI/Princeton University , Princeton, NJ.

Epithelial cells are characterized by their polarized architecture that enables them to exert their varied functions in embryonic and adult organisms. Epithelia exhibit a profound apical-basal polarity that is manifested in the cytoplasmic and surface organization of individual cells. Loss of apical-basal polarity is often associated with tumor metastasis. The establishment and maintenance of polarity relies on the regulated transport of newly synthesized and recycled proteins to these specific domains. The basement membrane (BM), a specialized sheet of the extracellular matrix contacting the basal side of epithelial tissues, has a major role in the establishment and maintenance of epithelial cell polarity. However, little is known about how BM proteins themselves achieve a polarized distribution. An attractive model system for the study of epithelial structure and morphogenesis is the follicular epithelium, which envelops the germline during Drosophila oogenesis. To unravel the molecular mechanism regulating the polarized deposition of the BM, we previously performed a genetic screen in which we identified *Crag*, a DENN domain containing protein, as a regulator of polarized BM secretion (1). We recently isolated a new gene involved in this process, *pis*, encoding Phosphatidylinositol Synthase, which has a critical role in phosphatidylinositol 4,5-bisphosphate (PIP2) regeneration after its hydrolysis into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) by Phospholipase C (PLC). PIP2 regulates many cellular functions, such as intracellular trafficking and membrane and ion transport. Significantly, in follicular cells mutant for *pis*, BM components are secreted at the apical side of the epithelium leading to the formation of an “apical” BM. This defect is not generally observed in mutants affecting epithelial polarity. However, apical, junctional and basolateral polarity is not affected. Altogether, our data indicate a specific role for *pis* in the organization of epithelial architecture by regulating the polarized deposition of BM components. (1) Denef et al., 2008.

224B

Characterization of cytoplasmic Eyes absent function in Drosophila eye development. Charlene Hoi, Wenjun Xiong, Fangfang Jiang, Ilaria Rebay. University of Chicago, Chicago, IL.

Eyes Absent (Eya) is a dual-function transcription factor (TF) and protein tyrosine phosphatase (PTP) that lies at the center of the retinal determination gene network which is essential for Drosophila eye development. Eya’s two functions are spatially separated by the non-receptor tyrosine kinase, Abelson (Abl), which phosphorylates Eya to relocalize it from the nucleus, where it regulates eye specification at the level of transcription, to the cytoplasm, where it directs photoreceptor morphogenesis. Although both activities of Eya are necessary for eye development, understanding of Eya’s contribution to retinal development have mostly centered on its role as a TF. To gain insight into cytoplasmic signaling pathways that Eya may be involved with, we performed a genetic screen of phosphotyrosine signaling networks based on Src Homology 2 (SH2) and Phosphotyrosine binding (PTB) domains and identified four Jak/Stat components: hopscotch (Jak), stat92E (Stat), socs36E and socs44A. Biochemical and genetic assays confirm that Eya interfaces with Jak/Stat signaling members, however further studies need to be done to fully understand the biological implications of these interactions and their mechanisms. Given Eya's unique dual-functionality and dynamic cellular localization, we suspect that it may be pivotal in integrating information from multiple signaling pathways during development. Unraveling Eya's relationship with the Jak/Stat pathway will hopefully increase our insight into Eya’s broader role as a hub of signaling cross-talk during development.

225C

Vesicle trafficking during wing margin development: a role for Docked. Suresh K. Kandasamy, Justin Thackeray. Biology Dept, Clark University, Worcester, MA.

We show that the previously described gene *docked* (*doc*) corresponds to the gene model *CG5484*. The gene encodes a homolog of yeast Yif1, which is known to play a key role in transport of vesicles between the ER and Golgi. A viable allele, doc^{1}, shows a truncated wing phenotype very similar to that seen in the "oblique" class of *dumpy* (*dp*) alleles, and we find that there is a synergistic interaction between alleles of *doc* and *dp*. We observed genetic interactions between *doc* alleles and those of genes encoding the COPII vesicle components Sec13, Sec23 and Sar1, as well as the SNARE protein Syntaxin1A, strongly suggesting a role for Doc in trafficking of COPII vesicles. Loss of Doc function in the wing margin using a UAS-RNAi construct

produced wing nicks; this nicking is rescued by over-expression of Serrate or Delta, suggesting that the nicks are due to reduced trafficking of these transmembrane ligands. We also investigated whether the oblique wing phenotype observed in *doc*^{1} flies is due to reduced trafficking of Dumpy; we found that the wing phenotype seen in *dp*^{D} heterozygotes is indeed enhanced by several of the same vesicle trafficking mutants described above that interact with *doc*. It remains unclear why the oblique wing phenotype is the only visible defect observed in *doc*^{1}. One possible explanation we pursued is that this is because Dp is so large (estimated at 2.5MDa) it is especially sensitive to reduced efficiency in vesicle trafficking. However, we could observe no interaction between *doc*^{1} and alleles of several other genes encoding very large proteins, such as *sallimus* and *mucin14A*.

226A

Phosphoproteomic analysis of *Drosophila* embryos deficient in neural-specific glycosylation. Varshika Kotu[1,2], Peng Zhao[1,3], Toshihiko Katoh[1], Lance Wells[1,2,3], Michael Tiemeyer[1,2]. 1) The Complex Carbohydrate Research Center, University of Georgia, Athens, GA; 2) The Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA; 3) The Department of Chemistry, University of Georgia, Athens, GA.

Appropriate glycan expression is essential for development and normal tissue function. However, a complete mechanistic understanding of the pathways regulating glycoprotein glycosylation is lacking. A family of structurally related N-linked glycans known as HRP epitopes are specifically expressed in *Drosophila* neural tissue, providing a platform to understand the regulatory mechanisms controlling tissue-specific glycosylation. We previously generated and characterized a *Drosophila* mutant called *sugar-free frosting (sff)* which affects HRP-epitope expression in the embryonic nervous system. The *sff*mutation mapped to the *Drosophila* homologue of a serine/threonine kinase known as SAD-1 in other invertebrate and vertebrate species. In mid-stage *Drosophila* embryos, confocal analysis demonstrated that the *sff* mutation alters Golgi compartmental distributions such that glycoprotein glycosylation is shifted in favor of greater glycan complexity and decreased HRP-epitope expression. In order to further characterize the molecular mechanisms underlying altered neural glycan expression, we have undertaken differential phosphoproteomic analysis of *OreR* and *sff* mutant embryos. By LC-MS/MS, we identified phosphoprotein serine/threonine phosphorylation sites that were utilized in wild-type but not detected in the *sff* mutant and undertook the validation of these proteins as Sff/SAD kinase substrates by orthogonal approaches. Detection of genetic interaction with our mutant *sff* allele nominated three phosphoproteins (Bifocal, Rasputin and Liprin-alpha) as candidate substrates for Sff/SAD kinase in relation to glycoprotein glycosylation. Our on-going efforts are directed towards understanding the functional significance of these identified phosphoproteins and of Sff/SAD kinase signaling in the context of neural specific glycosylation.

227B

Dynamic feedback shapes steroid pulses in Drosophila. Morten E. Møller[1], E. Thomas Danielsen[1], Rachel Harder[2], Michael B. O'Conner[2], Kim F. Rewitz[1]. 1) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 2) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, Minnesota.

Pulses of steroid hormones act as temporal signals that drive the juvenile-adult transition, which transforms the developing organism to a reproductively mature adult. This transition, known as metamorphosis in Drosophila, is triggered by pulses of the steroid hormone ecdysone produced and released from the prothoracic gland (PG). Ecdysone is synthesized in response to prothoracicotropic hormone (PTTH) release from the brain through a series of enzymatic reactions mediated by P450 enzymes. Although PTTH stimulates ecdysone synthesis, that alone cannot account for mechanisms determining the duration of the pulse. We show an ecdysone-dependent feedback switch in the PG which is required for the rapid increase and following decline of the ecdysone titer. This switch consists of a feedforward and a feedback loop. Blocking the feedforward loop in the PG results in reduced levels of ecdysone and delayed puparation. The negative feedback is responsible for the following decline of the titer, together these processes are required for generating a "pulse" that drives developmental progression. The feedback is mediated through the ecdysone receptor (EcR) that induces the expression of a transcription factor called Broad, which regulates the expression of the ecdysone biosynthetic enzymes by binding onto their promoters. Different Broad isoforms are responsible for the transcriptional activation and repression that changes the capacity of the PG to produce ecdysone. In conclusion: These findings demonstrate a feedback mechanism in the PG involving EcR and Broad, which is required to establishes the temporal boundaries of the ecdysone pulse and developmental transition to adulthood.

228C

Negative Regulation of the Folded gastrulation Signaling Pathway by the Non-visual β-arrestin Kurtz. Emily J. Simon, Alyssa J. Manning, Stephen L. Rogers. Dept Biology, Univ North Carolina-Chapel Hill, Chapel Hill, NC.

Epithelial morphogenesis is an important developmental process that underlies gastrulation and formation of tissues and organ systems in *Drosophila* as well as human development. We are using *Drosophila*as a model to study epithelial apical constriction during mesoderm invagination and imaginal wing disc development. This process is regulated by the Folded gastrulation (Fog) pathway. Recently our lab identified Mist, a G-protein coupled transmembrane receptor, which is activated by the ligand Fog and which downstream triggers cell contraction. Currently how this pathway is inactivated or regulated is not understood. We hypothesize that Mist inactivation is mediated by a β-arrestin. In our model, GPRK2 phosphorylates Mist on its C-terminal cytoplasmic domain causing recruitment of a β-arrestin/*Drosophila*Kurtz, which contributes to termination of the contraction signal. Through immunoprecipitation we have shown that Mist interacts with Kurtz. We are using a novel

cell culture assay to study this pathway.*Drosophila* S2R+ cells undergo a dramatic acto-myosin based contraction upon application of exogenous Fog protein. Measuring the percentage of S2R+ cells contracted over time following Fog application shows that the percent contracted plateaus after about 5 minutes. Consequent washout of Fog results in nearly complete relaxation of cells within 30 minutes. Data thus far show that cells overexpressing Kurtz exhibit a reduction in the percentage of contracted cells as compared to wild type cells after Fog treatment for a fixed time period. Conversely, Kurtz knockdown via RNAi results in an increased percentage of contracted cells. These experiments support our model for Mist desensitization and suggest that Kurtz is active during Fog pathway attenuation. We will use our cell culture assay to examine percentage of cellular contraction and relaxation after other perturbations to the pathway, such as alteration of Kurtz and GPRK2 levels. Thus our data demonstrates that Kurtz is a key component in the regulation of this cell contraction pathway via interaction with the Mist receptor.

229A

Mechanism and function of the capa/capaR in the desiccation stress response in Drosophila. Selim Terhzaz, Pablo Cabrero, Louise Henderson, Julian A.T. Dow, Shireen-A. Davies. Institute of Molecular Cell and Systems Biology, University of Glasgow, Glasgow, United Kingdom.

Drosophila species occur in a wide range of habitats, including hot and dry conditions. Their ability to survive desiccation stress has been studied and the enhanced desiccation resistance in Drosophila is the result of reduced rates of water loss. Although the major routes for water loss are being through the cuticle and the spiracles, the excretory water loss involving the Malpighian renal tubules and hindgut makes a significant contribution to the total water loss in desiccated fruit flies. Fluid secretion by the Malpighian tubules of insects is under elaborate neuropeptide control, which modulates appropriate cell signaling and ion transport pathways. The endogenous D. melanogaster capa neuropeptides (Drm-capa-1 and -2) increase fluid transport by adult Malpighian tubules of Drosophila. Capa-1 and capa-2 act via elevation of intracellular calcium and nitric oxide/cGMP signaling, in tubule principal cells. We recently demonstrated the kinetics of capa-1-induced activation and desensitisation of its cognate G-protein coupled receptor, capaR. CapaR is highly expressed in tubules and plays a role in desiccation stress resistance for the whole fly. CapaR gene expression in tubules is reduced under desiccation stress, whilst tubules from desiccated flies show markedly inhibited basal and capa-1-stimulated rates of fluid transport. Capa peptide amounts in capa-expressing peptidergic Va neurons are increased in response to desiccation; and capa gene expression is increased by exposure of flies to desiccation or to high salt. Precise spatial targeting of capa RNAi to the Va neurons caused increased survival of whole flies to water stress, both for desiccation (water deficiency) and osmotic (high salt) stress but did not reveal a phenotype in response to starvation, oxidative or immune stress. Taken together, the capa/capaR signalling acts in the key fluid-transporting tissue to regulate responses to desiccation stress in the fly.

230B

Chmp1 may negatively regulate DER and Notch signaling. Meagan Valentine, Simon Collier. Dept Biomedical Sciences, Marshall University, Huntington, WV.

Chmp1 is a component of ESCRT-III, a conserved protein complex required for degradation of activated membrane receptors. The ESCRT complexes downregulate many pathways involved in development and growth, and several components, Chmp1 included, have been linked to human cancer. Chmp1 has not been studied in *Drosophila*, so we are investigating Chmp1 function with knockdown and over-expression. Our results suggest that Chmp1 negatively regulates Epidermal Growth Factor Receptor (DER) and Notch signaling. We used VDRC and TRiP RNAi fly lines to knock *Chmp1* down in the *Drosophila* wing. The result was wider wing veins. Since the DER pathway regulates wing vein size, we tested for interactions with both positive and negative regulators of DER signaling. Our results suggest that Chmp1 negatively regulates DER signaling. We are evaluating the effect of *Chmp1* knockdown on expression of Blistered (Bs), which is negatively regulated by DER signaling, by generating clones of *Chmp1* knockdown in the wing. Preliminary results suggest that *Chmp1* knockdown reduces Bs, supporting the conclusion that Chmp1 negatively regulates DER signaling. We created fly lines to over-express Chmp1 or His-Myc-tagged Chmp1 (HM-Chmp1). Both of these lines display activity, as they can partially rescue the *Chmp1* knockdown phenotype. Over-expression of Chmp1 in the wing results in wing vein deltas, suggesting that Notch signaling may be altered as well. Notch signaling affects wing vein size, so we are testing for interactions between *Chmp1* knockdown and Notch. So far, it seems that *Chmp1* knockdown reduces the frequency of notches normally observed with the hypomorphic N^{55e11} allele, suggesting that Chmp1 negatively regulates Notch signaling. We are also using HM-Chmp1 to investigate Chmp1 localization. HM-Chmp1 localizes apically and to the membrane in multiple *Drosophila* tissues. Overall, our results suggest that Chmp1 negatively regulates Notch and DER signaling. Likely, the role Chmp1 plays in growth is at least in part due to regulation of DER and Notch signaling through its involvement in ESCRT function.

231C

The Frizzled-dependent planar polarity pathway locally promotes E-cadherin turnover via recruitment of RhoGEF2. Samantha J. Warrington, David Strutt. University of Sheffield, Sheffield, United Kingdom.

Polarised tissue elongation during morphogenesis involves cells within epithelial sheets or tubes making and breaking intercellular contacts in an oriented manner. Growing evidence suggests that cell adhesion can be modulated by endocytic trafficking of E-cadherin (E-cad), but how this process can be polarised within individual cells is poorly understood. The Frizzled (Fz) dependent core planar polarity pathway is a major regulator of polarised cell rearrangements in processes such

as gastrulation, and has also been implicated in regulation of cell adhesion through trafficking of E-cad, however it is not known how these functions are integrated. We report a novel role for the core planar polarity pathway in promoting cell intercalation during tracheal tube morphogenesis in Drosophila embryogenesis, and present evidence that this is due to regulation of turnover and levels of junctional E-cad by the guanine exchange factor RhoGEF2. We further show that core pathway activity leads to planar polarised recruitment of RhoGEF2 and E-cad turnover in the epidermis of both the embryonic germband and the pupal wing. We thus reveal a general mechanism by which the core planar polarity pathway can promote polarised cell rearrangements.

232A

Nicotinamide Mononucleotide Adenylyltransferase (NMNAT) Maintains Active Zone Structure by Stabilizing Bruchpilot. Shaoyun Zang[1], Yousuf O. Ali[2], Ruan Kai[1], R Grace Zhai[1]. 1) University of Miami,1600 NW 10 Ave. R.M.S.B. Bldg. 6068, Miami, FL 33136; 2) Baylor College of Medicine, Jan and Dan Duncan Neurology Institute, 1250 Moursund, Houston TX 77025.

Active zones are highly specialized presynaptic sites for synaptic vesicle docking and fusion. Such efficient and precise neurotransmission relies on the structural integrity of active zones. However, the mechanism for maintaining the structural integrity of active zones is largely unknown. Chaperones have been implicated in synaptic function and it is likely that molecular chaperones, the primary machinery that maintains cellular protein homeostasis, play a role in facilitating the redistribution of synaptic proteins and maintaining synaptic structural integrity during neuronal activity. We examined the role of a newly identified chaperone NMNAT (nicotinamide mononucleotide adenylyltransferase) in active zone maintenance. Our previous work has shown that NMNAT is a neuroprotective factor required for maintaining neuronal integrity, including active zone integrity and the neuroprotective ability of NMNAT was attributed partly to its chaperone function. Enzyme-inactive NMNAT rescues active zone degeneration in nmnat null background, suggesting that the chaperone function of NMNAT is sufficient to maintain active zone structure integrity. We directly examined the specific role of NMNAT at the synapse, and identified a novel mechanism of active zone maintenance by NMNAT in which it stabilizes the primary active zone structure protein Bruchpilot (BRP). Loss of NMNAT induced a significant reduction in synaptic BRP levels, leading to accumulation of ubiquitinated BRP, clustering with stress-induced Hsp70 chaperone and a surprising redistribution of BRP from the synapse to the cell body, resulting in the subsequent degeneration of active zones. Moreover, we show that NMNAT interacts with BRP biochemically in an activity-dependent manner. Our findings suggest that NMNAT functions to stabilize BRP and shield it from activity-induced ubiquitin-proteasome-mediated protein degradation, thereby maintaining active zone structural integrity during neuronal activity.

233B

Regulation of cell migration during dorsal appendage morphogenesis. Sandra G. Zimmerman, Celeste A. Berg. Department of Gemone Sciences, University of Washington, Seattle, WA.

Cell motility is critical for normal development and homeostasis. Abnormalities in these processes can produce birth defects or drive cancer cell metastasis. An excellent model for studying the regulation of cell migration is dorsal appendage (DA) morphogenesis in the ovary of *Drosophila melanogaster*. The DAs form from two patches of follicle cells that lie dorsal to the oocyte; these cells reorganize into tubes and elongate by crawling over the squamous “stretch” follicle cells, which lie over the nurse cells. Mutations in the transcription factor Bullwinkle (BWK) lead to cell adhesion defects and aberrant cell migration, resulting in broad, moose-antler-like DAs. BWK, which functions in the nurse cells, acts upstream of tyrosine kinases SHARK and SRC42A in the overlying somatic stretch cells to regulate DA cell migration. Interestingly, *shark* RNA localizes in the stretch cells in patches over the nurse cell nuclei, perhaps to localize SHARK translation. This *shark* mRNA distribution, which is absent in *bwk* egg chambers, may localize SHARK protein, possibly to facilitate phosphorylation activity. A major unanswered question is: what are the other components of this pathway? To identify new factors that regulate cell migration through their function in the BWK-SHARK-SRC42A pathway, we used liquid chromatography coupled with tandem mass spectrometry and label-free quantitation to compare protein expression and phosphorylation in stretch cells from wild-type vs. *bwk* egg chambers. To purify stretch cells, we adapted a published protocol for magnetic bead cell separation for a new use with mass spectrometry. We identified >100 proteins with at least a 2-fold difference in relative abundance between wild-type and *bwk* egg chambers. We selected a small subset of the most interesting of these candidate proteins for *in vivo* functional analysis using RNAi, protein and RNA expression analysis, and clonal analysis. Characterization of these newly identified factors will delineate their function in the BWK-SHARK-SRC42A pathway and advance our understanding of the regulation of cell migration.

234C

Role of Dachs localization and ATPase activity in Fat signaling. Abhijit A Ambegaonkar, Cordelia Rauskolb, Kenneth Irvine. Waksman Institute of Microbiology, Rutgers, the State University of New Jersey, Piscataway, NJ.

Dachs, a myosin family protein, is a downstream effector of Fat signaling pathway that regulates planar cell polarity (PCP) in *Drosophila*. Dachs also interacts with Zyxin, a LIM domain protein which is a component of Hippo signaling pathway that regulates growth. Both Dachs and Zyxin are localized in the sub-apical region of the cell. However, Dachs is polarized towards the distal side of the cell membrane whereas Zyxin is present around the entire circumference of the cell. Earlier studies have identified a correlation between Dachs localization and Fat signaling. To confirm the importance of Dachs membrane

localization and distinguish it from other potential influences of Fat, we targeted Dachs to the membrane independent of Fat activity by fusing Dachs to Zyxin. When expressed under UAS control, the Zyxin-Dachs fusion protein was observed to localize around the entire circumference of the cell. Zyxin-Dachs overexpression resulted in strong overgrowth and upregulation of Hippo pathway target genes. Moreover, wing hair polarity and cell division orientation was randomized, indicating that PCP is disrupted. These results confirm that Dachs localization is sufficient for both PCP and Hippo signaling. We have also tested Zyxin-Dachs fusion protein with mutation in Dachs ATPase site (Zyxin-Dachs^{R424E}), which would abolish its motor activity. Expression of Zyxin-Dachs^{R424E} resulted in overgrowth, but PCP was not affected. These results suggest that Dachs myosin motor activity is required for its effect on PCP but not for Hippo signaling.

235A

The transcriptional TOR and AMPK target sugarbabe regulates amino acid and lipid catabolism. Torsten Buelow, Katrin Riemschoss, Ingo Zinke, Michael J. Pankratz. Molecular Brain Physiology and Behavior, LIMES Institute, University of Bonn, 53115 Bonn, Germany.

Varying availability and composition of food make it necessary for organisms to adapt their metabolism in order to find a balance between growth, maintenance and autophagy. Two key sensors gathering information about the internal nutrient resources are the protein kinases TOR (target of rapamycin) and AMPK (AMP-activated protein kinase). These sensors form a signaling network that combines energy status and amino acid availability.Over the last decade, much knowledge has been gained on the regulation of these metabolic core components. Far less is known about the genetic program and its components downstream to these key sensors that coordinate basic biochemical processes. The nutrient dependent transcription factor sugarbabe is expressed in the fat body and gut of Drosophila larvae, and is strongly upregulated upon amino acid starvation but not complete starvation. Here we show that sugarbabe is a transcriptional target of TOR and AMPK signaling and mediates metabolic adaptation to nutrient conditions by repressing genes of both amino acid and lipid catabolism. We altered TOR and AMPK activity by genetic and pharmaceutical means and found sugarbabe to be repressed by both protein kinases, leading to derepression of its target genes. Our results show an example where nutrient information is processed from its sensors to transcription factors that control specific biochemical pathways.

236B

Investigating the Role of PI4P in Lysosome-related Organelle Biogenesis in the *Drosophila* Eye. Lauren M. Del Bel[1,2], Ronit Wilk[1], Jason Burgess[1,2], Gordon Polevoy[1], Ho-Chun Wei[1], Julie Brill[1,2]. 1) Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Phosphatidylinositol (PI) 4-phosphate (PI4P) is an essential membrane lipid within cells. PI4P is mainly present at the Golgi where it recruits regulators of intracellular trafficking. In *Drosophila melanogaster*, the type II PI 4-kinase (PI4KII) is one of the kinases responsible for generating PI4P at the Golgi, while a single phosphatase (Sac1) keeps PI4P levels in check. By generating Drosophila mutants, our lab has discovered that PI4KII and Sac1 together control a pool of PI4P that is critical for eye pigmentation. We have found that *sac1* and *PI4KII* mutants have reduced eye pigment levels, due to an altered number and distribution of eye pigment granules. *Drosophila* eye pigment granules are a type of lysosome-related organelle (LRO), which is a specialized membrane-bound compartment within specific cell-types. LROs are generated by specialized protein sorting and membrane trafficking, which is carried out by intracellular trafficking complexes, such as the clathrin Adaptor Protein complex 3 (AP-3). We have found that *PI4KII* and *sac1* mutants genetically interact with other trafficking complex mutants, such as those affecting AP-3. Indeed, we have found that Sac1 and PI4KII are required for proper distribution of the AP-3δ subunit Garnet in the *Drosophila* retina. This evidence suggests a novel role for PI4P in pigment granule formation.

237C

Investigating Expanded localization and binding partners. Leonie Alexandra Enderle, Robyn Rosenfeld, Vladimir Belozerov, Helen McNeill. Research, SLRI, Toronto, Ontario, Canada.

The Hippo kinase pathway plays an important role in growth regulation during Drosophila development and is highly conserved between species. It is well known how the core pathway, consisting of the kinases Hippo and Warts and the adaptor proteins Salvador and Mats, negatively regulates the transcriptional coactivator Yorkie. Upstream regulation of the Hippo pathway, however, is less well understood. One upstream regulator is the FERM-domain protein Expanded which interacts with different components of the Hippo pathway at several levels. Expanded localizes to apical junctions where it can bind Yorkie to prevent it from entering the nucleus. Further, Expanded protein levels seem dependent on the correct localization of Expanded in the epithelium. We therefore seek to understand the mechanisms that regulate the apical and junctional localization of Expanded and their biological function. We are using the Drosophila larval eye imaginal disc to study Expanded localization in epithelia. We have investigated the behavior of different Expanded protein truncations and identified two distinct regions in the Expanded C-terminus that are required for junctional localization. Apical and junctional localization is regulated separately since truncations missing one or both of the identified domains were still enriched apically. Finally, we are performing Affinity Purification coupled to Mass Spectrometry with Expanded from cell lines and Drosophila embryonic tissue to identify novel binding partners.

238A

Endocytotic vacuolation and vacuole acidification act in concert during early-to-mid prepupal development of

Drosophila salivary glands. Robert Farkas[1], Denisa Benova-Liszekova[1], Zuzana Datkova[1,2], Daniel Vlcek[2], Milan Beno[1,2], Ludmila Pecenova[1,2], Otakar Raska[3], Pavel Juda[3], Lubos Kovacik[3], Ivan Raska[3], Bernard Mechler[3,4]. 1) Inst Experimental Endocrinology, Slovak Academy Sciences, Vlarska 3, 83306 Bratislava, Slovakia; 2) Department of Genetics, Faculty of Science, Comenius University, Bratislava, Slovakia; 3) Institute of Cellular Biology and Pathology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; 4) Department of Developmental Genetics, Deutsches Krebsforschungszentrum-ZMBH Allianz, Heidelberg, Germany.

Larval salivary gland (SG) disintegration occurs 14-16 hr after puparium formation and is induced by the ecdysone hormone produced at the end of the larval development. Early during prepupal development, the cytoplasm of the SG cells undergoes an intense vacuolation resulting from endosomal recycling accompanied by strong acidification of the vacuoles. Here we show that vacuolation can be precociously initiated in late wandering third instar larvae by expressing transgenes for shi, syntaxins, Rab5, and Rab11. However, by comparison to prepupal vacuoles the larval vacuoles were poorly acidified. By manipulating vhaSFD, vha13, vha100.1, vha55, vha 26, and vhaAC39-1 we were able to show that the acidification of small basally-derived endosomes was distinct from the acidification of large apically-derived endosomes. Moreover we obtained evidence that the optimal and evenly distribution of acidified endosomes resulted from regulated fusion between large apical and small basal endosomes, which carry metabolic iron. These data indicate that before implementation of program cell death the SG cells are highly active in endosomal trafficking to provide resources for anabolic reactions. (Supported by the GACR grants P302/11/1640 and P302/12/G157, grants from Charles University UNCE 204022 and Prvouk/1LF/1, VEGA 2/0170/10, EEA & NFM Norwegian Fund # SK-0086/3655/2009/ORINFM).

239B

The interplay between TNF signaling, apoptosis, and tissue damage-induced pain sensitization in *Drosophila* larvae. Juyeon Jo[1], Felona Gunawan[2], Daniel Babcock[1], Michael Galko[1]. 1) UT M.D. Anderson cancer center, Housotn, TX; 2) Rice University.

Nociception is the detection of painful stimuli and is a fundamental protective mechanism to prevent potential tissue damage. Recently we established a novel nociceptive sensitization model using *Drosophila*larvae where tissue damage induced by UV radiation results in both apoptotic epidermal cell death and thermal allodynia, or aversive withdrawal to previously innocuous temperatures. Although TNF signaling and apoptotic cell death were previously correlated in the development of allodynia it is not yet known whether TNF-mediated induction of allodynia functionally requires apoptosis or other canonical downstream members of the TNF signaling pathway. To clarify the functional relation between UV-induced apoptosis and allodynia, components of the canonical cell death pathway both upstream and downstream of the initiator caspase Dronc were knocked down in the epidermis and both cell death and UV-induced thermal allodynia were measured. Surprisingly, we found that only Dronc knockdown was capable of blocking allodynia (whereas all knockdowns blocked epidermal apoptosis). Therefore, we suggest that Dronc has a non-apoptotic function in the induction UV-induced allodynia. This conclusion is supported by the fact that Dronc is required for the ectopic allodynia caused by TNF misexpression in nociceptive sensory neurons, a context where no apoptotic cell death accompanies sensitization. When we tested possible downstream mediators of TNF signaling by nociceptive sensory neuron-specific RNAi knockdown we found that the kinase p38, the adaptors TRAF3 and TRAF6, and the rel-like transcription factor Dorsal are all required downstream of the TNF receptor, Wengen, for induction of UV-induced thermal allodynia. Our results reveal a surprising independence between TNF signaling and apoptosis in tissue damage-induced pain sensitization, suggest an apoptosis-independent role for Dronc in TNF production, and identify the conserved molecular architecture of downstream TNF signaling in a pain sensitization context.

240C

JNK Signaling Antagonism: The role of Raw during Drosophila dorsal closure. Molly C. Jud, Melissa Ratcliffe, Anthea Letsou. Human Genetics, University of Utah, Salt Lake City, UT.

A surprisingly small number of conserved signaling pathways are used in development, and their tight regulation is necessary for embryogenesis to occur normally. We study two sequentially acting signaling pathways necessary for dorsal closure in the fruit fly *Drosophila melanogaster*; the Jun-N-terminal kinase/AP-1 pathway (JNK/AP-1; MAPK family member) is specifically activated in leading edge (LE) epidermal cells and transcriptionally activates the Decapentaplegic pathway (Dpp; TGF-β family member). Of particular interest to our lab are antagonists of the JNK and Dpp signaling pathways, including the novel gene, *raw*, an antagonist of the JNK signaling pathway. *raw* is a member of a dorsal-open subgroup, known as the *raw* group; this group includes three other genes (*puckered*, *ribbon*, and *mummy*) and is characterized by three shared, loss-of-function phenotypes: (1) a dorsal closure defect observed as a dorsal hole or pucker, (2) hypotrophy of ventral denticle belts, and (3) ectopic *dpp* expression in the lateral epidermis beyond the LE. We have previously shown that *raw* is expressed broadly throughout embryogenesis and is required to suppress zygotic Basket (JNK)-independent Jun activity in embryos undergoing dorsal closure. We hypothesize that Raw functions to silence basal levels of epidermal JNK signaling and is therefore a master regulator in the complex circuitry of the developing Drosophila embryo. Here, we show biochemical evidence that activated phospho-Jun accumulates in *raw* and *raw bsk* mutant embryos relative to wild types. As Jun is active in *raw* embryos, even without zygotic *basket*, another kinase must be responsible for Jun activation in *raw* mutant embryos. Here we show: (1) *raw* is necessary to define a LE, (2) a maternal *basket*-encoded JNK likely activates Jun in the epidermis of *raw* mutants, and (3) multiple levels of JNK/Dpp antagonism are necessary to restrict *dpp* to LE cells.

241A

Lipid modification of secreted signaling proteins. Hui Hua Liu[1], Rayshonda Hardy[2], Steven Blais[1], Thomas Neubert[1], Marilyn Resh[2], Jessica Treisman[1]. 1) Kimmel Center for Biology and Medicine of the Skirball Institute, NYU School of Medicine, New York, NY; 2) Cell Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY.

Lipid modification of secreted proteins can modulate their trafficking, secretion, diffusion, or ability to bind and activate their receptors. Three signaling ligands of different families have been found to acquire lipid modifications that regulate their fates and functions. Hedgehog and Spitz are both palmitoylated on their N-terminal cysteine residues by the acyltransferase Rasp; we have shown that the recognition sequence for Rasp and its mammalian homologue Hhat lies within the first 10 amino acids of these proteins. A related acyltransferase, Porcupine, transfers two fatty acids to Wnt family members. We are investigating whether additional signaling proteins in different families can also be lipid modified. We expressed a set of secreted proteins in cultured cells and screened them for hydrophobicity using a detergent extraction assay. This screen identified members of the BMP and Activin families as potential candidates for lipid modification. We are focusing on Glass bottom boat (Gbb), a BMP7 homologue important for wing patterning, synapse growth and energy homeostasis. Both full-length Gbb and its mature secreted form appear more hydrophobic than predicted from their amino acid sequence. We are using mass spectrometric analysis of Gbb purified from a stable cell line to look for lipid modifications and identify their attachment sites.

242B

Spargel/ PGC-1 is the new terminal effector in the Insulin-Tor Signaling pathway. Subhas Mukherjee, Atanu Duttaroy. Biology, Howard University, Washington, DC.

Insulin and Tor signaling pathways converge to maintain growth so a proportionate body form is attained. Spargel is the Drosophila homolog of PGC-1, which is an omnipotent transcriptional co-activator in mammals. Spargel/PGC-1 is recognized for their role in energy metabolism through mitochondrial biogenesis. Some studies have indicated that spargel/PGC-1 is possibly involved in insulin-TOR signaling, although a comprehensive analysis is still lacking. Using genetic epistasis analysis, we demonstrated that spargel action is necessary for TOR and S6K to regulate cell size and cell growth in a cell autonomous manner, as well as the tissue-restricted phenotypes of TOR and S6K mutants are also rescued by spargel overexpression. We show that spargel overexpression sets back the mitochondrial numbers and increases ATP production, which helps the cells and tissue to attain normal size. With regard to its interaction with FoxO, an important player in the insulin-signaling pathway, excess spargel, can ameliorate the FoxO overexpression defects although at a limited capacity. We therefore conclude that spargel functions as a terminal effector in the insulin-TOR pathway and should be incorporated as a new member of this growth-signaling pathway.

243C

Acal, a new 'vessel' that negatively regulates JNK signaling. Luis Daniel Ríos-Barrera, Juan Rafael Riesgo-Escovar. Developmental Neurobiology Dept., Neurobiology Institute, Universidad Nacional Autónoma de México, Queretaro, Mexico.

The Jun N-terminal kinase (JNK) is part of a conserved signaling pathway that controls dorsal closure in the Drosophila embryo. Gain and loss of function conditions for the JNK pathway result in defects in dorsal closure, visible as dorsal holes in cuticle preparations. Here, we characterize a new 'dorsal open' gene named *acal* in JNK signaling. The *acal* transcription unit is conserved among arthropods; however its molecular function is unclear as it has no conserved open reading frames. By cellular fractionation and RT-PCR, it is present in the nucleus, and by Northern blot, the primary transcript is processed to fragments smaller than 100 pb. These results suggest a non-coding RNA. Mutations in *acal* are lethal and result in cuticular dorsal holes (hence its name, meaning 'boat' in the Nahuatl language). Mutant phenotype analysis by means of *puc-lacZ*, a reporter of JNK activity, revealed ectopic activation of the pathway. Similarly, heterozygosity for *basket*, the JNK gene, partially restored the *acal*homozygous phenotype, showing that *acal* inhibits JNK signaling during dorsal closure. *acal* is expressed in the epidermis during dorsal closure stages. Targeting *acal* expression to the ectoderm or to the lateral epidermis using the UAS-Gal4 system rescues the embryonic mutant phenotype. The expression pattern of *raw*, a negative regulator of JNK signaling during dorsal closure, is very similar to *acal*. Using in situ hybridization we found that epidermal *acal* expression disappears in *raw* mutants, suggesting *raw* acts upstream of *acal* during dorsal closure. We then turned our attention to thorax closure to study*acal* and *raw*. Thorax closure is a process analogous to dorsal closure during metamorphosis, also controlled by JNK signaling. Over-expression of *acal* or *raw* in the thorax using the UAS-Gal4 system results in a mild thoracic cleft phenotype. However, over-expression of both genes at the same time results in a significantly stronger phenotype. Taken together, our results show that *acal* is a novel negative regulator of JNK signaling downstream of Raw.

244A

The atypical cadherin Fat directly regulates mitochondrial function to control planar cell polarity and Hippo signaling. Anson D Sing[1,2], Yonit Tzatzkis[2], Maïlis Bietenhader[3], Lacramioara Fabian[4], Tasha Stoltz[3], Robyn Rosenfeld[1,2], Julie A Brill[1,4], G Angus McQuibban[3], Helen McNeill[1,2]. 1) Molecular Genetics, University of Toronto, Toronto, ON, Canada; 2) Samual Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; 3) Department of Biochemistry, University of Toronto, Toronto, ON, Canada; 4) Collaborative Program in Developmental Biology, Hospital for Sick Children, Toronto, ON Canada.

The cell adhesion molecule Fat (Ft) is a large cadherin that regulates both the growth restricting Hippo signaling pathway and a form of tissue organization known as planar cell polarity (PCP). How Ft coordinates these is unclear. We have found an

unexpected role for Ft in directly regulating mitochondrial function, and demonstrate a critical role for mitochondria in the regulation of PCP and Hippo signaling activity during *Drosophila* development. We show that the intracellular domain of Ft is cleaved to release a soluble fragment which contains multiple mitochondrial targeting sequences. This domain is imported into mitochondria, where it binds Ndufv2, a core component of mitochondrial Complex I that plays an important role in the regulation of reactive oxygen species (ROS). Loss of Ndufv2 leads to PCP defects in the eye and wing and increased expression of the PCP gene *four-jointed* as well as the downstream Hippo pathway targets, Crumbs and Expanded. Loss of Ft leads to increased ROS levels, and increased activity of the ROS target JNK. Importantly, expression of a mitochondrially targeted fragment of the Ft cytoplasmic domain reduces ROS and ATP levels. We propose that Ft import into mitochondria impacts Ndufv2-dependent mitochondrial function, which in turn signals further to coordinate Hippo pathway activity and PCP regulation.

245B

Control of lipid metabolism by gut Tachykinin hormones. Wei Song[1], Jan Veenstra[3], Norbert Perrimon[1,2]. 1) Department of Genetics, Harvard Medical School, Boston, MA 02115, USA; 2) Howard Hughes Medical Institute; 3) Université de Bordeaux, INCIA UMR 5287 CNRS, 33405 Talence, France.

The interaction between the digestive and central nervous system (CNS) - necessary to maintain energy homeostasis, coordinate feeding, digestion, and other metabolic activities - is mediated in part by a series of hormones produced by both enteroendocrine cells (EEs) of the gut and the CNS. In many instances, the same "brain-gut" hormones are expressed in both the CNS and EEs complicating their functional analyses. As a result, we know little about the function of hormones produced from the gut. Using cell type specific genetic manipulation in Drosophila, we demonstrate that Tachykinins (TKs), one of the most abundant "brain-gut" hormones produced by EEs, regulate gut lipid metabolism in enterocytes (ECs) through activation of a GPCR/PKA signaling pathway. Further, unlike the knockdown of TKs in the CNS, gut-derived TKs do not result in an abnormal olfactory responses or defects in locomotor activities, thus demonstrating that brain-gut hormones can have fundamentally different physiological functions. Our findings illustrate the importance of analyzing the roles of brain-gut hormones in a tissue-specific manner to fully appreciate their diverse roles in physiology.

246C

Dissecting the Fat/Dachsous pathway's role in planar cell polarity using chromatin immunoprecipitation to find targets of Atrophin. Kelvin Yeung[1,2], Helen McNeill[1,2]. 1) Research, Samuel Lunenfeld Res Inst, Toronto, Ontario, Canada; 2) Molecular Genetics, University of Toronto St. George Campus, Toronto, Ontario, Canada.

Planar cell polarity (PCP) is the phenomenon in which epithelial cells are polarized in the plane of the epithelium, orthogonal to the apicobasal axis. PCP is evident in several *Drosophila* tissues such as the orientation of hairs on the *Drosophila* wing and the proper rotation of photoreceptor clusters in the *Drosophila* eye. There are several signalling pathways that establish PCP; one of which is the Fat/Dachsous (Ft/Ds) signalling pathway. Atrophin (Atro, also known as Grunge) is a downstream component in the Ft/Ds pathway and Atro is a nuclear co-repressor. However the downstream target genes of Atro in the Ft/Ds pathway remain unknown. In order to identify Atro's target genes that play a role in PCP, we plan to use chromatin immunoprecipitation (ChIP) against Atro followed by microarray in developing*Drosophila* embryos. To assess the PCP role(s) of the potential Atro targets, we plan to check the wings and eyes of RNA interference and mutant flies for PCP defects. To perform an Atro ChIP, I made and tested an anti-Atro antibody. I used my antibody to perform ChIPs in *Drosophila* S2 cells. End point PCR and quantitative PCR results showed that the ChIPs were successful as a known target of Atro was enriched when compared with the negative controls. I also carried out an Atro ChIP in embryos and verified it with end point PCR.

247A

The effect adenosine receptor and adenosine transporter on energy homeostasis. Michal Zurovec, Roman Sidorov, Lucie Kucerova. Dept Physiology, Biology Centre, Inst Entomology, Ceske Budejovic, Czech Republic.

Adenosine (Ado) is an ubiquitous metabolite, which plays a prominent role as a paracrine signal of metabolic imbalance within tissues. We found that transport of extracellular adenosine into the cytoplasm stimulates ATP synthesis and induces catabolism of carbohydrates and lipids in cells in vitro, whereas adenosine receptor signaling seems to work antagonistically and decreases cellular metabolic activity by blocking a number of metabolic enzymes. We also observed a ballance between adenosine transport and adenosine receptor signaling, which is characteristic for different cell types. Interestingly adenosine receptor is also required for the survival of model cancer clones in vivo. There is more than 20 times lower rate of wts tumor clones in the absence of AdoR, suggesting that AdoR plays a protective role for cancer clones in flies.

248B

Investigation of novel epidermal growth factor receptor target genes implicated in *Drosophila* egg and wing development. Jacquelyn Gallo, Luke Dombert, Justin Hunter, Kristopher Krawchuk, Connor Zale, Lisa Kadlec. Department of Biology, Wilkes University, Wilkes-Barre, PA.

Signaling by the *Drosophila* epidermal growth factor receptor (Egfr) plays an important role in many aspects of development, including oogenesis, embryogenesis and proper development of both the eye and the wing. In the ovary, the Egfr pathway plays a key role in the establishment of the body axes during oogenesis. In the wing, Egfr signaling plays an important role in vein tissue specification. Microarray screens by our lab and others have been used to identify potential downstream

transcriptional targets of the Egf receptor using the Drosophila ovary as a model system. Our initial work compared gene expression using fly ovaries in which the activity of the Egfr-pathway was reduced (grk HK36), normal (OreR), or constitutively active (CY2/λTop). We are now employing a number of approaches to investigate the expression, biological function, and mechanism of action of several putative targets of interest. Target genes currently under investigation include several genes implicated in eggshell formation (e.g. *Dec-1*) and/or as part of chorion amplicons (e.g. CG18419 and *yellow-G2*), as well as a number of genes of unknown function (including CG13299, CG11381, CG13083 and CG14309). RT-PCR has confirmed the up-regulation of a number of targets, as originally seen by microarray. Several putative targets exhibit developmentally regulated expression in the ovary, and in some cases this expression has been shown to be altered in response to changes in levels of Egfr signaling. Screening of putative targets for biological function using UAS-RNAi suggests roles for several target genes of unknown function in eggshell production and/or integrity, wing morphogenesis, or both. A neutral red uptake assay indicates defects in vitelline membrane integrity in compromised eggshells. Additionally, we are using *in situ*hybridization to investigate target gene expression in wing imaginal discs, as well as to evaluate the effectiveness of our targeted RNA interference.

249C

Torso-like influences developmental timing in *Drosophila melanogaster* independently of the Torso RTK pathway. Travis K. Johnson[1,2], Tova Crossman[2], Karyn Foote[2], Michelle A. Bennett[2], Lauren Forbes Beadle[2], Anabel Herr[1,2], James C. Whisstock[1], Coral G. Warr[2]. 1) Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, 3800, Australia; 2) School of Biological Sciences, Monash University, Clayton, Victoria, 3800, Australia.

Membrane attack complex/perforin-like (MACPF) proteins are best known for their ability to lyse and kill target cells during the vertebrate immune response, however several MACPF proteins play poorly understood roles in development. The *Drosophila* MACPF protein Torso-like (Tsl) is critical for terminal patterning in the early embryo and restricts activation of the Torso (Tor) receptor tyrosine kinase by an unknown mechanism. Recently, Tor was shown to have a second role in the prothoracic gland (PG), as the receptor for prothoracicotropic hormone (Ptth), which initiates metamorphosis[1]. Here, we explored the possibility that *tsl* and other terminal patterning genes might also be required for this process. We looked for expression of known maternal terminal class genes (*tsl, trunk, fs(1)Nasrat, fs(1)polehole and closca*) in the PG and found only *tsl* is expressed here. To test if *tsl* participates in Tor signalling in the PG, we generated *tsl* null mutants via ends-out gene replacement. *tsl* nulls phenocopied loss of *tor* exhibiting a delay in the time to pupariation, and this was rescued by expression of a *tsl* transgene. However, in *tsl; tor* double mutants the delay was greatly increased when compared to loss of *tor* or *tsl* alone, suggesting the effect of loss of *tsl* is additive rather than epistatic to loss of *tor*. Furthermore, we found that ectopic Ptth was highly active in both the PG and the embryo independently of *tsl*, producing faster development and an expansion of terminal regions respectively. Taken together we conclude that *tsl* is acting independently of Ptth/Tor in the PG to influence developmental timing. 1. Rewitz et al. (2009) Science 326, 1403-1405.

250A

Friend of Echinoid (Fred) and Echinoid (Ed) regulate EGFR trafficking. Qian Nie, Susan Spencer. Department of Biology, Saint Louis, MO.

The Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase that regulates signaling pathways critical for cell proliferation and differentiation in epithelial tissues. The amount of EGFR available for signaling is regulated by a balance of receptor recycling to the plasma membrane and degradation in the lysosome. We have found that the immunoglobulin cell adhesion molecules Echinoid (Ed) and Friend of Echinoid (Fred) can regulate the level of EGFR on the plasma membrane. Ed and Fred amino acid sequences are closely related, but Fred lacks the PDZ binding domain found at Ed's C-terminus. Here, using truncated and chimaeric forms of Ed and Fred, we examine the importance of Ed's PDZ-binding domain in regulating internalization from the plasma membrane. We also examine whether Fred's effects on EGFR internalization require Ed. A possible model of how Fred and Ed regulate EGFR internalization will be discussed.

251B

Characterization of Dis3 in *Drosophila melanogaster*. Amanda Raimer[1], Mark Snee[2], Hemlata Mistry[1], James Skeath[2]. 1) Department of Biochemistry, Widener University, Chester, PA; 2) Department of Genetics, Washington University School of Medicine, St. Louis, MO.

The exosome is the complex responsible for RNA degradation in the cell; Dis3 is a 3' to 5' exoribonuclease subunit of the exosome. Dis3 functions in both the nucleus and the cytoplasm, while its homolog Dis3-like is apparently restricted to the cytoplasm. Dis3 function has been implicated in accurate RNA degradation. However the mechanism that determines the cellular and temporal specificity of RNA degradation is unclear. Furthermore, it is uncertain how particular RNAs are targeted for destruction. To better understand the importance of Dis3 function *in vivo*, sixteen homozygous lethal *Dis3* alleles have been generated. Each allele will be molecularly characterized through sequencing the coding region and intron-exon junctions to specifically identify missense mutations. The stage of arrest in development will also be determined by comparing embryonic and larval development of the mutant lines to that of a *Dis3* RNAi line. The mRNA and protein expression patterns of *Dis3* in both embryos and larval imaginal discs will be compared using *in situ* hybridization and immunostaining, respectively. Finally, GST-tagged wild-type and exoribonuclease-defective Dis3 proteins will be used to identify potential Dis3 targets and the mechanism by which RNA degradation is modulated. This research will lead to a better understanding of Dis3 function

in *Drosophila*, and begin to uncover its importance in many developmental processes.

252C

Importance of tyrosine phosphorylation for Echinoid's function. Peter P Saengthien, Erin J Andrews, Susan A Spencer. Saint Louis University, St. Louis, MO.

Echinoid (Ed) is an Ig-domain cell adhesion protein implicated in Notch, EGFR, and Hippo-pathway signaling. The intracellular domain of Echinoid has been shown to be important to Ed's function in these pathways, but how it acts is poorly understood. One of Ed's functions is to limit Epidermal Growth Factor Receptor (EGFR) activity in developing *Drosophila* eye. EGFR signaling has also been shown to promote tyrosine phosphorylation of Ed both *in vitro* and *in vivo*. Based on the Netphos phosphorylation prediction program, nine of Ed's eighteen intracellular tyrosine residues are good candidates for phosphorylation. To test whether tyrosine phosphorylation is important for Ed function, we mutated these tyrosines to phenylalanines to create an unphosphorylatable form of Ed, EdYF. We have used this construct to examine the possible effects of tyrosine phosphorylation on Ed subcellular localization and activity in cultured S2 cells and in transgenic animals.

253A

Motor neuron regulates Indirect muscle patterning through EGF ligands. Kumar Vishal, Lindsay Grainger, Mary Turvy, Joyce Fernandes. Dept Zoology, Maimi Univ, Oxford, OH.

Unlike embryonic myogenesis, many aspects of adult myogenesis require innervation. One example is seen during development of the thoracic indirect flight muscles (IFMs) where denervation affects IFM is reduces myoblast proliferation and also causes loss of the organizer cell specific marker dumbfounded. These results suggest that motor neurons may act through organizer cells to regulate myogenesis (Fernandes and Keshishian, 2005). However, the natures of signals involved in this communication remain to be elucidated. Our overall goal is to understand how EGF signaling is involved in cell -cell communication during IFM myogenesis. We find that have shown that blocking EGF receptor in the receiving cells (organizer cell and myoblasts) alters muscle patterning. Disrupting the pathway in organizer cells led to a reduction in the number of one groups of IFMs (DLMs), 6DLMs are seen in 5% of the manipulate animals. Blocking the pathway in myoblast causes a less severe reduction in the number of DLM fibers, 6DLMs are seen in 60-65% of experimental animals. Dorsoventral muscles (DVM) profiles are also altered in both cases. These results suggest that the organizer cell is the primary receiving cell during IFM myogenesis. Based on these results, in this study we are testing the role of motor neuron as a primary signaling cell. We will manipulate the EGF ligands in the motor neuron using an RNAi approach. Effects of this manipulation will be examined on adult muscle profile. We will also examine muscle patterning during pupal stages to determine what aspects of patterning are disrupted. Myoblast proliferation will be monitored by BrdU incorporation assay, whereas fusion of myoblasts to form nascent fibers will be studied using antibodies to the transcription factor, erect wing. Our preliminary studies to block EGF ligand vein, suggest that muscle patterning is disrupted in 50% of cases. Since, IFM myogenesis shares a striking similarity to vertebrate skeletal muscles on their dependence on innervation; this study will help in a better understanding of the neuronal control of myogenesis.

254B

RhoGAP68F regulates endocytic recycling to facilitate epithelial flattening and tissue elongation. Beatriz Hernandez de Madrid, Lina Greenberg, Victor Hatini. Anatomy and Cell Biology, Tufts University, Boston, MA.

Epithelial elongation is a conserved morphogenetic process that shapes the morphology of the primary body axis as well as tissue and organs. Epithelial elongation requires coordinated assembly and disassembly of cell-cell junctions but the mechanisms involved are incompletely understood. During metamorphosis the leg disc dramatically elongates from a short and wide disc to a long and narrow tube providing a model to study tissue elongation. Leg elongation is mediated by coordinated changes in cell shape, cell-cell contacts, and the flattening of the epithelium from pseudostratified to simple. In an RNAi screen designed to uncover new regulators of tissue elongation, we had selected the Rho family regulator RhoGAP68F for further analysis. We find that RhoGAP68F is required to promote epithelial flattening. Functional analysis in vivo revealed that overexpressed mCherry::RhoGAP68F strongly colocalized with Rab4 recycling endosomes, caused their dramatic enlargement and clustering, and reduced their normal accumulation near the apical surface. Analysis in Schneider 2 cells revealed that RhoGAP68F reduced the speed and displacement of the Rab4 endosomes. The Rab4 endosomes colocalized with the septate junction (SJ) protein FascilinIII (FasIII) and both Rab4 and FasIII were required for leg elongation. Our findings suggest that RhoGAP68F inhibits the recycling of SJs back to the plasma membrane to diminish lateral cell-cell contact in order to facilitate epithelial flattening. Our current studies are designed to test the role of RhoGAP68F in trafficking of SJs components to the plasma membrane and the remodeling of SJs.

255C

***In vivo* Time Lapse Confocal Analysis of the *RhoA* Head Involution Defect and Molecular and Genetic Characterization of Five Extant *RhoA* Mutant Alleles.** Melissa Maloof[1], Rachel Stottlar[1], Pria Chang[1], Laura Johansen[1], Katherine Sinclair[1], Maureen Filak[1], Fafa H. Koudoro[1], Rahul Warrior[2], Susan R. Halsell[1]. 1) Biology, James Madison University, Harrisonburg, VA; 2) Developmental and Cell Biology, University of California, Irvine, CA.

RhoA signal transduction functions in myriad morphogenetic processes throughout the *Drosophila* life cycle. Characterized *RhoA* mutant alleles are homozygous embryonic lethal with a characteristic defect in head involution. This work

presents analysis of the head involution defect using time-lapse confocal microscopy. Cells and their actin cytoskeleton were visualized using an actin-binding GFP-moesin fusion protein driven by the *spaghetti-squash* promoter (SGMCA; Edwards et. al. 1997. Dev. Biol. 191, 103). Wild type and null *RhoA* mutants were analyzed. To date, data indicates the dorsal ridge forms normally and begins its anterior-ward movement, but is ultimately impeded on the dorsal side of the embryo when the procephalon and clypeolabrum fail to retract; this is consistent with the dorsal anterior hole observed in cuticle preparations of *RhoA* mutant embryos. Phenotypic and molecular characterization of five EMS induced *RhoA* mutations (*233* and *246*, Ward, Evans and Thummel, Genetics. 165:1397;*3.5.1*, *4.4.2* and *7.23.1*) will also be described. Four of the five alleles exhibit apparent complete loss of function phenotypes; they are 100% embryonic lethal and all show consistent anterior dorsal holes in the cuticle. In addition, three of these alleles have been sequenced and each shows a mutation consistent with a null phenotype. All are single G to A transitions and alter either the start codon, a splice donor site or introduce a premature stop codon. The fifth allele, however, may be a hypomorphic mutation. This is based on the observation that the mutation is not 100% lethal embryonically and the dead embryo cuticle phenotype is variable, with the majority of embryos exhibiting defects in the head skeleton by no dorsal anterior hole. Genetic and molecular characterization of this allele continues.

256A

Ack1 regulates a macromolecular complex involved in nucleotide synthesis. Todd Strochlic, Alana O'Reilly, Jeffrey Peterson. Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA.

Ack1 (activated cdc42-associated kinase 1) is a poorly characterized non-receptor tyrosine kinase implicated in tumor growth and metastasis in humans. To gain insight into the biological functions of Ack1, we analyzed the role of the homologous protein, DAck, in *Drosophila melanogaster*. *DACK*-deficient female flies display reduced fertility and defects in oogenesis characterized by disruption of plasma membranes between germline cells. These phenotypes can be rescued by transgenic expression of wild-type DAck but not a kinase-dead mutant, indicating that DAck kinase activity is critical for oogenesis in the fly. In *Drosophila* egg chambers, DAck localizes to unusual cytoplasmic filaments that also contain two metabolic enzymes: cytidine triphosphate synthase (CTPS) and inosine- 5'-monophosphate dehydrogenase (IMPDH). These enzymes catalyze the rate-limiting steps in the biosynthesis of CTP and GTP, respectively, and consequently we have named this macromolecular assembly of enzymes FINS (filaments involved in nucleotide synthesis). In addition to its role as an essential nucleotide, CTP is required to generate CDP-linked intermediates in the synthesis of membrane phospholipids. Female flies with reduced levels of CTPS are sterile and exhibit membrane defects that phenocopy those observed in *DACK*-deficient flies, suggesting that DAck may modulate the function of the FINS complex. Indeed, FINS in *DACK*-deficient flies are smaller and fragmented, indicating a role for DAck in maintaining the functional integrity of this complex. Importantly, the FINS complex is evolutionarily conserved. We have detected autophosphorylated (activated) Ack1 as a component of FINS in mammalian cells grown in nucleotide-depleted conditions, suggesting that FINS assemble in response to increased nucleotide demand and that CTPS and IMPDH are likely active in these structures. Taken together, these results implicate Ack1 in the regulation and coordination of nucleotide metabolism in both flies and mammals.

257B

Wnt/Wingless signaling, Earthbound, and Erect Wing are required for late stages of indirect flight muscle development. Hassina Benchabane, Ai Tian, Yashi Ahmed. Department of Genetics, Geisel School of Medicine at Dartmouth.

The Wnt/Wingless signaling pathway directs fundamental processes during development, and is required for homeostasis of adult tissues and maintenance of stem cells. Hence, the activity of the Wnt pathway and the transcription of its downstream target genes must be tightly regulated to ensure proper development and to prevent human disease. Because the majority of Wnt responses are context-specific, mechanisms have to be in place to restrict signaling and the activation of target genes to specific tissues and developmental stages. In a forward genetic screen in Drosophila, we recently identified two novel tissue-specific cofactors of the Wnt pathway, Earthbound1 (Ebd1) and Erect Wing (Ewg), which promote Wnt signaling in myoblasts and muscles, and are required for proper development of indirect flight muscles (IFMs). Inactivation of *ebd1* or *ewg*, or disruption of Wnt signaling in muscle cells, leads to a loss of IFMs in adults. We further investigated the role of Ewg, Ebd, and Wnt signaling in muscle development. We find that in *ebd1* and *ewg* mutants, as well as in mutants with disrupted Wnt signaling, IFMs are formed correctly initially, but degenerate during pupation. We show evidence that programmed cell death is involved in this degeneration. These findings indicate that Ewg, Ebd1 and Wnt signaling are required for later stages of IFM development.

258C

TH8, a new ADAMTS like protease in Wg signaling pathway. Go-Woon Kim, Jong-Hoon Won, Ok-Kyung Lee, Orkhon Tsogtbaatar, Su-Jin Nam, Yeon Kim, Kyung-Ok Cho. Department of Biological Sciences, KAIST, Daejeon, Republic of Korea.

Proper regulation of cell division and cell survival is crucial for preventing cancer or abnormal cell death. Evidence is accumulating that ADAMTS (a disintegrin and metalloproteinase with thrombospondin domains) family of metalloproteases play roles in the promotion or suppression of cancer formation and metastasis. We have recently discovered a novel Drosophila metalloprotease named TH8, whose sequence has high homology to ADAMTS family proteins. To understand the function of TH8 protein, we have generated deletion mutants in the th8 gene by imprecise excision of P element, and found that loss of th8 function results in lethality. Similar phenotype was also obtained by RNAi expression. At cellular level, the loss of th8 function causes apoptosis, indicating that TH8 is essential for inhibiting cell death. To understand the underlying

mechanism of TH8, we carried out a genetic screen to search for suppressors of th8 over-expression-induced lethal phenotype. One of the suppressor lines had mutation in the wntless (Wls) gene that plays an important role in secretion and uptake of wingless (Wg), suggesting that TH8 also participates in the same process. Indeed, loss of TH8 function decreased Wg signaling and, genetic interactions between TH8 and Wg signaling components such as Dishevelled or Van Gogh/Strabismus were observed. Activation of TH8 strictly depended on Wls, demonstrating the one of biochemical functions of Wls is the cleavage of TH8 prodomain either directly or indirectly. We propose that TH8 is one of essential components in regulating Wg secretion or uptake.

259A

Revisiting the role of Wnt signaling in sensory organ development in the Drosophila wing. Ezgi Kunttas-Tatli, Kellie Kravarik, Sandra Zimmerman, Amy Fuller, Brooke M. McCartney. Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

The colon cancer tumor suppressor Adenomatous polyposis coli (APC) negatively regulates Wnt signal transduction through its activity in the destruction complex. APC binds directly to the main effector of the pathway, β-catenin (Armadillo in Drosophila), and targets it for proteosome-mediated degradation. The disruption of *APC* (both *APC1* and *APC2*) is implicated in the initiation of >80% of human colon cancers. In addition to its role in Wnt signaling, APC acts as a cytoskeletal regulator, although it is less clear how the disruption of these cytoskeletal functions contributes to tumorigenesis. To understand how disruption of *APC* affects epithelial tissues, we are using the developing wing epithelium as a model. We previously showed in the larval wing disc that the complete loss of *APC* in clones results in apical constriction and invagination through activation of canonical Wnt targets, RhoI and Myosin II. We are currently investigating the long-term effects of *APC* loss and Wnt pathway activation on the development of the wing epithelium and its sensory organs. We have found that activation of the Wnt pathway by either loss of *APC*, expression of a stabilized form of Armadillo (Arm^{S10}), or manipulation of Shaggy (GSK3β) leads to the development of ectopic sensory organs in the anterior and posterior blade consistent with previous results. Activation of excessive Wnt signaling at the anterior margin results in cell fate changes and spacing defects that may reveal novel Wnt dependent changes in gene regulation. Surprisingly, *APC* null clones in the posterior compartment exhibit innervated sensory organs in contrast to the non-innervated sensory organs at the wild type posterior margin. We are currently testing the hypothesis that this innervation and the cell fate transformations at the anterior margin are the result of changes in the expression of Wnt targets Senseless, Achaete and Scute.

260B

An *in vivo* kinome and phosphatome RNAi screen in the *Drosophila* wing imaginal disc identifies a novel regulator of Wnt/Wg secretion. Tirthadipa Pradhan, Sharan Swarup, Esther Verheyen. Simon Fraser Unversity, Burnaby, Canada.

Wingless (Wnt/Wg) proteins are secreted molecules which act in an evolutionary conserved pathway to regulate cell proliferation and cell fate specification. The key step in the pathway is the regulation of the levels of cytoplasmic β-catenin. β-catenin acts as a transcriptional regulator, which upon pathway activation accumulates in the cytoplasm and subsequently translocates to the nucleus where it interacts with the Tcf/Lef family of transcription factors to direct target gene expression. In the absence of the Wnt/Wg, the levels of β-catenin are kept low in the cytoplasm through constitutive degradation via a protein destruction complex composed of Axin, Adenomatous Polyposis Coli (APC), Glycogen synthase kinase-3 (GSK-3), Casein kinase1 (CK1).

Phosphorylation events are known to regulate multiple steps of the Wnt/Wg pathway. The key components such as β-catenin, Dishevelled, LRP5/6, APC, Axin and TCF are phosphorylated in the pathway. The ubiquitous kinases GSK-3β and CKIα and phosphatases such as PP1 and PP2 regulate multiple steps of these phosphorylations by distinct mechanisms. However the significance of most of these phophorylation events is not well understood. To fill the gap in our knowledge we did an *in vivo* RNAi screen in the *Drosophila* wing imaginal disc. Our screen has yielded a number of novel regulators of the Wnt/Wg pathway. Subsequent characterization of the one of the phosphatases by loss of function and overexpression analysis revealed its novel role in Wnt/Wg secretion. We found that in its absence Wg gets trapped in the secreting cells. Furthermore, loss of this phosphatase causes reduction in Wntless (Wls) levels *in vivo*. We are in the process of performing further genetic and biochemical interaction studies with the members of Wnt/Wg secretion machinery. Taken together, our data provides new insight into novel regulators of Wnt/Wg pathway and a better understanding of Wnt/Wg secretion.

261C

Regulation of Wnt signaling by the tumor suppressor APC does not require the ability to enter the nucleus nor a particular cytoplasmic localization. David M. Roberts[1], Mira I. Pronobis[2], John S. Poulton[2], Eric G. Kane[1], Mark Peifer[2]. 1) Department of Biology, Franklin & Marshall College, Lancaster, PA; 2) Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Wnt signaling plays key roles in both development and disease. The tumor suppressor Adenomatous polyposis coli (APC) is an essential negative regulator of Wnt signaling that is inactivated in over 80% of all colon cancer cases. APC regulates Wnt signaling by contributing to a multi-protein complex (the destruction complex) that targets the Wnt effector protein βcatenin for phosphorylation and subsequent proteasomal destruction. However, several studies have suggested additional roles for APC in negatively regulating Wnt signaling, postulating that APC can also act in the nucleus to either modify activity of Wnt-responsive promoters or to actively export βcatenin out of the nucleus to facilitate its destruction. In addition, we previously

suggested that an additional function of APC might be to position the destruction complex at the appropriate subcellular location. Here, we directly test these models by generating APC variants with localization tags that force APC to different cytoplasmic locations while simultaneously preventing its nuclear entry. These APC localization variants were then assessed for function in human colon cancer cells and *Drosophila* embryos. Strikingly, all tethered APC variants rescued βcatenin destruction and down-regulated Wnt target genes in colon cancer cells, and most restored Wg/Wnt regulation in *Drosophila* embryos null for APC. These data suggest that APC does not have required nuclear functions, nor does it position the destruction complex to a precise subcellular location to function in Wnt signaling.

262A

The interaction between Tankyrase and Axin modulates Wingless signaling during development. Ofelia Tacchelly Benites, Zhenghan Wang, Eungi Yang, Michael Randall, Yashi Ahmed. Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH.

The Wnt/Wingless pathway directs cell fate decisions, cell proliferation, and apoptosis during development in metazoans. Deregulation of the Wnt/Wingless pathway is involved in a number of developmental diseases and cancers. Targeted proteasomal degradation of the transcriptional activator beta-catenin controls the levels of Wnt/Wingless pathway activity, and this degradation depends on the activity of the destruction complex. Axin is a rate-limiting component of the destruction complex and an increase in Axin levels inhibits Wnt/Wingless signaling in many contexts. Recently, the poly-ADP-ribose polymerase Tankyrase has been shown to promote Axin turnover in cultured carcinoma cells. This interaction is thus an attractive therapeutic target for diseases in which beta-catenin regulation is lost. However, understanding the importance of Tankyrase in regulating Axin and Wnt/Wingless signaling in vivo has not been successful in vertebrates, because of functional redundancy in vertebrate Tankyrase genes. There is only one fly Tankyrase gene and it is highly conserved, thus making Drosophila an ideal system to study the significance of Tankyrase mediated Axin regulation. Using a Drosophila model, we have shown that loss of Tankyrase and deletion of the Tankyrase binding domain of Axin lead to an aberrant stabilization of Axin and loss of Wingless signaling. Here, we provide the first in vivo evidence that Tankyrase and the Tankyrase binding domain of Axin are required to promote multiple developmental processes that are dependent on Wnt/Wingless signaling.

263B

Context-dependent Transcriptional Cofactors Regulate Specific Wnt Target Genes. Ai Tian, Hassina Benchabane, Nan Xin, Yashi Ahmed. Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH.

The Wnt/Wingless signal transduction pathway is repetitively used during metazoan development to direct an array of cellular processes such as cell proliferation, fate specification, differentiation and apoptosis. To ensure proper development, the Wnt pathway must therefore regulate distinct target genes in different developmental/cellular contexts. The collaboration of tissue-specific transcription factors is proposed to be a mechanism underlying the contextual activation of Wnt/Wingless target genes. Using a genetic screen in Drosophila for context-specific Wingless pathway components, we identified two context-specific transcriptional regulators, Earthbound1 (Ebd1) and Erect-wing (Ewg). We established that Ewg binds DNA and recruits Ebd1 to chromatin, while Ebd1 promotes the association between TCF and Arm. Using a bioinformatics approach, we identified potential Ewg target genes whose expression is misregulated in *ewg* mutants. Putative Ewg and Tcf binding sites in these target genes, as well as the sequences surrounding these sites, are highly conserved across Drosophila genomes. Notably, some candidate Ewg target genes are also misregulated in *ebd* mutants, as well as upon Wingless pathway inhibition. We moreover find that some of their putative enhancers bind Ewg directly. Analyzing how Ewg, Ebd1, Arm and TCF cooperate to activate these enhancers will shed further light on the mechanisms of context-specific regulation of target genes.

264C

Drosophila Tankyrase Regulates Axin Through Cell Membrane Recruitment and Proteolysis. Zhenghan Wang, Ofelia Tacchelly Benites, Eungi Yang, Geoffrey Noble, Megan Johnson, Michael Randall, Yashi Ahmed. Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH.

Aberrant activation of the Wnt signal transduction pathway triggers the development of colorectal carcinoma. Recently identified small molecule inhibitors of the poly-ADP-ribose (pADPr) polymerase Tankyrase attenuate Wnt signaling in cultured colon carcinoma cells by stabilizing the negative regulatory component Axin, and thereby provide a promising new therapeutic strategy. However, functional redundancy in vertebrate Tankyrase genes has impeded efforts to identify the in vivo contexts in which Tankyrase regulates Axin and Wnt signaling. Here, using a Drosophila model, we provide the first in vivo evidence that Tankyrase and the pADPr-directed E3 ubiquitin ligase RNF146/Iduna function together to promote Axin proteolysis in all epithelial cells throughout development. By preventing supraphysiologic increases in Axin, Tankyrase and RNF146 promote Wnt signaling in multiple developmental contexts. Newly developed Axin antisera reveal that in contrast with the prevailing model, endogenous Axin is enriched at the cell membrane both in the presence and in the absence of Wnt stimulation, and unexpectedly, that Tankyrase mediates not only the turnover of Axin, but also its recruitment to the cell membrane. Thus through dual roles in the cell membrane recruitment and proteolysis of Axin, Tankyrase prevents supraphysiologic Axin levels in all epithelial cells, and thereby also promotes signaling in cells responding to Wnt exposure.

265A

Control of stalk cell number and morphology. Antoine Borensztejn[1], Anne-Marie Pret[2], Kristi Wharton[1]. 1) Brown

University, Department of Molecular Biology, Cell Biology, and Biochemistry, Providence, RI; 2) CNRS, Centre de Génétique Moléculaire, Gif-Sur-Yvette, France.

The control of cell number is essential for the proper formation of organs during development. Once formed the maintenance of cells is key to the architecture and functioning of different tissues. During Drosophila oogenesis, each egg chamber is separated from the previous one by a single column of ~8 stalk cells. The number of stalk cells and the morphology of the stalk has been suggested to be under the control of apoptosis (Assa-Kunik et al. 2007), however the timing and regulation of the proposed apoptotic events is not understood. In this ongoing study, we have investigated the precise control of stalk cell number and the pathways implicated in this mechanism. Our previous work (Borensztejn et al. 2012) and that of Assa-Kunik et al. 2007 have shown that the Jak/Stat ligand, Upd, and signaling via the pathway effect stalk cell number. What is the precise role of apoptosis in regulating stalk cell number ? Do Jak/Stat and Notch pathways control this proposed stalk cell apoptosis ? Results will be presented that contribute to the overall understanding of the mechanisms controlling cell number and their contribution to shaping organ morphology.

266B

Apoptotic priming is regulated during *Drosophila* development. Yunsik Kang, Arash Bashirullah. Sch Pharmacy, Univ Wisconsin, Madison, Madison, WI.

Resistance to apoptosis is a hallmark of cancer cells meditated in part by an increased threshold for initiating caspase activation. Despite its importance in disease, however, the role of apoptotic thresholds under normal physiological conditions remains poorly understood. Here we demonstrate that apoptotic thresholds vary dramatically during development and, as a result, not all developing cells are primed to trigger apoptosis. “Primed” cells initiate caspase activation and apoptosis in response to expression of death activator proteins like the IAP-antagonist *reaper* (*rpr*) or to loss of the IAP *diap1*. In contrast, we identified “unprimed” cells that are resistant to ectopic expression of IAP-antagonists. Surprisingly, these “unprimed” cells are also resistant to *diap1* knockdown, challenging the notion that IAPs are the final barrier to initiation of apoptosis. We show that “unprimed” cells are 50-fold more resistant than “primed” cells and that these “unprimed” resistant cells are characterized by reduced levels of core death genes like *Ark* and *Nc* (the *Apaf1* and *caspase-9* homologs, respectively). Importantly, increasing expression of *Ark* and *Nc* is sufficient to prime previously resistant cells to respond to death activators. Conversely, reducing levels of *Ark* and *Nc* is sufficient to confer apoptotic resistance to “primed” cells. We show that apoptotic priming precedes, and is essential for, programmed cell death. Finally, our data suggests that apoptotic priming is regulated by ecdysone in a tissue- and stage-specific manner during major developmental transitions. Thus, regulation of apoptotic priming provides a novel and critical cellular protection mechanism during development.

267C

Autophosphorylation of DBT Occurs in its C-terminal Domain and is required for its Antiapoptotic Function. John C Means, Jin-Yuan Fan, Ed Bjes, Jeffrey Price. University of Missouri-Kansas City, Kansas City, MO.

DOUBLETIME (DBT), the key circadian protein kinase responsible for PERIOD (PER) protein phosphorylation, undergoes autophosphorylation in Drosophila S2 cells. Several of the autophosphorylation sites were mapped to the C-terminal domain of DBT by mass spectrometry and analysis of DBT mutant proteins. In particular, a mutant form of DBT (DBT-Cala), in which 6 serines and threonines in a part of the C-terminal domain evolutionarily conserved in the Drosophilids are mutated to alanine, exhibits reduced autophosphorylation and enhanced stability in S2 cells treated with the general phosphatase inhibitor okadaic acid. Vertebrate orthologs of DBT (casein kinase I δ/ε) also autophosphorylate their C-terminal domains, leading to reduced kinase activity in vitro. However, autophosphorylated DBT does not exhibit reduced kinase activity in vitro. Studies addressing a role for DBT autophosphorylation in the circadian clock are thus far not conclusive. Analysis of DBT electrophoretic mobility in circadian mutants demonstrates an accumulation of slow-mobility forms of DBT, suggesting that alterations in circadian proteins with which DBT is known to interact can lead to autophosphorylation of DBT. Since autophosphorylation of DBT in S2 cells leads to reduced DBT levels, it is possible that it may trigger degradation of DBT in some circadian cells when its circadian partner protein (PER) is eliminated. The phosphatase(s) which normally antagonize DBT autophosphorylation are potentially important circadian clock components. In addition, DBTWT prevented UV induced apoptosis in S2 cells by direct binding and phosphorylation of caspases, with degradation of DBT also produced in response to UV. The catalytic activity of DBT is required to inhibit apoptosis, because expression of a catalytically inactive DBT dominant negative induced caspase activation. This antiapoptotic function was dependent on phosphorylation of the C-terminal domain of the protein, since the DBT-Cala was unable to protect S2 cells from apoptosis and was not degraded in response to UV treatment.

268A

The regulation of Dronc by Hippo Pathway. Shilpi Verghese[1], Aidan Fenix[1], Madhuri Kango-Singh[1,2,3]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) Pre-Medical Programs, University of Dayton, Dayton OH; 3) Center for Tissue Regeneration and Engineering at Dayton, University of Dayton, Dayton OH.

Hippo pathway regulates organ size by maintaining a fine balance between cell death and proliferation by regulating the transcription of several target genes including *diap1*, *myc*, *ex*, *bantam miRNA*, *head involution defective* (*hid*), *Drosophila Nedd-2 like caspase* (*dronc*) and *cyclin E*. Loss of Hippo signaling causes proliferation by increased activity of its transcriptional co-activator Yorkie (Yki); whereas gain of Hippo signaling by hyperactivation of genes like Hippo causes cell death via Jun N-

terminal Kinase (JNK) and Caspase mediated cell death pathways. We found that loss of *warts* (*wts*) induces *dronc*transcription suggesting that Dronc is normally activated by Hippo signaling unlike other reported target genes. We will test the mechanism of *dronc* regulation by Hippo signaling. Mammalian Yorkie homologs (YAP, TAZ) act both as transcriptional co-activators/repressors. YAP regulates apoptosis through *p73*- a *p53* family transcription factor. The *p53* family [*p73*, *p63*, *p53*] regulates growth, apoptosis and DNA damage response. *Drosophila p53*(*Dmp53*) is the sole *p53* family gene in flies, and *Dmp53* regulates *dronc* transcription for the regulation of irradiation-dependent and independent compensatory proliferation. The Hippo pathway may regulate *dronc* transcription through or independent of Yki to regulate organ size. Using GAL4-UAS and transgenic RNAi approaches, we tested for interaction between *Dmp53*, Hippo pathway and Dronc to investigate the mechanism by which Hippo pathway controls *dronc* transcription. Over expression of full length *Dmp53* enhances the cell death caused by Hippo over-expression while loss of *Dmp53* and *hpo* (using RNAi) in the wing pouch (using nubGAL4) down-regulates *dronc* transcription suggesting that *Dmp53* acts downstream of Hippo pathway. We present our studies of the interaction between the Hippo pathway and *Dmp53* in the regulation of *dronc* transcription.

269B

Modeling of spreading cell death by necrosis neurons to adjacent cells in *Drosophila*. Yong Yang, Lin Hou, Lei Liu. Peking University, Beijing, China.

Necrotic cells often spread damage to adjacent tissues in diseases such as ischemic stroke and traumatic brain injury. However, the signaling mechanisms of dying cells on their neighbors are poorly understood. To model this cellular response, we made a transgenic fly line that induced neuronal necrosis specifically in a few neurons by expressing a leaky cation channel. Namely, this system contains *UAS-GluR1^{Lc}* (the leaky channel) driven by *sevenless-Gal4* (*sev>GluR1^{Lc}*), which is expressed in three of the eight photoreceptor neurons in each ommatidium of eyes. We found that calcium overloading through*GluR1^{Lc}* expression indeed caused neuronal necrosis and reduced eye size of adult flies. Moreover, we found that spreading cell death took place in adjacent neurons but not glial cells through caspase-dependent and JNK-dependent apoptosis (JNK activation was determined by an *in vivo* reporter, *puc-lacZ*). Further genetic tests showed that the caspase-dependent apoptosis was mediated by *hid*; and the JNK-dependent apoptosis was regulated by ROS through metabolic pathways. In addition, we found that the key spreading factors from neuronal necrosis were *eiger* and ROS, because genetic manipulations of their levels affected both JNK activation and eye size of *sev>GluR1^{Lc}*. To determine the sequential events among ROS, *eiger* and JNK activation, we performed a tissue culture *in vitro*assay. In response to ectopically added hydrogen peroxide (H_2O_2), *eiger* was up-regulated and JNK signaling was elevated. Interestingly, *eiger* RNAi abolished the effect of H_2O_2 on JNK. Together, these results suggest that releasing of *eiger* and ROS from necrotic neurons synergistically activates JNK in the adjacent neurons with *eiger* pathway to be the dominant signal. Our model provides the first genetic evidence to demonstrate how necrotic neurons may influence their neighboring cells.

270C

Polyploidy Rewires The Spindle Assembly Checkpoint. Benjamin M Stormo[1], Ruth Montague[2], Sarah Paramore[2], Don Fox[1,2]. 1) Department of Cell Biology, Duke University, Durham, NC; 2) Department of Cancer Biology and Pharmacology, Duke University, Durham, NC.

Many types of human cancers are known to be polyploid, however whether polyploidy is a result or a cause of genome instability is not known. However, recent studies of mitotic polyploid cells in both Drosophila and mice have shown these cells are particularly susceptible to errors in separating their chromosomes during mitosis. Studying developmentally programmed polyploid cell divisions that occur in Drosophila rectal papillae, we found polyploid cells show significantly levels of unaligned chromosomes and chromosomal bridging. We hypothesized that these mitotic errors are caused by an aberrant Spindle Assembly Checkpoint (SAC). The SAC is a complex of proteins that binds to unattached kinetochores during metaphase and inhibits the Anaphase Promoting Complex (APC). We suspected the SAC might not function in polyploid cells, leading to genomic instability. By treating developing papillae with microtubule poisons, we find the SAC remains intact, but appears less robust, in polyploid cells. Further, we find the SAC regulator Mad2 fails to localize to papillar kinetochores, and that loss of Mad2 has no effect on recruitment of unaligned chromosomes. However, we do detect a polyploid-specific role for Mad2 that is independent of the kinetochore. Through live cell imaging, we find mad2 null animals have a high rate of chromosome bridging, specifically in polyploid cells, suggesting cytoplasmic Mad2 regulate anaphase timing without localizing to the kinetochore. These results suggest study of papillar cells may help to resolve the long-standing controversy regarding Mad2 function outside of the kinetochore. Taken together, our work suggests a novel SAC configuration in polyploid cells that is less robust, providing a link between polyploidy and genomic instability.

271A

Identification of novel regulators of apoptosis during metamorphosis. Gina Castelvecchi[1], Yunsik Kang[1], Anne Sapiro[2], Sarah Ives[1], Arash Bashirullah[1]. 1) Division of Pharmaceutical Sciences, University of Wisconsin-Madison, Madison, Wisconsin, United States of America; 2) Department of Genetics, Stanford University, Stanford, California, United States of America.

The transformation from larvae to pupae during metamorphosis heavily relies on the massive and rapid destruction of obsolete larval tissues. This process is triggered, in part, by the stage- and tissue-specific activation of apoptosis in response to expression of IAP antagonists *reaper* (*rpr*) and *head involution defective* (*hid*). Animals carrying loss-of-function mutations in critical regulators of apoptosis like the initiator caspase *Nedd2-like caspase* (*Nc*), the effector caspase *Drosophila ICE* (*drice*)

and the *apaf-1-related killer* (*Ark*) die during metamorphosis, presumably as a result of defects in remodeling the future adult. To identify novel regulators of apoptosis, we conducted a large-scale EMS mutagenesis screen. First, we generated over 8,600 new lethal mutations on the third chromosome and selected those that died exclusively during metamorphosis. We then conducted dominant *GMR-rpr* and *hs-rpr* modifier screens and identified 17 complementation groups among the ~900 newly identified metamorphosis-specific lethals. We identified loss-of-function alleles of *Nc* and a gain-of-function allele of *diap-1*, validating the efficacy of the screen for identifying regulators of apoptosis. The strongest and most frequently hit complementation group maps to a novel and evolutionarily conserved gene we named *bulsa* ("immortal" in Korean). Mutations in *bulsa* block all endogenous programmed cell death during metamorphosis while the overexpression of *bulsa* is sufficient to trigger caspase activation, demonstrating that *bulsa* is a critical regulator of apoptosis. We will present our initial characterization of *bulsa* and our progress in identification of the remaining loci identified in our screen.

272B

Identification of genes that mediate steroid- and TNF-triggered non-apoptotic cell death. Gautam Das, Tsun-Kai Chang, Sudeshna Dutta, Charles Nelson, Emily Clough, Cheng-Yu Lee, Daniel Caffrey, Eric Baehrecke. Cancer Biology, University of Massachusetts Medical School, Worcester, MA.

Programmed cell death is important for development, elimination of abnormal cells, and is altered in disorders including cancer. Although much is known about apoptosis, less is known about non-apoptotic cell death involving autophagy and necrosis. Here we investigate steroid-triggered cell death during development where DNA binding proteins influence target genes that control cell death. The steroid-response protein E93, a helix-turn-helix transcription factor, is necessary and sufficient for non-apoptotic cell death. We use genome-wide E93 DNA binding combined with gene expression analyses in E93 mutants to identify target genes, and show that E93 binds to a novel DNA sequence motif in target genes that control cell death. Significantly, we present evidence for tumor necrosis factor-triggered non-apoptotic cell death that is mediated by E93 and a novel target gene. This is the first evidence of genes that appear to contribute to caspase- and autophagy-independent programmed cell death under physiological conditions during development.

273C

Invadolysin, a novel and essential metalloprotease, is involved in the activation of apoptosis. Michal M. Janiszewski, Christopher G. Mills, Catherine M. Rose, Cristina Aguilar, Samantha J. Littler, Margarete M. S. Heck. University/BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom.

Induction of programmed cell death in *Drosophila* requires the activity of three closely linked genes: *reaper*, *hid*, and *grim*. It has been previously established that these proteins activate apoptosis by inhibiting the anti-apoptotic activity of the *Drosophila* IAP1 (dIAP-1) protein. Here we show that invadolysin, a novel and essential metalloprotease, plays a crucial role in the activation of apoptosis. Invadolysin shares residues in common with other IAP antagonists in flies at what we are predicting to be N-terminus of cleaved full-length protein. In a genetic modifier screen, *invadolysin* mutants strongly suppressed reaper-, hid- and grim-induced apoptosis. Significantly, RT-PCR analysis of lines overexpressing *reaper*, *hid*, or *grim* all showed an increase in the level of both invadolysin transcript and protein. In addition, invadolysin accumulates upon heat-shock activation of *hid* and *reaper.* Finally, *dcp-1* and *dronc* caspase mutants suppress and *diap-1* mutant enhances an invadolysin-induced rough eye phenotype, which could suggest a genetic interaction between pro- and anti-apoptotic genes and *invadolysin*. As invadolysin is highly conserved amongst eukaryotes, we also analyzed the localization of invadolysin in HeLa cells undergoing apoptosis. Staurosporine-induced apoptosis revealed relocalization of invadolysin from the cytoplasm to the nucleus with a strong concentration in apoptotic bodies, similar to what is observed with cleaved caspase-3 staining. Taken together, our data suggest that invadolysin may be involved in the activation and/or regulation of apoptosis.

274A

Uncovering novel targets of Escargot-inhibited cell death in the *Drosophila* ovary by RNA-seq. Victoria Kathryn Jenkins, Kim McCall. Department of Biology, Boston University, Boston, MA.

Cell death, required for proper homeostasis and development, is controlled by the interactions of a wide variety of pro- and anti-death genes. The *Drosophila* ovary is the site of two cell death events which illustrate aspects of apoptotic, autophagic, and potentially other non-canonical types of cell death. Starving the fly of protein can induce the death of entire pre-vitellogenic "mid-stage" egg chambers. All egg chambers, as they approach maturation, undergo the developmentally required "late-stage" death of non-oocyte germline cells ("nurse cells"). Overexpression of the anti-apoptotic protein DIAP-1 or the transcription factor Escargot causes a cytologically identical "undead" phenotype in starved fly ovaries, causing nurse cells to remain unkilled in both mid-stage and late-stage death events. DIAP-1 has long been known to block caspase (apoptotic protease) activation, but an anti-death function of Escargot (or another member of the Snail family of transcription factors) has not yet been described in *Drosophila*. It is not known how overexpression of *escargot*, normally required for gastrulation, cell cycle regulation, and the epithelial-to-mesenchymal transition, can mimic overexpression of *diap-1*. In order to identify targets of Escargot that may regulate cell death in the ovary, we have performed RNA-seq on ovaries of well-fed versus protein-starved flies overexpressing *escargot* or *diap-1*, both driven by the germline*NGT;nanos-Gal4* promoter, compared to controls. Ten females of each genotype were provided either yeast paste or apple juice agar for 48 hours prior to sacrifice at 6 days post eclosion. Ovary mRNA was extracted and sequenced as 50 bp single reads by an Illumina HiSeq 2000, yielding 29.0 +/- 4.7 million reads per group. The resulting transcriptomes were compared to find differences in gene expression, splice isoform

usage, and other mechanisms that Escargot may use to influence both known and potentially novel cell death genes, as well as to uncover the signaling events resulting in an undead phenotype similar to that seen in flies overexpressing DIAP-1.

275B

Engulfment Receptors in Programmed Cell Death in the Drosophila Ovary. Tracy L. Meehan, Allison Timmons, Jon Iker Etchegaray, Jeffrey Taylor, Olivia Rudnicki, Sarah Yunes, Kim McCall. Department of Biology, Boston University, Boston, MA.

Programmed cell death is essential for an organism's development and homeostasis to dispose of unwanted or diseased cells. Upon starvation, the nurse cells undergo apoptosis and are engulfed by the surrounding follicle cells. We studied the role of known engulfment receptors, Draper and integrins, in the role of starvation-induced cell death. Draper is the Drosophila homolog of the C. elegans engulfment receptor, CED-1. Integrins, on the other hand, have historically been studied for their roles in migration and cell signaling; only recently have they been shown to play a role in engulfment in D. melanogaster. Both drpr null flies and dsRNA drpr knockdown specifically in the follicle cells resulted in defective mid-oogenesis death. The follicle cells in these mutant egg chambers did not enlarge appropriately and died prematurely. Draper's main role was found to be activating JNK, as expression of activated hep (JNK kinase) suppressed the Draper mutant phenotype. To determine if integrins are required for engulfment in the ovary, we knocked down integrin βPS, the main β subunit in Drosophila, effectively knocking out the majority of all integrins in follicle cells. These egg chambers showed defective egg chambers very similar to those found in drpr RNAi flies. We are currently working on immunocytochemistry experiments to determine if there are subtle differences between βPS and Draper mutants. Previous work has shown that integrins can activate the JNK pathway, which activates Draper in our system. To determine if integrins work through the JNK pathway to up-regulate Draper in the ovary, we stained βPS mutant egg chambers with Draper and Draper mutant egg chambers with integrins. We found that Draper is still up-regulated in βPS mutant egg chambers and integrins are still up-regulated in Draper mutant egg chambers. We are currently screening candidate genes from the JNK and known engulfment pathways and the α integrin subunits to understand the complete pathway occurring in mid-oogenesis engulfment. Future studies will investigate how integrins and Draper interact.

276C

Inhibiting both autophagy and caspases does not abolish nurse cell death in late stage egg chambers. Jeanne S. Peterson, Alla Yalonetskaya, Kim McCall. Dept Biol, Boston Univ, Boston, MA.

During late oogenesis egg chambers degrade and eliminate their 15 nurse cells as part of normal development. This process of degradation involves at least two types of cell death, apoptosis and autophagy, as indicated by anti-caspase immunostaining, TUNEL and LysoTracker staining. In addition, mutations affecting either caspase dependent cell death or autophagy partially reduce nurse cell removal, leaving behind end stage egg chambers with persisting nurse cell nuclei (PNs). To determine whether apoptosis and autophagy work in parallel to degrade and remove nurse cells as is the case with salivary glands during the pupal stage (Berry and Baehrecke, 2007), we made mutants doubly affecting both caspases and autophagy and found no significant increase in the number of late stage egg chambers containing PNs nor in the number of PNs per egg chamber. This indicates that there is another form of cell death functioning in the ovary to remove all nurse cell remnants from late stage egg chambers. To examine this further, we are investigating the morphological changes that occur to nurse cells during developmental cell death. In particular, we have found that nurse cell nuclei show dramatic involutions, and the nuclear lamina persists until late in the process of cell death.

277A

A non-cell-autonomous contribution of somatic cells to programmed cell death of the germline in Drosophila. Claire E. Schenkel, Jon Iker Etchegaray, Kim McCall. Biology, Boston University, Boston, MA.

Programmed cell death is an important process in human development and disease. Apoptosis, autophagic cell death, and necrosis are the most well-known forms of cell death, but recent research has begun to characterize other forms of cell death, including phagoptosis—cell death initiated by a phagocytic mechanism. Our laboratory investigates the genetic control of cell death using the ovary of Drosophila melanogaster as a model. Each egg chamber includes germline-derived nurse cells and an oocyte, surrounded by somatic follicle cells. During normal egg chamber development, the nurse cells transfer their cytoplasmic contents to the oocyte as they initiate programmed cell death, and by time the egg chamber is mature, the nurse cell nuclei are completely gone. The mechanisms controlling this developmental cell death are mysterious; it occurs independently of the major cell death pathways. Research in the lab has shown that the phagocytic receptor Draper is required non-cell-autonomously for the removal of nurse cell nuclei. When draper was knocked out or knocked down in the follicle cells, persisting nurse cell nuclei were highly visible in all stage 14 egg chambers. To determine which cells required Draper activity, we knocked down draper in subsets of follicle cells, and found that a group called the stretch follicle cells are the most crucial in this process. Further experiments have aimed to identify whether draper-mediated engulfment mechanisms play an active role in the nurse cell death process or affect clearance only. We first aimed to determine if the nurse cells were able to transfer their nuclear contents in draper mutants. This release of contents does occur, but noticeably later in development in draper mutant flies than in controls, indicating that follicle cell mechanisms are involved in the death process. Future experiments will examine acidification patterns and nuclear lamin morphology to further characterize the role of the follicle cells in nurse cell death. These studies will reveal the contribution of follicle cells to the death and clearance of nurse cells.

278B

The contribution of follicle cells to non-apoptotic programmed cell death of nurse cells during late oogenesis. Allison Timmons, Claire Schenkel, Jon Iker Etchegaray, Jeffrey Taylor, Olivia Rudnicki, Kim McCall. Biology, Boston University, Boston, MA.

Programmed cell death (PCD) is an essential process in animal development and tissue homeostasis which ensures that aged, damaged, or excess cells are eliminated. In the *Drosophila* ovary, PCD occurs as a normal part of development. During late oogenesis, germline derived nurse cells (NCs), which provide nutrients, proteins, mRNAs, and organelles for the developing oocyte, transfer their contents into the oocyte and undergo PCD. Interestingly, disruption of apoptosis or autophagy only partially inhibits PCD of the NCs, indicating that other mechanisms contribute to the process. One possibility is that the surrounding epithelial follicle cells (FCs) non-autonomously contribute to the death of the NCs during late oogenesis. We have found that disruption of the engulfment receptor *draper*, *ced-12*, or the JNK signaling pathway in the stretch FCs leads to a persisting nuclei phenotype, indicating that these genes are required for NC death and/or clearance. Overexpression of *draper* or a constitutively activated JNKK in the FCs is sufficient to kill the NCs. Furthermore, Draper staining is reduced in JNK pathway mutants, suggesting that they interact to eliminate the NCs. LysoTracker staining shows that the acidification of the NCs that normally occurs during late oogenesis is absent in *draper* mutants, suggesting that Draper may play a role in the death of the NCs. In order to identify other genes involved in PCD and/or clearance in late oogenesis, we are conducting a candidate RNAi screen. We also plan to perform epistasis experiments to determine the pathways that lead to NC death and clearance during late oogenesis. Further investigations are underway to distinguish the role of the FCs in the death vs. clearance of NCs. Developmental PCD of the NCs in the *Drosophila* ovary is a unique example of PCD that may lead to a greater understanding of the careful coordination between death and clearance, as well as forms of PCD that are non-apoptotic.

279C

Molecular characterization of cell competition and compensatory cell proliferation in Drosophila. Li He. Genetics, Harvard Medical School, Boston, MA.

One of the most fascinating questions in biology is how organs achieve and maintain their final sizes, which is continuously regulated by coordinated cell death and proliferation. Two evolutionary conserved and highly correlated mechanisms, cell competition and compensatory cell proliferation, have emerged as playing fundamental roles in this process. Cellular competition is the process by which cells possessing unequal fitness, which can survive if kept alone, compete with each other during tissue growth. Competition between the two cell populations involves active cell killing of the less fit “losers” by the fitter “winners”. Activation of apoptosis in the loser cells in turn triggers compensatory proliferation of the winners, thus maintaining proper organ size. Besides the size-control function, cell competition has also been found to maintain the tissue quality by eliminating mutant cells with oncogenesis potential. In addition, deregulation of cell competition may also promote cancer initiation or metastatic colonization. Studies in the past ten years have implicated a number of signaling pathways such as Jun-kinase, Wnt, Hedgehog, Decapentaplegic(Dpp), and growth regulators such as Myc, Yorkie and ribosomal proteins in these processes. However, despite these advances, we still do not fully understand how loser cells are recognized and eliminated and how winner cells are induced to proliferate. Since cell competition and the ability of dying cells to secrete ligands also occur in tissue culture, we propose to first develop system level methodology to characterize the signaling mechanisms of these processes in drosophila tissue culture and verify the result in vivo using a new mosaic RNAi method for twin spot analysis.

280A

Overexpression of DNA polymerase theta (Pol theta) in Drosophila melanogaster causes reduced hatch rate and sensitivity to nitrogen mustard. Anna Dukhovich, Kelly Beagan, Mitch McVey. Biology Department, Tufts University, Medford, Ma.

DNA polymerase theta (Pol theta), encoded by the *mus308* gene, participates in the repair of DNA double strand breaks in *Drosophila melanogaster* by a mechanism called alternative end joining. Previously, we reported that *Drosophila* with mutated versions of the Pol theta protein are more sensitive to interstrand crosslinking agents (Chan, 2008). The role of Pol theta mainly has been studied by mutating or knocking out *mus308*, but the effect of overexpression has not been considered. Recently, the protein’s overexpression was noted to be significant in DNA stability: in a clinical study of untreated breast cancer patients, Pol theta expression was 3- to 26-fold higher in tumor than in normal tissues (Lemée et al., 2010). The purpose of this project was to investigate the effects of Pol theta upregulation in*Drosophila*. Pol theta was overexpressed by utilizing the UAS-Gal4 system, using either ubiquitin-Gal4 or nanos-Gal4 drivers. The overexpressors were analyzed for phenotypic defects and, in the case of ubiquitous overexpression, tested for sensitivity to interstrand crosslinking agent nitrogen mustard. We found that both types of Pol theta overexpressors have a lower hatch rate, and that Pol theta ubiquitous overexpressors are slightly more resistant to nitrogen mustard. From these results, the effect of Pol theta upregulation in *Drosophila* is presently unclear, but it appears that appropriate Pol theta levels are important for genomic stability. Further investigation of this protein in fruit flies can then be related to the role of Pol theta and its overexpression in human cancer.

281B

DR-*white* measures double-strand break repair pathways in *Drosophila melanogaster*. Jeannine R. LaRocque, Margot Le Neveu, Anthony Do. Department of Human Science, School of Nursing and Health Studies, Georgetown University, Washington,

DC 20057.

A DNA double-strand break (DSB) can result from both exogenous sources and endogenous cellular byproducts. Failure to repair these breaks results in genomic instability that may lead to cell death, mutations, cancer, and aging. DSBs are repaired by several mechanisms: error-free homologous recombination (HR) where an identical sequence is used as a template for repair, non-homologous end joining (NHEJ) where the ends of the DSB are modified and ligated, and single strand annealing (SSA) if the DSB occurs between two DNA repeats. In both yeast and mammalian cells, HR between diverged sequences is suppressed, preserving genomic integrity by preventing aberrant recombination products. DSB repair and the contribution of each repair pathway in the context of a whole genetically tractable organism has yet to be delineated. To address this, two novel reporter assays, DR-*white* and DR-*white.mu*, were integrated into the Drosophila genome. Phenotypic and molecular analyses using DR-*white*can detect induced DSB repair by NHEJ, HR, and SSA. DR-*white.mu* measures gene conversion tract lengths associated with HR and can also be used to detect NHEJ, HR, and SSA in the context of diverged sequences.

We found that Drosophila repair simple DSBs predominantly by HR. Interestingly, gene conversion tract lengths are longer than those reported in mammalian cells. Additionally, HR repair between diverged sequences is suppressed, similar to levels measured in human cells. This work establishes this assay as a useful tool for measuring DSB repair in a whole organism and has the potential to address many future questions, including suppression of recombination between diverged sequences and the link between aging and DNA repair.

282C

The Smc5/6 complex confers resistance to caffeine and genotoxic stress and plays a role in cell cycle regulation and cell survival in *Drosophila melanogaster*. Xiao Li[1], Ran Zuo[2], Stanley Tiong[2], Francesca Di Cara[2], Kirst King-Jones[2], Sarah C. Hughes[1], Shelagh D. Campbell[2], Rachel Wevrick[1]. 1) Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Department of Biological Sciences, University of Alberta.

The SMC5/6 complex consists of Smc5, Smc6 and Non-Smc-Element (Nse) proteins and is important for genome stability in many species. We identified inactivating mutations in *CG5524* and *MAGE*, homologs of genes encoding Smc6 and Nse3 in yeast, from a genetic screen for mutants with reduced resistance to caffeine. *Smc5* mutants are also caffeine-sensitive and Mage physically interacts with*Drosophila* homologs of Nse proteins, indicating that the structure of the Smc5/6 complex is conserved in *Drosophila*. Unlike their yeast counterparts, the *Drosophila* Smc5/6 complex is not essential under normal circumstances, although the mutants are hypersensitive to genotoxic agents such as ionizing radiation, camptothecin, hydroxyurea and MMS, consistent with a conserved role of the Smc5/6 complex in genome stability. We also show that they are not compromised for pre-mitotic cell cycle checkpoint responses. Rather, caffeine-induced apoptosis in these mutants is exacerbated by inhibition of ATM or ATR checkpoint kinases but suppressed by Rad51 depletion, suggesting a novel functional interaction involving homologous DNA repair pathways that deserves further scrutiny. We hypothesize that caffeine treatment and the loss of Smc5/6 synergistically misregulate Rad51 to cause apoptosis in *Drosophila*. In addition, overexpression of MAGE in *Drosophila* developmental eyes results in a small eye phenotype that can be suppressed by co-overexpression of cycE and overexpression of MAGE in S2 cells enhances their resistance to genotoxic agents, suggesting a role in cell cycle regulation and cell survival. Whether the other components of the Smc5/6 complex are involved is under investigation. Our insights into the SMC5/6 complex provide new challenges for understanding the role of this enigmatic chromatin factor in multi-cellular organisms.

283A

Mu2 cooperates with p53 to regulate fusion of dysfunctional telomeres in *Drosophila*. Sarah R. Oikemus, Hannah Pham, Michael Brodsky. Dept PGF&E, Univ Massachusetts, Worcester, Worcester, MA.

p53 plays a conserved role in animals linking the canonical DNA damage response pathway to the cellular machinery that regulates apoptosis, cell cycle control and DNA repair. Animals homozygous for mutations in the ATM homolog, *telomere fusion* exhibit defective telomere protection, leading to chromosome fusions and p53-dependent apoptosis. We find that *p53* promotes non-homologous end-joining (NHEJ) of dysfunctional telomeres and DNA breaks. Genetic analysis demonstrates that *p53* specifically regulates DNA repair choice, only promoting NHEJ when homologous recombination (HR) is available as an alternative repair pathway.

Similar to *p53*, *mu2*, the *Drosophila* ortholog of MDC1 (*mediator of DNA damage checkpoint 1*), also promotes fusion of unprotected telomeres. Simultaneous loss of both *mu2* and *p53* does not have an additive effect suggesting that they act in the same pathway to promote telomere fusions. Analysis of the repair products from endonuclease-induced DNA breaks indicates that loss of *mu2* affects three repair pathways, NHEJ, HR and single strand annealing. Again similar to *p53*, *mu2* only affects repair when HR is available, suggesting a role in DNA repair pathway choice. A previous large-scale screen identified Mu2 as a potential p53 interacting protein. Using an *in vitro* pull down assay, we have mapped the interaction sites to the BRCT repeat domain of Mu2 and the N-terminal activation domain of p53.*in vivo*, we find that Mu2 specifically localizes to unprotected telomeres. We propose that Mu2 acts to recruit p53 to dysfunctional telomeres and DNA breaks and that this interaction helps to regulate DNA repair choice.

284B

Regulation of the translesion DNA polymerase eta by the E3 ubiquitin ligase NOPO. Heather A. Wallace[1], Julie A. Merkle[2], Laura A. Lee[1]. 1) Cell and Developmental Biology, Vanderbilt University, Nashville, TN; 2) Howard Hughes Medical Institute,

Department of Molecular Biology, Princeton University, Princeton, NJ.

We previously identified a *Drosophila* maternal effect-lethal mutant that we named "*no poles*" (*nopo*). Embryos from *nopo* females undergo mitotic arrest with barrel-shaped, acentrosomal spindles during the rapid S-M cycles of syncytial embryogenesis due to activation of a Chk2-mediated DNA checkpoint. Syncytial embryos lacking NOPO exhibit a shorter interphase during cycle 11, suggesting that they may enter mitosis prior to completion of DNA replication. NOPO is the *Drosophila* homolog of mammalian TNF Receptor Associated Factor (TRAF)-interacting protein (TRIP), which has been implicated in TNF signaling. NOPO and TRIP contain RING domains that closely resemble those of known E3 ubiquitin ligases. We sought to elucidate the mechanism by which NOPO/TRIP promotes genomic stability by performing a yeast two-hybrid screen to identify potential substrates/interactors. We identified members of the Y-family of non-canonical DNA polymerases that facilitate replicative bypass of damaged DNA (translesion synthesis) as TRIP interactors. We have shown that *Drosophila* NOPO similarly interacts with *Drosophila* Y-family polymerase eta in cultured cells. Furthermore, we observe enhanced ubiquitination of DNA polymerase eta by TRIP and NOPO E3 ligases in cultured cells. We generated a null mutation in *DNApol-eta* to determine its role during *Drosophila* embryogenesis. Mutations in human Polη result in a variant form of xeroderma pigmentosum, a disease characterized by increased UV sensitivity and skin cancer risk. We found that both *DNApol-eta* and *nopo*-derived embryos show increased sensitivity to UV irradiation. Additionally, *DNApol-eta* embryos exhibit *nopo*-like spindle defects. We show that the decreased hatch rates and spindle defects observed in *nopo*-derived embryos are suppressed by overexpression of DNApol-Eta. These findings suggest that NOPO ubiquitinates DNApol-Eta and may act as a positive regulator of its activity during early embryogenesis.

285C

An mCherry-tagged Gemini Bac transgene provides a biosensor throughout *D. melanogaster* development and a tool for studying Geminin function. Robert C. Eisman, Samantha Young, Melissa A.S. Phelps, Amelia D. Tomlinson, Stacy L. Holtzman, Brian R. Calvi, Thomas C. Kaufman. Dept Biol, Jordan Hall A505, Indiana Univ, Bloomington, IN.

Geminin is a conserved metazoan protein that prevents multiple rounds of DNA replication in a single cell cycle by binding Cdt1 and preventing the assembly of the DNA replication initiation complex. Additionally, normal Geminin function requires a cyclical increase in protein levels during the S and G2 phases of the cell cycle and subsequent degradation of the protein pool. In this study we have made two transgenic fly lines with *gem* Bac clones using the P[acman] system that express either an unmodified GEM or a recombineered GEM::mCherry fusion protein. Both transgenic fly lines survive when homozygous for the Bac in a WT genetic background and rescue *gem* mutant phenotypes, but four copies of gem reduce female fertility and perturb normal cleavage divisions in syncytial embryos. When GEM levels are high mitosis proceeds, but we find chromosome congression and alignment is aberrant, chromatin bridges are common at Anaphase, normal histone modifications are altered, and many nuclei fall out prior to cellularization. In addition to providing a biosensor throughout *D. melanogaster* development, these new *D. melanogaster* transgenic lines, in conjunction with publicly available *gem* mutant stocks, provide a cell cycle marker throughout development and a new tool for future investigations of GEM function and DNA replication in live animals and fixed tissues.

286A

Loss of the Werner's Syndrome exonuclease sensitizes flies to replication stress and promotes tumorigenesis. Mitch McVey[1], Elyse Bolterstein[1], Rachel Rivero[1], Robert Salomon[2]. 1) Tufts University, Medford, MA; 2) Tufts Medical Center, Boston, MA.

Werner's Syndrome (WS) is an autosomal recessive disease that causes accelerated aging and increased susceptibility to cancer in affected patients. WS is caused by mutations in *WRN*, a member of the RecQ family of helicases that contains both a helicase and exonuclease domain and plays critical roles in DNA replication, repair, and the maintenance of genome integrity. The Drosophila melanogaster homolog, WRNexo, consists of only the exonuclease portion of WRN, which provides a unique opportunity to study the exonuclease functions in DNA repair separate from the helicase. We have created a*wrnexo* null mutant in which the entire gene locus is deleted. Flies lacking Wrnexo are not sensitive to the topoisomerase I inhibitor camptothecin or to the double-strand break-inducing agent bleomycin, but are sensitive to hydroxyurea. This suggests that Wrnexo may be important for resolving stalled replication forks but not for repair of two-ended breaks. Furthermore, *wrnexo* mutant embryos have reduced hatch rates and display defects during nuclear divisions in early embryogenesis, consistent with a role for Wrnexo under conditions of high replicative stress. Finally, we have observed that *wrnexo* mutants have increased frequencies of tumor formation in their testes, similar to what is observed in flies lacking the RecQ helicase DmBlm. Currently, we are attempting to determine whether Wrnexo and DmBlm act together or separately during various DNA replication and repair processes. Our ultimate goal is to characterize the division of labor between RecQ orthologs in various organisms.

287B

Functional dissection of Mcm10: exploring the essential functions of a replication factor. Michael C. Reubens, Tim W. Christensen. Biology, East Carolina University, Greenville, NC.

Life depends on a series of highly orchestrated and regulated biochemical processes collectively known as the cell cycle. It is through these heavily regulated stages that cells grow, divide, and accurately transmit their genetic material. The preservation of cellular identity and genomic stability through these stages requires that DNA replication take place with high fidelity, and

that chromatin states are accurately passed from one generation to another to ensure the proper transcriptional state of the resulting cells. It has become more apparent that the processes of DNA replication and the establishment of epigenetic chromatin states are more intimately linked than once thought. A protein common to both processes, Mcm10, has become an interesting avenue of research in an attempt to better understand the dynamic link between these two processes. By utilizing an established collection of 29 independent Mcm10 mutant fly lines consisting of twenty two missense point mutations, four truncation alleles, two homozygous lethal alleles, and one hypomorphic allele we have begun to elucidate regions of this conserved protein that are either essential for, or dispensable for, given biological functions. Analysis of a hypomorphic allele demonstrates that reduced protein levels result in abnormal chromosome condensation phenotypes. Our truncation alleles have suggested that the only the N-terminal 388 amino acids of the protein are required for viability; however, the C-terminal 388 amino acids are required for female specific fertility, and the extreme C-terminal 65aa are required for proper endoreplication. The C-terminal 388 amino acids have been shown to contain a region important for the interaction with HP1 which overlaps with regions important for genomic stability, heterochromatin formation, and contains two independent homozygous lethal alleles. It is our hope that further analyses using this mutant collection will shed light on the essential nature of Mcm10 in Drosophila, and aid in a better understanding of replication, chromosome biology, and potentially oncogenesis.

288C

Investigating the Interaction of RecQ4 and Mcm10 in Drosophila melanogaster. Wayne A. Rummings, Tim W. Christensen. Biology, East Carolina University, Greenville, NC.

Instability of the genome through misregulation of the highly orchestrated events of the cell cycle is thought to play an important role in the development and progression of cancer and has also been implicated in the aging process. RecQ4 is one of the five RecQ helicases found in humans. It is a 1208 amino acid protein with a highly conserved Superfamily II (SFII) helicase domain that is important for maintaining cell viability. Mutations in the helicase domain of the conserved protein lead to distinct clinical diseases with increased cancer rates and premature ageing. The protein also has a unique N terminus with a 200 a.a. sequence that shares homology with yeast DNA replication initiation factor, Sld2. RecQ4 is the least characterized RecQ protein and recent studies have shown its role not only in DNA unwinding but with DNA damage repair and telomere maintenance. Given these potential roles, especially in replication, efforts have focused on elucidating specific protein-protein interactions that provide insight into the cellular processes in which RecQ4 may be involved. The mini-chromosome maintenance protein (Mcm10), a highly conserved protein first discovered in Saccharomyces cerevisiae, has essential roles in DNA replication and heterochromatin formation. Work in 293T cells and in Xenopus extracts shows a direct interaction between the two proteins with Mcm10 mediating RecQ4's association with the Mcm2-7 helicase and GINS complex. Taken together, it is of interest to determine if an interaction exists between RecQ4 and Mcm10 in the genetic model organism, Drosophila melanogaster. To confirm the interaction of the two proteins a yeast two-hybrid approach will be implemented to analyze the protein interaction. In addition genetic interactions will be tested using flies with mutations in both proteins. The use of these studies will aid in dissecting the cellular functions of these essential proteins along with increasing our understanding of the mechanisms of the disease states resulting from their associated defects.

289A

Genome damage triggers non-canonical cell death during Drosophila polyploid mitosis. Heidi Bretscher, Don Fox. Duke University, Durham, NC.

Maintaining a stable genome prevents damaged DNA, altered cellular function, and ultimately diseases such as cancer. Genome instability is monitored by a checkpoint regulated by the tumor suppressor p53, which prevents cells with damaged genomes from progressing into mitosis, where such damage can contribute to chromosome number imbalance (aneuploidy). This p53-dependent checkpoint is inhibited in murine trophoblast cells, several larval Drosophila tissues as well as certain cancerous cells, allowing cell cycling despite DNA damage. All of these cell types undergo endoreplication. During endoreplication cells alternate between G and S phases thus increasing in ploidy but not cell number. In contrast to endoreplicating cells in cancer, most programmed endocycling cells are terminally differentiated and do not re-enter the mitotic cell cycle, preventing study of connections between polyploidy, DNA damage, and aneuploidy. We previously established a model to address mechanisms by which polyploidy promotes genome instability during mitosis. In the Drosophila rectum, we found endoreplicated cells can re-enter mitosis as polyploid cells, but that such divisions are error-prone. Given this new connection between endoreplication and genome instability, we next examined the status of the p53 checkpoint in endoreplicating and mitotic rectal cells. Like other endoreplicating cells, we find Drosophila rectal cells tolerate significant DNA damage. p53 over-expression does not sensitize rectal cells to cell death, indicating this canonical death pathway is silenced in rectal cells. However, when rectal cells with severely damaged genomes enter mitosis, they undergo cell death. Unlike in diploid cells, we find such death is caspase- and p53- independent. However, this death is dependent on re-entry into the mitotic cell cycle. Our data suggest genome damage in naturally occurring polyploid cells can trigger Mitotic Catastrophe (MC), a poorly understood cell death mechanism. Lack of this mechanism could thus contribute to expansion of cancerous polyploid cells.

290B

Interactions between purine synthesis and cell death pathways. Denise V. Clark, Ashley M. DiPasquale. Dept of Biology,

Univ New Brunswick, Fredericton, NB, Canada.

Mutations affecting *de novo* synthesis of purine nucleotides have a pleiotropic phenotype. Surviving adults have wing and leg defects, reduced bristles, and reduced red eye pigmentation. More severe mutations cause arrest in prepupal and pupal stages, often with development of necrosis in wing and leg discs. We are interested in determining the pathways that lead to the development of this phenotype. We previously found that the pupal lethality and necrosis phenotypes have a link with apoptosis, since they are dependent on caspase activity, and since prepupal wing discs show apoptotic nuclei prior to development of the necrosis. These phenotypes do not appear dependent on *p53* or the apoptosis effector region containing the *reaper*, *grim* and *hid* genes [1]. To explore further this link between reduced purine synthesis, lethality, and apoptosis, we examined other apoptosis pathway genes for interactions with reduced purine synthesis. To reduce purine synthesis, we focused on the *ade2* gene, which encodes the 4th step in the purine de novo synthesis pathway, and a deletion allele $ade2^{1-6}$. For apoptosis, we focused on Drosophila inhibitor of apoptosis protein 1 (DIAP1, or *thread*). Counter to our predictions, over-expression of DIAP1 enhances the early pupal arrest and degree of necrosis, whereas under-expression in *thread* mutant heterozygotes partially rescues both of these phenotypes. This result is leading us to explore the role of other cell death pathways in the development of the purine synthesis phenotype. In addition to DIAP1, we also explored the role of HtrA2, a mitochondrial serine protease that can activate DIAP1, in the purine syndrome phenotype. *HtrA2* RNAi did not suppress pupal necrosis; however, it suppressed the lethality of $ade2^{1-6}$. Further characterization of the lethal phenotype of $ade2^{1-6}$showed death of at least half of the mutants in third instar larvae. Our results suggest that HtrA2 may have a role in mediating the response to reduced purine synthesis during larval development. [1] Holland et al (2011) Genetics Jun;188(2):359-67.

291C

***Cdc20/fizzy* maintains neural stem cells by suppressing necrotic cell death.** Cheng-Yu Lee[1,2,3,4], Chaoyuan Kuang[4,5]. 1) Center for Stem Cell Biology, Life Sciences Institute; 2) Division of Molecular Medicine and Genetics, Department of Internal Medicine; 3) Department of Cell and Developmental Biology; 4) Program in Cellular and Molecular Biology,; 5) Medical Scientist Training Program, University of Michigan Medical School, Ann Arbor, MI 48109.

Mechanisms preventing precocious differentiation are indispensable for stem cell maintenance, but nothing is known about the cell survival mechanisms required for preserving a steady stem cell pool. Here, we show that Cdc20/Fizzy (Fzy), a conserved activator of the Anaphase-Promoting Complex/Cyclosome (APC/C), functions to maintain neural stem cell (neuroblast) viability in *Drosophila* larval brains independently of its well-established role in promoting cell proliferation. While a novel *fzy* mis-sense mutation has no effects on the maintenance of stem or precursor cell identity, it leads to programmed necrosis in neuroblasts as indicated by ultrastructural changes and molecular marker expression. Consistently, removing genes critical for the activation of apoptosis or autophagy does not suppress the loss of neuroblasts in *fzy* mutant brains. The point mutation occurs in the WD40 domain of Fzy but is not associated with the surfaces required for recruiting canonical Fzy substrates, suggesting that a novel APC/C-Fzy substrate is responsible for loss of neuroblasts. Importantly, neuroblasts lacking the APC/C function also undergo premature necrotic cell death. Finally, inactivating c-Jun N-terminal Kinase (JNK) signaling or removing Apoptosis inducing factor (Aif) function significantly prolongs survival of the fzy mis-sense mutant neuroblasts. Thus, Fzy suppresses neuroblast necrotic cell death by antagonizing multiple downstream pathways via an APC/C-dependent mechanism during Drosophila larval brain neurogenesis.

292A

Drosophila p53 isoforms differentially regulate apoptosis and apoptosis-induced proliferation. Bertrand Mollereau[1], Marie-Laure Dichtel-Danjoy[1], Dali Ma[1], Pierre Dourlen[1], Gilles Chatelain[1], Francesco Napoletano[1], Marion Robin[1], Marlene Corbet[1], Clemence Levet[1], Hind Hafsi[2], Pierre Hainaut[2], Hyung Don Ryoo[3], Jean Christophe Bourdon[4]. 1) LBMC UMR5239, Ecole Normale Superieure, Lyon, France; 2) International Agency for Research on Cancer, Lyon, France; 3) Department of Cell Biology, New York University School of Medicine, New York, NY, USA; 4) European Associated Laboratory University of Dundee/Inserm U858, Department of surgery and Molecular Oncology, Dundee, DD1 9SY UK.

Epithelial tissues have the intrinsic capability to repair and regenerate following irradiation or genetically induced cell death. However, how epithelial cells respond to injury and recover is not well understood. In the past few years, studies from metazoan models such as Drosophila forged the concept of apoptosis-induced proliferation, a process by which damaged cells entering apoptosis signal the surrounding unaffected cells to divide so to recoup the tissue loss. Importantly, the findings made in Drosophila have greatly impacted the understanding of tumor repopulation during cancer irradiation and also the process of regeneration in vertebrates. In Drosophila, apoptotic cells play an active role in proliferation, where the caspase Dronc (caspase 9 homolog) and p53 induce mitogen expression and growth in the surrounding tissues. The Drosophila p53 gene structure is conserved and encodes at least two protein isoforms: a full-length isoform (Dp53) and an N-terminally truncated isoform (DΔNp53). Historically, DΔNp53 was the first p53 isoform identified and was thought to be responsible for all p53 biological activities. Here, we investigated the roles of Dp53 and DΔNp53 in apoptosis and apoptosis-induced proliferation. Understanding the roles of Drosophila p53 isoforms in apoptosis and in apoptosis-induced proliferation may shed new light on the roles of p53 isoforms in humans, with important implications in cancer biology. The ability of apoptotic cells to secrete mitogenic signals may be of major significance in regeneration processes and in the development of tumors.

293B

Anastasis: An unexpected route to rescue dying cells, and its physiological and pathological implications. Ho Lam Tang,

Ho Man Tang, Denise Montell. Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD.
The programmed cell death process known as apoptosis (Greek for "falling to death") plays critical roles in normal embryonic development and adult homeostasis. Impaired apoptosis causes cancer whereas excess apoptosis contributes to major diseases such as neurodegeneration. Apoptosis is thought to be irreversible after mitochondrial permeabilization and effector caspase activation because mitochondrial dysfunction, alone can lead to cell death and caspases cause massive destruction of structural and functional cellular components including the genome. However, this assumption has not been fully tested. Here, we report the discovery of reversal of late stage apoptosis in tissue culture cells, and we called this process anastasis (Greek for "rising to life"). Dying cells can reverse apoptosis, survive, and proliferate, even after they pass through critical checkpoints generally thought to be the point of no return, including mitochondrial permeabilization and caspase activation. Simply removing the apoptotic inducers by washing is sufficient to allow the vast majority of dying cells to arrest the apoptotic process and survive. Notably, while many cells recover completely, some of the cells that reverse apoptosis acquire permanent genetic alterations and undergo oncogenic transformation at a higher frequency than controls. We have developed a Drosophila anastasis biosensor to detect and track reversal of apoptosis, and identify anastasis in live Drosophila after environmental insult that induces apoptosis. We propose that anastasis could participate in various physiological and pathological conditions. For example, anastasis could be an unanticipated mechanism to protect cells that are difficult to replace, such as mature neurons in the aging brain or cardiomyocytes in adult heart cells. At the same time, the DNA mutations that persist following anastasis represent a form of stress-induced mutagenesis, which may result carcinogenesis and evolution of drug resistance following chemotherapy.

294C

Generation of transaldolase knockdown Drosophila in the apoptosis study and screening for the apoptosis modifiers. Yi-Chun Chen, Tzu-Li Yen, Ju-Ching Yu, Horng-Dar Wang. Institute of Biotechnology, HsinChu, Taiwan.
Transaldolase is the rate-limiting enzyme in the non-oxidative branch of pentose phosphate pathway. While deficiency of transaldolase has been implicated in enhancing apoptosis in cell culture, yet there is no reported genetic phenotypic study of transaldolase knockdown mediated apoptosis in Drosophila. Here, we showed the novel phenotypes, the posterior bulgy eyes and the wrinkled wings, upon RNAi knockdown of transaldolase by eye-specific GMR-Gal4 and wing-specific MS1096-Gal4 drivers respectively. In order to examine whether the phenotypes by the knockdown of transaldolase are due to apoptosis, we knockdown each of the apoptosis-related genes, p53, hid, and Dronc, simultaneously in the transaldolase knockdown flies. Blocking the expression of the apoptosis-related genes rescues the phenotypes by the knockdown of transaldolase back to normal, suggesting the specific phenotypes are triggered by apoptosis. Biochemical analysis by acridine orange confirms the phenotypes are consequences of apoptosis. We have generated the viable GMR-Gal4;UAS-TalRNAi homozygous line to screen about 400 RNAi knockdown fly lines for the modifiers either suppressing and enhancing the bulgy apoptotic eye by transaldolase knockdown. We have identified a number of novel modifiers which genetically interact with the transaldolase knockdown induced apoptosis. Future large-scale screening will further provide more new novel molecular targets for the treatment and prevention of metabolic diseases and cancer.

295A

JAK/STAT signaling controls loss of polarity and apoptosis for elimination of supernumerary polar cells in the *Drosophila* ovary. Anne-Marie Pret[1,2], Antoine Borensztejn[1,3], Alba Torres[1,4], François Agnès[1,4]. 1) Centre de Génétique Moléculaire, CNRS UPR3404, Gif-sur-Yvette, France; 2) Université de Versailles-St Quentin, Versailles, France; 3) Université Pierre et Marie Curie, Paris, France; 4) Université Paris-Sud, Orsay, France.
Apoptosis is a widespread form of cell death, which allows precise destruction of cells preserving tissue architecture and integrity. *Drosophila* polar cells (PCs) are specialized pseudo-epithelial cells at ovarian follicle antero-posterior extremities, which are produced in excess (up to 6 cells) and restrict to exactly 2 cells by apoptosis. Reduction of PC number to 2 is necessary for subsequent recruitment of the correct number of border cells and their migration with PCs to the oocyte where they will form the micropyle, the sperm entry point into the oocyte. We have shown that supernumerary PC apoptosis is induced by cell autonomous and non-cell autonomous JAK/STAT-dependent activation of a canonical apoptosis cascade involving transcriptional activation of *hid*, leading to downregulation of Diap1 and consequent activation of executor caspases. Using cell polarity markers, we show that supernumerary PC elimination first involves full envelopment by neighboring PCs, accompanied by apical constriction with stereotyped anisotropy, concomitant with apical detachment followed by rounding up and shrinking. Our current work is aimed at establishing the molecular link(s) between JAK/STAT signaling, loss of PC polarity and PC apoptosis.

296B

How Do Endocycling Cells Block Apoptosis? Bingqing Zhang, Brian R. Calvi. Biology, Indiana University, Bloomington, IN.
Eukaryotic cells employ multiple checkpoints to preserve genome integrity. Apoptosis, which is one type of programmed cell death, is triggered by excessive DNA damage and considered a major barrier to genome instability and cancer. An important remaining question is how cell cycle programs and checkpoints differ among cells in development. Using *Drosophila melanogaster* as a model system, we have found that endocycling cells, which only go through G and S phases, do not apoptose in response to DNA damage. Also unlike mitotic cycling cells, endocycling cells do not engage apoptosis after over-expression of p53, but do apoptose after over-expression of the pro-apoptotic genes. This suggests that apoptosis is repressed because

p53 cannot induce transcription of its target genes at the H99 locus. In support of this, qPCR and promoter reporters indicated that H99 gene expression is repressed in endocycling cells. Chromatin immunoprecipitation (ChIP) using antibodies against modified histones demonstrated an increase in silencing marks and depletion of activating marks in the endocycling cells. In addition, initial results from our genetic screen showed that knockdown of several genes that encode epigenetic silencing proteins sensitize salivary gland endocycling cells to p53 over-expression. Recent genome annotation suggests that p53 encodes different protein isoforms. Our preliminary data implicate that different p53 protein isoforms have different abilities to induce apoptosis. We are currently using a combination of genetic and biochemical methods to further characterize the regulation and function of these p53 isoforms. This study is providing general insights into the developmental regulation of the cellular response to stress and the decision to activate the apoptotic pathway.

297C

Regulation of life or death fate in Drosophila neural stem cells. Richa Arya, Ying Tan, Hsiao-Yu Huang, Francisca Rodriguez, Tatavik Keshishyan, Megumu Yamada-Mabuchi, Kristin White. CBRC, MGH/HARVARD, CHARLESTOWN, MA.

Whether to survive or die is a critical decision cells make during development. Although the canonical apoptotic pathways are well characterized, very little is known about how these pathways are activated only in "doomed" cells. Apoptosis is a major process that shapes the developing nervous system in many animals. Our lab is studying how the spatial and temporal regulation of developmental apoptosis takes place in the Drosophila neural stem cells or neuroblasts(NBs). We found that the apoptotic activators reaper (rpr) , grim, and sickle (skl) are required for the normal death of NBs in the abdominal region of the ventral nerve cord. We have genetically identified a 25kb NB specific cis-regulatory region (NBRR) for rpr, grim, and skl that is necessary for the elimination of these cells. Based on evolutionary conservation and available ChIP data, we selected a 5kb portion of this region to generate GFP-reporter flies (NBRR1-GFP). NBRR1-GFP is expressed in a subset of abdominal NBs in the late embryo, in cells that also express rpr and grim. This strongly supports the idea that this region includes cis-regulatory sequences for the regulation of rpr and grim in doomed NBs. To identify the upstream regulators that could directly and/or indirectly regulate the NBRR, we performed an open ended RNAi screen for transcriptional regulators that are required for the apoptosis of embryonic abdominal NBs. We have identified a number of candidates. Based on GO function these candidates fall in various functional categories from CNS development to chromatin remodelling. In the study we have described the apoptotic genes and the regulatory region necessary for NB apoptosis. Currently we are asking how various upstream regulators identified in our screen are involved in initiating the death of specific cells during development.

298A

Dpp signaling counteracts JNK-dependent apoptosis caused by epithelial disruption. Jorge V. Beira[1,2], Jean-Paul Vincent[1]. 1) Developmental Biology Division, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, NW7 1AA, London, United Kingdom; 2) Research Department of Cell and Developmental Biology, University College London, Gower Street, London, United Kingdom.

A dynamic balance between cell proliferation and cell death is crucial to maintain tissue homeostasis throughout embryonic development and adult life. Homeostatic apoptosis contributes to eliminate abnormal or defective cells, ensuring they do not spread uncontrollably. While the cellular machinery executing apoptosis has been relatively well characterized, our understanding of the upstream signals regulating homeostatic apoptosis is still fragmentary. For example, how the removal of apical determinants from the embryonic epidermis leads to apoptosis remains poorly understood. We have shown that JNK is a key mediator in this process: it is activated by epithelial disruption, as shown with a JNK sensor, and it itself leads to activation of reaper expression. Interestingly, JNK signaling does not trigger reaper expression throughout the epidermis. In the dorsal epidermis no reaper is expressed despite high level JNK signaling. We provide evidence that this could be because Dpp signaling, which is highly active in this region of the embryo, prevents rpr transcription. Our data suggest that a simple gene regulatory network integrating JNK- and Dpp-dependent inputs regulates reaper transcription and apoptosis. This network forms a bi-stable switch that enables JNK signaling to direct distinct outcomes, cell death or migratory activity, according to the local environment. The interplay between these conserved pathways and the apoptotic machinery could have implications for the elimination of pre-tumoral cells in vertebrates.

299B

A novel screen to identify regulators of cell competition in Drosophila. Justin A. Bosch, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

Cell competition is a phenomenon observed in Drosophila that results in the removal of cells from a developing tissue. The molecular mechanism of cell competition is not well understood and requires identifying novel molecules that allow cells to detect and respond to competitive ability. We have devised a novel genetic assay in Drosophila, named CoinFLP, to systematically screen for genes affecting cell competition by gene overexpression or RNAi knockdown. This system uses the Gal4/UAS system to misexpress a gene of interest in the Drosophila eye, and the FLP/FRT system to ensure this gene misexpression occurs only in a subset of the tissue. Two possible recombination events in the eye imaginal disc produce patches of cells that either express Gal4 or not. To facilitate easy scoring of cell competition phenotypes, Gal4 expressing cells are marked as white in the adult eye with UAS-whiteRNAi. Initial screening efforts have focused on genes encoding secreted or transmembrane proteins, since cell competition is thought to involve extracellular cell-cell communication.

300C

"Divide and rule": cell mixing induced by winner cells is required for loser cell elimination during cell competition. Romain V. Levayer, Eduardo Moreno. IZB institute für Zellbiologie, University of Bern, Bern, Bern, Switzerland.

Cell competition is the mechanism by which suboptimal cell are removed from a growing tissue through apoptosis. The molecular mechanism driving the recognition of surviving cells ("winners") and the cell that will die ("losers") has become an intensive field of research in the past years. An increasing number of pathways modulating cell fitness and driving cell competition has been characterized in Drosophila, yet we still know very little regarding the core downstream events leading to cell elimination. Several observations in vivo and in cell cultures have shown the requirement of close contact between loser and winner cells in order to drive loser cell elimination. For instance, apoptotic cells are preferentially localized at the boundary of loser clones, and loser cell elimination is more efficient when surrounded by multiple winner cells. Yet, we still lack clear evidences showing that direct physical contact between loser and winner cells is required to drive loser elimination. Other early observations also remained unexplained so far, including: 1.The absence of competition across compartment boundary, 2.The fragmentation of loser clones. Here, we propose that loser cell elimination is controlled by the surface of contact shared with winner cells. The winner/loser surface of contact is actively increased during cell competition by the activation of loser/winner cell mixing, which is induced upstream of apoptosis. This model would explain the restrictive effect of compartment on cell competition (by preventing cell mixing) and the appearance of fragmented clones during cell competition. We will present evidences supporting this model based on : 1. Systematic quantification of clone shape in the wing disc during cell competition and in absence of apoptosis, 2. Live imaging of competition performed in the pupal notum, 3.Effect of cell mixing regulators on cell competition.

301A

Analysis of Yorkie activity in *scribble* mutant cells challenged with different cell competitive environments. Indrayani Waghmare[1], Shilpi Verghese[1], Alyssa Lesko[2,3], Amit Singh[1,4,5], Madhuri Kango-Singh[1,4,5]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) University of Dayton Honors Program, Dayton, OH; 3) Department of Chemistry, University of Dayton, Dayton, OH; 4) Pre Medical Program, University of Dayton, Dayton, OH; 5) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

The Hippo pathway is responsible for regulating organ size through regulating the expression of a diverse array of target genes, and is conserved from flies to humans. Recent studies suggest a role fro Hippo signaling in maintaining tissue homeostasis and cell-cell interactions. *scribble* (*scrib*) is a neoplastic tumor suppressor gene that regulates growth and maintains apical-basal polarity. *scrib* acts downstream of Fat to regulate Warts activity in the Hippo pathway. Loss of function of *scrib* shows distinct phenotypes of survival and growth depending on the genetic background making it ideal to study local cell-cell interactions. Somatic clones of $scrib^{-/-}$ cells face cell-competition through JNK mediated apoptosis. We studied somatic clones of $scrib^{-/-}$ in various competitive backgrounds that improved survival (over expression of P35, Hippo pathway loss of function, Ras gain of function) or reduced growth rate of the surrounding cells (*Minute/+*). We found that additional mutations in $scrib^{-/-}$ cells caused them to behave like super-competitors. Further, we found that the super-competitive trait is coupled with regulation of Hippo pathway target genes. We hypothesize that the different growth phenotypes are generated by local cell-cell interactions due to differential regulation of Yki activity levels between the mutant clone and the surrounding wild-type cells. To test this hypothesis we have studied the Yki mediated regulation of target genes during super-competition. We have also tested the requirement of differential Yki activity in the growth response of the mutant cells. Our results suggest that Yki activity levels determine the nature of competitive interaction.

302B

The bHLH proteins Emc and Da control cell cycle progression through the transcriptional regulation of the Cdc25 phosphatase string, during Drosophila development. Irene Andrade-Zapata, Antonio Baonza. Centro de Biología Molecular Severo Ochoa, Madrid, Spain.

The Helix-Loop-Helix (bHLH) family of transcription factors are key regulatory molecules that control multiple developmental processes, including cell differentiation and cell cycle control. They form heterodimers interacting through its HLH domain and are subdivided into groups, attending to their function, distribution, and DNA binding properties. Class V HLH proteins lack any basic domain, and as a consequence, heterodimers of class V proteins with other bHLH proteins are unable to bind DNA. Drosophila has a single class V protein, Extramacrochaetae (Emc), that is homologue to inhibitor of DNA binding (Id) proteins in vertebrates. It has been proposed that the function of this gene is necessary to maintain a proliferative state during organ development. Emc is known to form heterodimers with the class I protein Daughterless (Da). Recently, Bhattacharya and Baker (2011) have proposed that a cross-interacting regulatory network links expression of Da, which regulates its own expression, with expression of Emc, which antagonizes Da function. These authors suggest that most phenotypic effects, including cell proliferation defects, of mutating *emc* are due to the up-regulation of Da in *emc* mutant cells. However, the mechanisms by which this network regulates cell proliferation remain still unknown. In this work, we found that the reduction of *emc* or the over-expression of Da produces an accumulation of cells on G2 phase of the cell cycle. The main activator of the G2/M transition in eukaryotic cells is the *string* (Cdc25) phosphatase. We present evidences that indicate that the arrest in G2 phase of *emc* mutant cells and*da* over-expressing cells is a consequence of a reduction of *string* expression. Our results indicate that Da binds to string promoter, and function as a transcriptional repressor. We provide the first molecular mechanism to explain how the HLH proteins Emc and Da control cell proliferation during development.

303C

Nutrition/TOR signaling promotes growth via the conserved Pol I transcription factor, TIF-IA in Drosophila. Abhishek Ghosh, Savraj S. Grewal. Clark H. Smith Brain Tumor Centre, SACRI, University of Calgary, Calgary, AB, T2N 4N1, Canada.

The conserved Target of Rapamycin (TOR) kinase signaling pathway links nutrition to growth in Drosophila. The upstream components of the TOR pathway are known. In contrast, the downstream effectors via which TOR promotes growth are less clear. We are exploring the role of ribosomal RNA (rRNA) synthesis as a growth regulatory target of the nutrition/TOR pathway in Drosophila. Studies in yeast, Drosophila and cultured cells showed that the conserved RNA polymerase I (Pol I) transcription factor TIF-IA links nutrition/TOR to rRNA synthesis. We have found that amino acid starvation leads to reduced TIF-IA transcript and protein levels in Drosophila larvae. These effects of starvation on TIF-IA levels are phenocopied in tor null larvae, but not in S6 kinase (a known TOR effector) mutant larvae. These results suggest that TOR promotes rRNA synthesis via controlling TIF-IA gene expression. Currently, we are investigating how TOR stimulates TIF-IA transcription. In addition to cell autonomous growth, TOR activity in specific tissues such as fat and muscle is promotes systemic growth. For example, in Drosophila, TOR activity is required in the fat body (adipose tissue) to trigger an endocrine response leading to the release of Drosophila insulin like peptides (dILPs) from brain. These dILPs circulate and promote growth in peripheral tissues via the PI3K/Akt signaling pathway. We have found that genetic knockdown of TIF-IA in either fat or muscle leads to reduced larval growth and delayed development, phenocopying loss of TOR. In addition, fat and muscle specific TIF-IA knockdown larvae have altered expression of brain-derived dILPs and Foxo target genes, consistent with reduced systemic insulin signaling. These data suggest that TIF-IA activity and hence, ribosome synthesis may be required for the non-autonomous, endocrine effects of TOR signaling. Overall, this study highlights the role of TIF-IA as a key effector of TOR signaling in the control of tissue and organismal growth.

304A

Transcriptional Mediators of Growth and Survival Downstream of the Target of Rapamycin (TOR) Pathway. Lauren E. Killip, Savraj Grewal. Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada.

Nutrition is essential for growth and survival in animals. The conserved Target-Of-Rapamycin (TOR) kinase signaling pathway is the central nutrient-sensing pathway that controls metabolism to promote either cell growth or survival. When nutrients are abundant, TOR is active and stimulates metabolism to drive cell, tissue and body growth. Upon nutrient deprivation, TOR is inactivated and the animals switch their metabolism to promote survival. Our lab studies mechanisms downstream of TOR that mediate the metabolic switch between growth and survival. One potential mechanism is the regulation of gene expression. We have focused on identifying transcription factors that regulate metabolic gene expression downstream of TOR in Drosophila. One transcription factor required for TOR-dependent growth is DREF. We found that DREF levels are decreased in conditions of reduced TOR signaling and that loss of DREF leads to decreased organismal growth. These effects are due in part to a requirement for DREF function in cell-autonomous growth. We also uncovered a non-autonomous role for DREF activity in the larval fat body where the tissue-specific loss of DREF leads to reduced systemic insulin signaling, slow larval growth and smaller final size. This result phenocopies the effects of starvation and loss of TOR and is consistent with previous findings that fat-body specific activation of TOR couples nutrition to insulin release from the brain. In addition, we showed that DREF is required for expression of many ribosome biogenesis genes, suggesting that DREF may link nutrient availability and TOR activation to tissue growth by stimulating protein synthesis. We are also exploring transcriptional mechanisms that may mediate responses to nutrient starvation. We have identified several transcriptional and translational regulators whose expression, in contrast to DREF, is strongly upregulated upon starvation. We are currently exploring the role of these transcription factors in mediating homeostasis following nutrient deprivation to promote organismal survival.

305B

Scribble acts in the Drosophila Fat-Hippo pathway to regulate Warts activity. Shilpi Verghese[1], Indrayani Waghmare[1], Hailey Kwon[1], Katelin Hanes[1], Madhuri Kango-Singh[1,2,3]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) Pre-Medical Programs, University of Dayton, Dayton OH; 3) Center for Tissue Regeneration and Engineering at Dayton, University of Dayton, Dayton OH.

Hippo pathway regulates organ size from flies to mammals through the transcriptional co-activator Yorkie (Yki). The pathway controls gene expression and growth regulation by controlling the nuclear availability of Yki by several alternate mechanisms (e.g., sequestration of Yki in the cytoplasm by Warts (Wts) phosphorylation following hyper-activation, or by binding of Expanded (Ex) and Yki resulting in its membrane localization). Several Hippo pathway components (like, Fat (Ft) and Ex) localize to cell junctions organized by three distinct protein complexes that maintain epithelial sheet integrity and aid in signaling interactions. Amongst the junctional proteins, Crumbs (Crb), atypical Protein Kinase C (aPKC), Scribble (Scrib) and Lethal giant larvae (Lgl) are known to interact with Hippo pathway to regulate growth. However the molecular mechanisms of these interactions are largely unknown. *scrib* is a neoplastic tumor suppressor gene known to regulate growth and apico-basal polarity in cells. Loss of *scrib*causes neoplastic tumors while *scrib* mutant cells challenged with wild type cells get eliminated attributing differential growth properties to *scrib* mutant cells. Recent studies have shown that *scrib* interacts with the Hippo pathway and loss of *scrib* affects expression of Hippo target genes. Furthermore, both in flies and mammalian model systems, Scribble has been shown to act upstream or parallel of Warts and Scribble requires Yki to regulate its growth functions.

However, the mechanism by which Scribble regulates growth via Hippo pathway remains unclear. Using the GAL4-UAS system and transgenic RNAi approach, we show that Scrib acts downstream of Ft. We also show that Ft requires Scrib to interact with Ex and Dachs (D), and for regulating Wts levels and stability, thus placing Scrib in the Hippo pathway network.

306C

Drosophila *RNase Z^{L}* is involved in cell growth and cell cycle progression. Xie Xie, Edward Dubrovsky. Biological Sciences, Fordham University, Bronx, NY.

The RNase Z enzyme is a highly conserved endoribonuclease expressed in all living cells. Previously, we reported the identification and biochemical analysis of dRNaseZ, the *Drosophila* homolog of the long form of dRNase Z^{L}. Knockdown of dRNaseZ by RNAi impaired larval growth and development causing death during the second larval molt. To clarify further the role of dRNaseZ in fly development, we have now isolated and characterized the knockout allele, *RNZ^{ED24}*. KO flies can be rescued by a dRNaseZ-expressing transgene controlled by UAS, HS, or native promoters. Using fully functional V5-tagged genomic transgene, we followed the expression of dRNaseZ at the protein level, and found dRNaseZ is highly abundant in dividing cells. By utilizing a conditional rescue system, we studied the requirement of dRNaseZ in adult stage and found dRNaseZ KO affect flies fertility. Combining FLP/FRT technique and conditional rescue system, we found RNaseZ is required for cell growth in endoreplicating tissue. RNaseZ KO affects protein synthesis through tRNA nuclear accumulation. While in mitotic tissues, RNaseZ is required for cell proliferation. RNaseZ KO cells are arrested at G2/M transition. We therefore conclude that dRNaseZ protein is required for endoreplicating cell growth and mitotic cell proliferation.

307A

Activated STAT regulates growth and induces competitive interactions independently of Myc, Yorkie, Wingless and ribosome biogenesis. Tamara Zoranovic[1], Aloma Rodrigues[1], Aidee Ayala-Camargo[1], Savraj Grewal[2], Tamara Reyes-Robles[1], Michelle Krasny[1], D. Christine Wu[3], Laura Johnston[3], Erika Bach[1]. 1) Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY, USA; 2) Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada; 3) Department of Genetics and Development, Columbia University, New York, NY, USA.

Cell competition is a conserved mechanism that regulates organ size and shares properties with the early stages of cancer. In Drosophila, wing cells with increased Myc or with optimum ribosome function become supercompetitors that kill their wild-type neighbors (called losers) up to several cell diameters away (1,2). Here, we report that modulating STAT activity levels regulates competitor status. Cells lacking STAT become losers that are killed by neighboring wild-type cells. By contrast, cells with hyper-activated STAT become supercompetitors that kill losers located at a distance in a manner that is dependent on hid but independent of Myc, Yorkie (3-5), Wingless (6) signaling, and of ribosome biogenesis (7-9). These results indicate that STAT, Wingless and Myc are major parallel regulators of cell competition, which may converge on signals that non-autonomously kill losers. As hyper-activated STATs are causal to tumorigenesis and stem cell niche occupancy, our results have therapeutic implications for cancer and regenerative medicine.

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308B

Polyploid Hindgut Cells in *Drosophila* Undergo Multipolar Mitosis and Tolerate Aneuploidy. Kevin Schoenfelder[1], Ruth Montague[2], Sarah Paramore[2], Donald Fox[1,2]. 1) Duke University Program in Genetics and Genomics, Durham, NC 27710; 2) Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC 27710.

Polyploid cells, which contain duplications of their genome, account for most of the biomass of the planet, yet the role and consequences of polyploidy are poorly understood. Polyploidy may be generated by many mechanisms, including entry into an alternate version of the cell cycle that skips M phase, known as the endocycle. Our lab's preliminary data demonstrate that the endocycle can result in the amplification of centrosomes, which organize the accurate segregation of chromosomes during mitosis. In 1902, Theodor Boveri proposed that extra centrosomes cause multipolar cell division, subsequent chromosomal imbalance (aneuploidy), and cancer. Since his hypothesis, the viability of such multipolar divisions has been controversial. In either diploid mammalian tumor cell lines or in the Drosophila brain, multipolar metaphases generally resolve into bipolar anaphases, calling into doubt the multipolar cancer generation hypothesis. Centrosome amplification has also been implicated in producing aneuploidy in polyploid cells. However, it remains unclear whether polyploid cells exhibit multipolar division, and whether such divisions yield viable daughter cells. Our lab has addressed this issue in the Drosophila hindgut, a developmental model of polyploid cell division. We find such polyploid cells naturally amplify centrosomes and undergo a surprisingly high rate of multipolar division. Further, genetically increasing the number of cells with extra centrosomes in the developing polyploid hindgut raises the frequency of multipolar polyploid mitosis, yet adult hindgut tissue from these adults remains morphologically and functionally intact, with no detectable cell inviability. These results suggest that 1) the endocycle inhibits mechanisms that normally block multipolar division, and 2) in contrast to diploid cells, polyploid cells tolerate aneuploidy induced by multipolar cell division.

309C

Interplay between the dividing cell and its neighbors temporally and spatially regulates *adherens* junction formation during cytokinesis in epithelial tissue. Sophie Herszterg[1], Andrea Leibfried[2], Floris Bosveld[1], Charlotte Martin[1], Yohanns Bellaiche[1]. 1) Polarity Division and Morphogenesis Team, Institut Curie, CNRS UMR 3215, INSERM U934, 26 rue d'Ulm, 75248 Paris Cedex 05, France; 2) Present address: Developmental Biology Unit, European Molecular Biology Laboratory, 69117 Heidelberg, Germany.

Epithelial tissue proliferation requires the formation of new *adherens* junctions (AJs) to maintain tissue polarity, integrity and architecture. How AJs are formed upon cell division is largely unexplored. We found that AJ formation is coordinated with cytokinesis and relies on an interplay between the dividing cell and its neighbors. During the contraction of the cytokinetic ring, the neighboring cells locally accumulate Myosin II and produce the cortical tension necessary to set the initial geometry of the daughter cell interface. Yet, the neighboring cell membranes impede AJ formation. Upon midbody formation and concomitantly to neighboring cell withdrawal, Arp2/3-dependent F-actin polymerization oriented by the midbody maintains AJ geometry and regulates AJ final length and the epithelial cell arrangement upon division. We propose that cytokinesis in epithelia is a multicellular process, whereby the cooperative actions of the dividing cell and its neighbors define a two-tiered mechanism that spatially and temporally controls AJ formation while maintaining tissue cohesiveness.

310A

Chromosome condensation and the evolution of Drosophila karyotypes. Shaila Kotadia, William Sullivan. Molecular, Cell and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA.

Chromosomes must be cleared from the metaphase plate during anaphase to prevent collisions between lagging chromosomes and the cleavage furrow. How cells adapt to clear particularly long chromosomes remains largely unexplored. In yeast and mammals, successful clearance of lagging or long chromosomes from the plate occurs by either a delay in cytokinesis initiation or increased compaction of anaphase chromosomes. In contrast, we find that *Drosophila melanogaster* neuroblast stem cells containing long armed chromosomes elongate during late anaphase and telophase, thus creating more space to ensure proper chromosome clearance. We found that the extent of elongation directly correlates with the length of the chromosome arm. While these studies reveal a novel pathway in *D. melanogaster* for chromosome clearance prior to cell cleavage, the tested karyotypes relied on translocations and other lab generated chromosome rearrangements. Thus, the mechanism of clearing naturally long armed chromosome karyotypes still remained a mystery. Therefore, we examined *Drosophila virilis* and *americana*, which have greater than twice the normal arm length of *D. melanogaster*. To our surprise, *D. virilis* and *americana* compact their long chromosomes in anaphase to ensure clearance from the metaphase plate. Therefore, Drosophila evolution solves the issue of clearance by compaction rather than elongation or a delay in cytokinesis. We will present our studies examining the role of condensin in regulating anaphase arm length in Drosophila chromosome evolution.

311B

Insulin signaling controls mitose/ endocycle switch through Notch signaling during drosophila oogenesis. Patrick Jouandin, Stéphane Noselli. Insitut Biologie Valrose (iBV), Nice, Alpes Maritimes (06), France.

The insulin/ insulin-like growth factor signaling (IIS) pathway is evolutionary conserved among metazoans and couples growth, metabolism, stress response, lifespan and reproduction with nutrient availability. During drosophila oogenesis, IIS is important for germline and somatic follicular cells (FCs) growth and development, controlling germline cyst development, vitellogenesis, GSC division rates in response to neural insulin. In addition, IIS is responsible for the coupling of germline growth with FCs proliferation. However, the IIS effect on FCs proliferation seems indirect and mediated by the germline. Hence, the cell autonomous role of IIS within the follicular epithelium remains unclear. Following a proliferation phase, FCs undergo a stereotyped mitotic cell cycle/ endocycle (M/ E) switch that is critical for oogenesis. This process is achieved by a transient activation burst of the Notch (N) pathway activating Hindsigth (Hnt) which in turn inhibits Cut expression. This Cut down regulation is necessary and sufficient to promote the entry into endocycle. We investigated the cell autonomous role of IIS during this process. Interestingly, *Drosophila Insulin Receptor* (*dinr*) mutant clones entered the M/ E switch, but were unable to achieve it properly. Instead, *dinr* mutant clones were characterized by a lasting N activation concomitant with Cut misexpression. The results suggest that following poor diet conditions, IIS is required to maintain FC competence for further normal development, through an intermediate, 'switch-like' state.

312C

dLipin interacts with the insulin signaling pathway in the control of fat metabolism and growth. Michael Lehmann, Sandra Schmitt. Dept Biological Sci, Univ Arkansas, Fayetteville, AR.

Lipins are central regulators of adipose tissue development and fat storage in both fruit flies and mammals. Loss of the single lipin homolog in *Drosophila*, dLipin, and the mammalian lipin 1 paralog both lead to severe underdevelopment of the fat tissue and diminished organismal stores of neutral fats (triacylglycerides, TAG). This phenotype was in part explained by the discovery that lipins are phosphatidate phosphatases that catalyze the penultimate step of the glycerol-3-phosphate pathway leading to TAG. However, lipins can also translocate into the cell nucleus where they participate in gene regulation as transcriptional co-regulators. Nuclear translocation of lipin 1 is controlled by TOR-dependent phosphorylation of the protein in response to insulin signaling. We found that, in *Drosophila*, *dLipin* shows a strong genetic interaction with the insulin

pathway and TOR, resulting in enhanced fat body defects and reduced viability. Lack of TOR induces migration of dLipin into the nucleus indicating that under fasting conditions dLipin has primarily gene regulatory functions. Intriguingly, in genetic mosaic animals, fat body cells lacking dLipin were not only deficient in fat droplets, but also showed a cell-autonomous growth defect. This induced us to more closely examine insulin signaling in fat body cells containing reduced levels of dLipin. We found a strong reduction of PIP3 in these cells, the second messenger that mediates insulin/PI3K signaling. At the same time, levels of PIP2, the substrate of PI3K and direct precursor of PIP3, were unchanged. In addition, we found that animals lacking dLipin have substantially elevated sugar levels in their hemolymph. Together, these data indicate that lack of dLipin causes insulin resistance and suggest a novel function of the protein, which is to provide feed back regulation of the insulin signaling pathway. Elucidation of the mechanism by which dLipin controls the insulin response will help us understand how organisms coordinate cell growth and the creation of energy stores.

313A

CENP-E is required for chromosome bi-orientation in meiosis in Drosophila females. Tranchau L. Hoang[1], Sarah J. Radford[2], Kim S. McKim[1,2]. 1) Genetics Department, Rutgers University, Piscataway, NJ; 2) Waksman Institute, Rutgers University, Piscataway, NJ.

Defects in chromosome segregation in meiosis lead to aneuploidy, which causes the death of embryos or diseases such as Down syndrome, Turner's syndrome, and Klinefelter's syndrome in humans. For accurate chromosome segregation to occur, chromosomes interact with a bipolar array of microtubules called the spindle. During mitosis, microtubules emanate from the organizing centers at the poles called centrosomes. The microtubules attach to chromosomes at the kinetochores, protein complexes that assemble at the centromeres. These connections facilitate chromosome bi-orientation and accurate segregation. There are no centrosomes in oocytes; therefore, how chromosomes interact with the spindle is not known. CENP-E, a kinesin motor protein at the centromeres, is required for chromosome congression during mitosis. We are testing if CENP-E is required for chromosome bi-orientation during meiosis. The *Drosophila melanogaster* genome encodes two homologues of CENP-E, which are CANA and CMET. The *cana* and *cmet* genes are adjacent to each other in an inverted orientation, suggesting the possibility of recent duplication and redundancy. CENP-E mutants were generated by screening for imprecise excision of a *P* element, which was inserted between the *cana* and *cmet* genes. Even though *cmet* mutants are lethal, *cana* mutants are viable and fertile. In CANA-depleted oocytes, chromosomes were oriented properly. However, CMET-depleted oocytes had mis-oriented chromosomes at metaphase I. In addition, oocytes of double *cana cmet* knock down showed chromosomes with the same mis-orientation defect at metaphase I as in CMET-depleted oocytes. In general, the bipolar spindle looks normal in *cana cmet* mutant oocytes. These results indicate that CMET but not CANA is required for accurate alignment of chromosomes during meiosis. For future research, we would like to investigate the mechanism and proteins that CMET interacts with to ensure the bi-orientation of chromosomes in meiosis.

314B

Discovery of B Chromosomes in *Drosophila Melanogaster* That Causes Female Specific *4th* Chromosome Nondisjunction. Elisabeth Bauerly[1], Stacie Hughes[1], R. Scott Hawley[1,2]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Molecular and Integrative Physiology, Kansas University Medical Center Kansas City, Kansas 66160, USA.

B chromosomes are small, nonrecombi¬nant chromosomes that are not required for the viability of a species and are transmitted in a non-Mendelian manner. B chromosomes have been hypothesized to be a mechanism used to drive evolution, as they create cells with novel karyotypes and similar chromosomes in humans are linked to a number of developmental abnormalities. We have identified a strain of*Drosophila melanogaster*, which carried the meiotic mutant *matrimony (mtrm*), in which we frequently observe 12 to 15 B chromosomes, a number that is much higher than what is typically seen in similar organisms. These B chromosomes can be stably maintained in an otherwise completely wild type background at about half the amount as what it is seen in the original mutant strain, despite the fact that they cause a significant amount of *4th* chromosome nondisjunction in females (but not in males). As shown by fluorescent in situ hybridization they are largely, if not entirely, composed of 4th chromosome heterochromatic sequences and indirect immunofluorescence indicates that they contain centromeres. Presumably as a consequence of their heterochromatic content, these chromosomes show a significant effect on position effect variegation. Insights on these B chromosomes in a highly tractable genetic system such as *Drosophila melanogaster* could provide insight on chromosome formation and chromosome segregation, as well as side effects that arise when extra or unstable chromosomes are present.

315C

Discovering new genes required for mitosis and meiosis by analysis of interactions with the kinesin Subito. Daniel J. DiSanto, Arunika Das, Kim S. McKim. Waksman Institute, Rutgers University, Piscataway, NJ.

Accurate segregation of chromosomes during meiosis and mitosis is essential to an organism's growth and development. We are interested in chromosome segregation during Drosophila female meiosis, which differs from mitosis and male meiosis in that it does not use centrosomes as microtubule organizing centers. Since these processes are so similar, many of the proteins involved in mitosis are also used during meiosis. Taking advantage of this fact, we devised a genetic screen to select for mutants that enhanced the mitotic phenotype of a homozygous null subito (sub) mutant (synthetic lethality). Subito is a kinesin-6 protein used during both mitosis and meiosis that associates with and may bundle anti-parallel microtubules. Subito

null mutants are viable but build defective mitotic spindles. By screening for synthetic lethal mutants in a sub null background, we isolated 17 mutations in genes that may function in the same pathway as Subito. Five of these mutants are alleles of Incenp and ial, genes previously known to be synthetic lethal with sub (including one homozygous viable Incenp allele). We are studying one of the new mutations isolated in the EMS screen, currently referred to as 27.89. Since 27.89 is homozygous lethal, germline clones were generated to view its phenotype during meiosis in stage 14 oocytes. 27.89 clones failed to develop mature oocytes, a phenotype of genes essential for the germline mitotic cell divisions such as Incenp and ial. Using a combination of genetic mapping and whole genome sequencing we will identify the 27.89 gene. This will reveal why it exhibits synthetic lethality with sub and what role it plays in meiosis.

316A

Heterochromatin Proteins Required for Association of Achiasmate Homologous Chromosomes in Drosophila Oocytes. Christopher C. Giauque, Sharon E. Bickel. Biological Sciences, Dartmouth College, Hanover, NH.

Physical association of homologous chromosomes throughout meiotic prophase I is essential for their bipolar orientation on the metaphase I spindle and accurate segregation during anaphase I. In most cases, homologous chromosomes undergo recombination and recombinant homologs are held together by arm cohesion along the sister chromatids. However, in Drosophila oocytes, 6-10%; of *X*chromosomes fail to achieve a crossover and *4th* chromosomes never recombine; yet, these achiasmate chromosomes are still able to segregate correctly. Genetic and cytological analyses of achiasmate chromosomes indicate that homology-dependent interactions within their pericentric heterochromatin are required for their proper segregation. However, little is known about the role of heterochromatin proteins in this process. We have used a UAS-GAL4 strategy to knock down heterochromatin proteins in the germ line starting at stage 2 followed by FISH to monitor the pericentric heterochromatin association of achiasmate *FM7a/X* homologs. Defects in achiasmate homolog association increase significantly when HP1A is knocked down. In addition, reduction of HP1A (in Su(var)205^5/+; heterozygotes) causes a small but significant increase in *FM7a/X* missegregation in Drosophila oocytes. We also have utilized two different RNAi hairpins to reduce the methyltransferase Su(var)3-9, which modifies H3K9 and thereby recruits HP1A to heterochromatin. For both hairpins, we observe a similar (and significant) increase in defects. Finally, we have begun to investigate a possible role for the piRNA binding protein Piwi, which is known to physically interact with HP1A and play a role in heterochromatin formation. Our preliminary data indicate that induction of a Piwi RNAi hairpin starting at stage 2 of oogenesis causes defects in *FM7a/X* pericentric heterochromatin interactions at all subsequent prophase I stages examined. These experiments argue that normal chromatin organization within pericentric heterochromatin is required for maintaining the association of achiasmate homologs during meiotic prophase I in Drosophila oocytes.

317B

Analysis of synaptonemal complex initiation. Mercedes R. Gyuricza[1], Kathryn B. Landy[1], Sanese K. White- Brown[2], Kim S. McKim[1]. 1) Waksman Institute, Rutgers University, NJ; 2) UMDNJ, Piscataway, NJ.

Accurate chromosome segregation is essential for proper production of gametes during meiosis, and requires both synapsis to hold homologous chromosomes together and cohesion to hold sister chromatids together. Synapsis is the process by which a proteinaceous structure, known as the synaptonemal complex (SC), is assembled between homologous chromosomes along the chromosome axis. The chromosome axis is composed of several proteins including both SC and cohesion proteins. We have found that SC formation is dependent upon cohesion proteins found at the axis, SMC1 and SMC3. However, when cohesion protein Rad21 is knocked-down, no effect is seen on the SC. We are looking into how other cohesion regulators effect the formation of the SC as well. Synapsis has been shown to initiate first at the centromere, and then at 6-8 sites on the chromosome arms. To test if the synapsis initiation sites correlate with cross over sites, we have examined two *Drosophila* homologs of the budding yeast cross over protein Zip3, CG31053 and CG12200. Our genetic evidence shows that both of these proteins are acting redundantly. Upon expression of CG12200 RNAi in a CG31053 mutant background, non-disjunction levels were increased and crossing over reduced compared to either RNAi or mutant individually. We are currently creating an antibody to CG12200 to determine if it localizes to crossover sites and can serve as a future crossover marker in *Drosophila*.

318C

Topoiosomerase II is required for the proper separation of heterochromatic regions during female meiosis. Stacie E. Hughes[1], R. Scott Hawley[1,2]. 1) Stowers Inst Med Res, Kansas City, MO; 2) Department of Molecular and Integrative Physiology, Kansas University Medical Center, Kansas City, Kansas.

Heterochromatic regions are essential and sufficient for the segregation of achiasmate chromosomes during meiosis I in *Drosophila melanogaster* females. Heterochromatic threads connecting achiasmate chromosomes have been observed during prometaphase I in oocytes and may be part of the mechanism by which heterochromatin ensures proper achiasmate chromosome segregation. How these heterochromatic threads are established and resolved and the mechanism by which heterochromatin properly segregates achiasmate chromosomes are unknown. Decreasing the levels of *topoisomerase II*(*top2*) by RNAi in the later stages of female meiosis results in a defect in the separation of heterochromatic regions after spindle assembly. In many late-stage oocytes only a single large focus could be observed for fluorescent in situ hybridization probes to heterochromatic regions of all four chromosomes. In other oocytes, the heterochromatic regions were stretched into long and abnormal projections. Despite these aberrant heterochromatic configurations we could observe spindles in *top2* RNAi oocytes,

though some lacked tapered poles or were elongated to accommodate the DNA projections. Based on CID localization, centromeres appear to be located at the tip of these DNA projections. Finally, achiasmate chromosomes exhibit a near complete failure to move precociously towards the spindle poles during prometaphase I. These data suggests that Topoisomerase II is involved in the resolution of DNA entanglements in the heterochromatin during meiosis I. These entanglements may be part of the mechanism ensuring proper alignment and segregation of the achiasmate chromosomes and likely give rise to the heterochromatic threads observed in prometaphase I. The studies indicate that Topoisomerase II plays important roles in meiosis other than resolving replication intermediates during DNA replication, such as properly separating and orienting chromosomes during meiosis I.

319A

SUN is required to maintain centromere cohesion and for proper chromosome segregation during meiosis in both male and female *Drosophila melanogaster*. Badri Krishnan[1], Sharon Thomas[1], Hirotsugu Yamada[1], Rihui Yan[1], Bruce McKee[1,2]. 1) Biochemistry and Cellular and Molecular biology, University of Tennessee, Knoxville, TN; 2) Genome Science and Technology program, University of Tennessee, Knoxville, TN.

Cohesion between sister chromatids is essential for connecting homologous chromosomes during meiosis I and sister centromeres during meiosis I and II. Sister chromatid cohesion at the centromere is also essential for mono-orientation and bi-orientation of sister centromeres during meiosis I and II respectively. In the absence of adequate understanding of the cohesin complex in *Drosophila melanogaster*, knowledge of meiotic cohesion and chromosome segregation is derived from studies of *Drosophila* specific cohesion genes like *ord* and *solo*. We have identified a novel gene called *sun*, *sisters unbound*, which is essential for proper chromosome segregation during meiosis in both male and female *Drosophila melanogaster*. We performed genetic crosses to determine the frequency of non-disjunction in *sun*mutants. In order to investigate the segregation pattern of chromosomes and the status of centromeric cohesion during meiosis in *sun* mutants, we used Fluorescent-In-Situ-Hybridization (FISH) and immunostaining. Finally, to determine the cellular localization of SUN protein, we created transgenic lines carrying a construct of *sun* with the fluorescent tag Venus. We found that *sun* mutants cause high frequencies of both homologous and sister chromatid non-disjunction (NDJ) in both sexes, loss of sister centromere orientation during meiosis I and II and disruption of centromeric cohesion by late prophase I. SUN protein co-localizes with CID (Centromere Identifier) to the centromeric region in spermatocytes until anaphase II and in oocytes during prophase I. Our study indicates that SUN at the centromere helps in maintaining sister centromere cohesion and establishing sister centromere orientation patterns during meiosis I and meiosis II.

320B

Exploring SOLO Working Mechanism in Drosophila Meiosis Cohesin Complex. Qian Ma, Bruce McKee. Univ of Tennessee, Knoxville, Knoxville, TN.

In eukaryotes, sister chromatids are closely aligned due to cohesion, a process essential for chromosome pairing and segregation during both mitosis and meiosis. Chromatid missegregation and mutation of cohesion proteins are associated with cancers, infertility, Down syndrome, and Cornelia de Lange Syndrome (CdLS). A conserved cohesin complex in a ring structure is composed of four subunits, including each of these four members or their homologs, SMC1, SMC3, SCC1/RAD21/REC8, and SCC3/SA. However, it is still unclear either the complex components or the working mechanism in Drosophila meiosis cohesion. Sisters on the loose (SOLO) is a newly reported meiotic protein required for centromere cohesion, and cohesin complex localization by recruiting cohesin subunit SMC1 in Drosophila meiosis. Our study utilized site-directed mutagenesis to carry out structure-function analysis of SOLO. Sequence alignment indicates SOLO shares conserved C terminal residues with SCC1/RAD21/REC8 family members, which are important for their interactions with SMC1 in cohesin complex. To test whether SOLO C terminus residues work similarly as SCC1/RAD21/REC8, by interacting with SMC1, we designed a series of mutations at the SOLO C terminal conserved residues using the Invitrogen Gateway system. With C terminus conserved residue mutations, SOLO localization is disrupted, and accurate chromosome segregation is compromised during meiosis in both males and females. In addition, we found centromere pairing is disrupted in SOLO C terminus mutant flies during meiosis. Furthermore, Yeast-Two-Hybrid was performed in order to test the direct interactions between SOLO, specifically N and C terminus domains, and cohesin proteins, SMC1 and SMC3. We found SOLO interacts with SMC1 with its C terminus domain while interacting with SMC3 by its N terminus domain, from both yeast growth and β-Galactosidase assay. These results support the idea that SOLO works as the SCC1/Rec8 homolog in Drosophila meiosis cohesin complex.

321C

Recombination and the Function of Chromosome Pairing Sites. John R. Merriam. Dept Molec/Cell/Dev Biol, Univ California, Los Angeles, CA.

That chromosome rearrangement heterozygosity reduces crossing over in the vicinity of the breakpoints is no surprise. Rearrangements at some breakpoint locations, however, are notable for reducing or eliminating crossing over for interval(s) distal to a break. In(1)delta-49 is perhaps the best known example. This feature has been used to map specific “pairing” sites that serve as gateways to eliminating crossing over distally (Hawley 1980, Szauter 1984 and Sherizen et al 2005). The mechanism of such sites is not clear, however, since normal chromosome synapsis, seen as the formation of the synaptonemal complex along the entire chromosome, is not affected even with multiply rearranged chromosomes (Gong et al 2005). - New data will be presented that map such a site on the left arm of chromosome 3 by comparing several pericentric inversion

heterozygotes. Located between 65D and 69F, the ca. 13% crossovers observed distal to the 69F break are completely eliminated by the inversion with the break at 65D. - A model will be presented that proposes the function of such sites is for them to act as probabilistic boundaries in determining whether an exchange that commits the bivalent to segregation has been established. Normally this occurs by a crossover in the middle third of the chromosome arm, as most crossovers are found there. Crossing over in the distal and proximal thirds is much less frequent than would be expected on physical distance alone, increasingly so towards either pole. "Assurance" is the term that describes both recognition of the requirement for a committing crossover as well as activation of a mechanism that promotes crossing over more in keeping with physical distance over the remaining distal and proximal intervals in the absence of a committing crossover. Heterozygosity for a rearrangement breakpoint seems to activate the assurance program, a phenomenon recognized as the interchromosomal effect (Schultz and Redfield 1951) but blocks further crossovers within its boundary interval, perhaps by interference with breakpoints mimicking bona fide crossovers.

322A

Role Of Cohesins In Drosophila Male Meiosis. Avik Mukherjee[1], Bruce McKee[1,2]. 1) Genome Science and Technology, University of Tennessee, Knoxville, TN; 2) Department of Biochemistry Cell and Molecular Biology, University of Tennessee, Knoxville, TN.

Meiosis is driven by the pairing and proper segregation of both sister chromatids and homologous chromosomes. Cohesion between sister chromatids plays multiple roles in pairing and segregation of homologs as well as sister chromatids. It depends, in both mitosis and meiosis, on a conserved protein complex, cohesin, that forms a ring around duplicated sister chromatids and prevents them from separating prematurely. Insight into the structure and role of cohesin in Drosophila meiosis is thus far very limited. The Drosophila genome encodes single orthologs of SMC1 and SMC3 and two orthologs of RAD21 (RAD21 and C(2)M) and SCC3/SA (SA and SNM) cohesin proteins. Previous work has shown that in Drosophila male meiosis pairing depends on two chromosomal proteins (Stromalin in meiosis (SNM) and Mod(Mdg4) in Meiosis (MNM)) that stably maintain homolog pairing throughout meiosis I. SNM is a paralog of one of the core cohesin genes SA/SCC3, raising the possibility of a central role of cohesin in homolog pairing. Mutations in c(2)m are meiosis specific and are related to synapsis in female meiosis but is not essential for sister chromatid cohesion. The protein Ord is required for centromeric cohesion and colocalizes with SMC1 protein at the centromeres of meiotic chromosomes. Another gene, solo, is required for both homolog and sister chromatid segregation in both sexes and colocalizes on chromosomes with cohesin proteins. We are investigating the role of cohesin in Drosophila male meiosis by using germ-line specific RNAi against the genes encoding the core cohesin proteins SMC1 and SMC3 which are long coiled-coil proteins that, together with SCC1/RAD21, are thought to form a tripartite ring that topologically constrains sister chromatid pairs.The effect of cohesin mutation on meiotic pairing and cohesion will be presented.

323B

The role of mcm5 and mad2 in the Pachytene checkpoint in Drosophila females. Anshu A. Paul, Kim S. McKim. Waksman Institute of Microbiology, Rm 206, Genetics, Rutgers, New Brunswick, New Brunswick, NJ.

The meiotic recombination pathway in Drosophila females is monitored by the presence of several checkpoints. Checkpoints serve as error correction mechanisms present at different stages of meiosis to monitor the fidelity of various ongoing processes. One of these checkpoints, called the Pachytene checkpoint, oversees the processes that lead up to the formation of crossovers. In the presence of a defect, the checkpoint is not satisfied and it leads to a delay in meiotic progression which allows the cell additional time to repair its defects and generate crossovers. I am characterizing the roles of two candidate genes, mcm5 and mad2, as they relate to the Pachytene checkpoint. The gene mcm5, which was previously known to function in DNA replication, transcription activation, and chromosome condensation, is also one of the precondition genes in the meiotic recombination pathway. Furthermore, it was found to be similar in sequence to the gene mei-218, which is involved in the Pachytene checkpoint. Similarly, the gene mad2, which was previously known to function in mitotic checkpoint activation, may have a role in meiotic checkpoint activation. In order to determine the function of these genes in the Pachytene checkpoint, I combined each of these mutations with a mei-9 mutation, which typically fails to satisfy the checkpoint and has a meiotic prophase delay. If the double mutants have a meiotic delay, indicating the activation of the Pachytene checkpoint, then mcm5 and mad2 are not involved in checkpoint activation. On the other hand, if there is no delay in my double mutants, indicating that the checkpoint was not activated despite the presence of a crossover defect, then mcm5 or mad2 function in checkpoint activation. My results showed that mcm5, even when combined with the crossover defective mutant mei-9, did not result in a Pachytene delay, suggesting that mcm5 does indeed play a role in the activation of the Pachytene checkpoint. Experiments are currently being performed on the mad2 mutants to characterize their function in the Pachytene checkpoint.

324C

Temporal Analysis of DSB Formation in Meiotic Prophase Heterochromatin. Marissa C. Pelot[1], R. Scott Hawley[1,2]. 1) Stowers Institute for Medical Research, Kansas City, MO 64110; 2) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160.

Heterochromatin, DNA that is generally characterized as gene-poor, and enriched for repetitive sequences, has, in the past, had a reputation as "junk" DNA. However, it is now known to play important roles in a number of biological processes. Because heterochromatin is highly enriched for repetitive sequences, the repair of double-strand DNA breaks (DSBs) within it presents

a special challenge. Imprecise repair of DSBs in highly repetitive regions could result in the loss or gain of DNA or formation of aberrant chromosomes. These aberrant products can contribute to cancer and other human diseases. In somatic cells, DSBs only occur in response to damage, such as that done by ionizing radiation (IR). In meiosis, however, cells enter a program that incorporates DSB formation as a necessary prerequisite for synapsis and recombination. DSBs are not formed in heterochromatin as part of the normal meiotic program, but meiotic heterochromatin is not refractory to DSBs induced by IR. Little is known regarding the kinetics or the resolution DSBs induced this way. Using Drosophila oocytes as a model, we have examined the kinetics of DSB formation induced by IR in early meiotic prophase, with surprising results. Preliminary data indicates a delay in the recognition of DSBs in meiotic heterochromatin compared to those formed in mitotic heterochromatin. DSBs in meiotic heterochromatin also interact with components of recombination machinery, though the mechanism for their resolution remains to be investigated. These studies may further elucidate the role of heterochromatin and DSB formation in human cancers.

325A

Regulation and Function of the Drosophila Shugoshin, MEI-S332. Belinda Pinto, Cristina Nogueira, Terry Orr-Weaver. Whitehead Inst, Cambridge, MA.

Accurate chromosome segregation requires the step-wise release of chromatid cohesion from the arms and centromeres. This pattern of release is facilitated by the Shugoshin family of proteins that protect centromeric cohesion. To execute this protective role, Shugoshins interact with the B' associated form of the PP2A phosphatase (PP2A-B'). The role of PP2A-B' during metazoan mitosis has been elucidated, but the role during metazoan meiosis is unclear. The function of Shugoshins in protecting centromeric cohesion is linked to centromeric localization from prometaphase to the metaphase-anaphase transition. A few proteins that regulate Shugoshin localization have been identified, but other as yet unidentified factors are likely to regulate the spatial and temporal localization of these proteins. Here, we examined the role and regulation of Shugoshins in centromeric cohesion through studies of the Drosophila homolog, MEI-S332. First, genetic interaction studies indicate that two B' phosphatase subunits, Wdb and dPP2A-B', make redundant contributions to the role of MEI-S332 in centromeric cohesion. Second, we developed a model using Drosophila mitotic cells to identify factors that regulate the centromeric localization of MEI-S332. We performed a high-throughput RNAi screen in Drosophila cell culture and identified a number of candidates that affect MEI-S332 localization at metaphase and anaphase. Validation of one these candidates uncovered a role for the proteasome in delocalizing MEI-S332 at anaphase. Studies to investigate the mechanism by which the proteasome regulates MEI-S332 localization are underway. Taken together, data from our studies of MEI-S332 will be valuable in understanding the mechanism of action of Shugoshins during meiotic and mitotic chromosome segregation.

326B

A Novel Role for Sister-Chromatid Cohesion Proteins in Promoting Heterochromatin Mediated Association of Achiasmate Homologs in Drosophila Oocytes. Brian C Seitz, Sharon E Bickel. Biological Sciences, Dartmouth College, Hanover, NH.

During meiosis in Drosophila females, a single crossover between homologous chromosomes is sufficient to ensure their proper orientation and segregation during the first meiotic division. Cohesion along the arms of sisters keeps recombinant homologs physically associated until anaphase I, when arm cohesion is destroyed. However, not all homolog pairs achieve a crossover. *X* chromosomes are achiasmate in 6-10% of Drosophila oocytes and *4th* chromosomes are always achiasmate; still, in the absence of a crossover, these chromosomes segregate with high fidelity. Work by several investigators has demonstrated that homology-dependent interaction between the pericentric heterochromatin of achiasmate homologs during prophase I is required for their accurate segregation at anaphase I. However, identification of chromatin-associated proteins that facilitate this physical interaction has remained elusive. Previous work from our lab (Subramanian and Bickel, 2009) revealed an unexpected role for the meiotic cohesion protein ORD in promoting pericentric heterochromatin-mediated association of achiasmate homologs. This led us to ask whether other cohesion proteins are required for this process. We used the Gal4/UAS inducible system to knock down the cohesion regulators Wapl and Pds5 during meiotic prophase. Using a FISH probe that hybridizes to the 359bp repeat in the pericentric heterochromatin of the *X*chromosome, we observed significant disruption of *FM7a/X* association when either Wapl or Pds5 was knocked down in the germ line. In addition, using our standard *X* meiotic segregation assay, we observed a small but significant increase in *FM7a/X* nondisjunction in Wapl knockdown oocytes. These genetic data are consistent with those reported by Vernì et al. (2000) for a *wapl* loss of function allele in *wapl/+* heterozygous females. Together, our results support the hypothesis that proteins involved in sister chromatid cohesion not only maintain the association of chiasmate homologs but also play an essential role in promoting the physical association of achiasmate homologs in Drosophila oocytes.

327C

Euchromatic homology is sufficient for pairing of rDNA-deficient X chromosomes in male meiosis. John E. Tomkiel, Andrew Bourgeios, Christina Morgan, Katie Hansen, Kayla Hill, Aboubakar Doura. Dept Biol, Univ North Carolina, Greensboro, NC.

Meiotic sex chromosome pairing in male Drosophila occurs at the rDNA, located in both the X and Y heterochromatin, and is required for both normal chromosome segregation and spermatogenesis. Deletion of rDNA pairing sites results in XY nondisjunction, meiotic drive, and sterility when in combination with certain T(1;Y) translocations. It is unknown if these

phenomenon are related to interactions between the X and Y specifically at the rDNA, or if it is pairing itself that is important. It is also unknown if other homology between the X and Y might substitute as a pairing site. We examined these questions by monitoring the ability of a collection of T(1;Y) chromosomes to pair and segregate from an rDNA-deficient X homolog. We found that euchromatic X homology was sufficient for pairing and segregation and for suppression of meiotic drive, but not all X segments behaved the same. This differential segregational ability of X segments suggests that there may be discrete pairing and/or conjunction sites distributed through X euchromatin. These may differ in nature from autosomal conjunction sites, as segregation ability did not correlate with the presence or absence of binding sites for Teflon, a protein required for autosomal conjunction. Because euchromatic X pairing sites would not normally function in hemizygous males, their conservation may indicate that the underlying mechanism of pairing in males and females is the same. We suggest that the location of sex chromosome pairing sites in heterochromatin may not be functionally important, but rather may merely reflect the location of remaining homology after evolution of heteromorphic sex chromosomes.

328A

Rejuvenation of cohesion during meiotic prophase is required for maintenance of chiasmata and accurate chromosome segregation. Katherine A. Weng, Charlotte A. Jeffreys, Sharon E. Bickel. Biological Sciences, Dartmouth College, Hanover, NH.

Accurate chromosome segregation in human oocytes requires that meiotic sister chromatid cohesion remain intact for decades and work in model organisms indicates that deterioration of meiotic cohesion over time may be a major determinant of age-related segregation errors. We are using Drosophila to investigate whether oocytes rely exclusively on cohesive linkages that are established during meiotic S phase or if maintenance of meiotic cohesion is an active process that requires rejuvenation throughout the extended period of prophase I. Deco is the Drosophila homolog of the yeast cohesion establishment factor, Eco1, which is required to establish cohesive linkages during S phase. To test the hypothesis that Deco is required for maintenance of meiotic cohesion, we used a Gal4/UAS inducible approach to knock down Deco after meiotic S phase in the female germline. We find that reduction of Deco after meiotic S phase causes premature disassembly of the synaptonemal complex (SC) and increased levels of meiotic nondisjunction (NDJ). Additionally, although chiasmata are formed, they are not maintained. Moreover, SC defects and increased NDJ also occur when cohesin subunits (SMC1, SMC3, SA) and the cohesin loader, Nipped-B are knocked down after meiotic cohesion is established. These data argue that rejuvenation of cohesion requires the loading of new cohesin complexes during meiotic prophase to stabilize chiasmata and ensure proper segregation of meiotic chromosomes. Our data also indicate that Deco-mediated cohesion rejuvenation during prophase I is necessary even in the absence of meiotic double-strand breaks (mei-W68 mutant) and therefore differs from the damage-induced cohesion re-establishment pathway that operates during G2 in yeast vegetative cells. We propose that programmed rejuvenation of cohesive linkages during prophase I represents a critical mechanism that allows metazoan oocytes to counteract the deterioration of cohesion caused by aging. Experiments to investigate the mechanism of cohesion rejuvenation are currently in progress.

329B

Role and regulation of BubR1 on acentric chromosome segregation. Nicolas Derive[1], Zohra Rahmani[2], Anne Royou[1]. 1) Institut Européen de Chimie et Biologie, CNRS, Université Bordeaux Segalen, 2 rue Robert Escarpit, 33607 Pessac, France; 2) Institut Jacques Monod, CNRS, Université Paris Diderot Paris 7, 4 rue Marie-Andrée Lagroua Weill-Halle, 75205 Paris, France.

Correct transmission of the genetic material during mitosis requires the proper chromosome attachment to the spindle microtubules. The centromere of the chromosome serves as an assembly site for a multiprotein structure called the kinetochore, the functional unit that binds the microtubules. The centromere is thus essential for proper chromosome segregation. However, we have recently identified a mechanism by which fragments of chromosomes lacking the centromere segregate properly. The observation of live Drosophila neuroblasts transiting mitosis with broken chromosomes revealed that acentric chromatids segregate faithfully. This is achieved through a DNA “tether” that attaches the acentric fragments to its centric partners. The integrity of the tether depends on BubR1 function, a protein that localizes on the kinetochore until anaphase, and accumulates on the “tether” throughout mitosis. To determined how BubR1 recruitment to the “tether” is regulated, we monitored dividing cells expressing fluorescent BubR1 constructs lacking specific domains. We found that the Glebs motif and more specifically the Glutamate 481 are required for BubR1 localization on the tether. Since E481 is essential for BubR1 interaction with Bub3 and its localization on the kinetochore, we investigated whether Bub3 localizes to the tether and more generally whether the “tether” is an assembly site for other kinetochore proteins. We found that five core kinetochore proteins did not localize on the tether ruling out the hypothesis that a neo-kinetochore forms on the tether. In contrast, Bub3 accumulated on the tether throughout mitosis. Moreover, BubR1 was not required for Bub3 localization to the tether suggesting that Bub3 acts upstream of BubR1 on the tether. All together these results suggest that the molecular pathway regulating BubR1 recruitment to the tether is similar but not identical to that of BubR1 localization to the kinetochore.

330C

Lipid droplets buffer the histone supply of *Drosophila* embryos. Zhihuan Li, Michael Welte. Department of Biology, University of Rochester, Rochester, NY.

In eukaryotic cells, DNA molecules wrap around histones to form nucleosomes, which protects the genome and regulates

gene expression. However, excess free histones bind DNA randomly, alter nucleosome structure, interfere with gene expression, and ultimately cause cell lethality. Early *Drosophila* embryos contain massive amounts of excess histones H2A, H2B and H2Av. It has been hypothesized these excess histones are not detrimental to the embryo because they are sequestered on lipid droplets, fat storage organelles in the cytoplasm. We previously showed that the histones are bound to lipid droplets via the novel protein Jabba and serve as a backup supply that supports the rapid cell cycles of early embryogenesis. We now demonstrate that lipid droplets are not merely histone storage sites, but also protect the embryo from histone overexpression. In the wild type, we detect the overexpressed histones on lipid droplets, suggesting that lipid droplets can indeed bind and likely sequester supernumerary histones. In *Jabba* embryos, lipid droplets cannot recruit histones. In the nuclei of *Jabba* mutants, increased levels of histones accumulate during syncytial stages, presumably because they cannot be retained on lipid droplets and thus prematurely enter nuclei. In *Jabba* embryos, histone overexpression results in anaphase bridges, numerous mislocalized nuclei, abnormal morphology during cellularization, and embryonic lethality. We propose that lipid droplets serve as a buffer in the embryos, storing histones for the massive demands in early cell cycles and sequestering free histones to ensure genomic stability.

331A

Maternal PIWI proteins are essential for embryonic mitosis and chromatin integrity. Sneha Mani[1], Heather Megosh[2], Haifan Lin[1], *First and second authors equally contributed to this work. 1) Cell Biology, Yale University, New Haven, CT; 2) Cell Biology, Duke University, Durham, NC.

PIWI proteins in Drosophila have been implicated in transcriptional and posttranscriptional gene silencing mediated by small non-coding RNAs. Although these proteins are known to be required for germline development, their somatic function remains elusive. Here, we examine the role of maternal Piwi, Aub and Ago3 during early embryogenesis—the first phase of somatic development.

In syncytial embryos, Piwi has a dynamic localization pattern that is embryonic stage-dependent; most of Piwi is localized in the cytoplasm during mitotic cycles 1-13 after which it is moves into the nucleus. Aub and Ago3 are diffusely cytoplasmic till Stage 9, after which they localize more obviously to the perinuclear region. Embryos depleted of any one of the three maternal PIWI proteins display various severe mitotic defects including abnormal nuclear morphology, cell cycle arrest, asynchronous nuclear divisions and aberrant nuclear migration. A more thorough examination of early embryonic cell divisions reveals roles for all three PIWI proteins in the assembly of mitotic machinery and in the regulation of progression through mitosis. Additionally, embryos depleted of maternal PIWI exhibit various deficiencies in markers of chromatin organization.

These observations suggest that maternal Piwi, Aub and Ago3 play a critical role in the maintenance of chromatin structure and cell cycle progression during embryogenesis, with compromised chromatin integrity as a possible cause of the observed cell cycle defects. Our study demonstrates the essential function of PIWI proteins in somatic development.

332B

An in situ analysis of Drosophila imaginal disc regeneration: pattern reorganisation occurs independently of cell proliferation. Sandra Diaz-Garcia, Antonio Baonza. Development and Genetics Dept, CBMSO-UAM (CSIC), Madrid, Spain.

One of the most intriguing problems in developmental biology is how an organism can replace missing organs or portions of their bodies after injuries. This capacity, known as regeneration, is conserved across different phyla. The imaginal discs of Drosophila melanogaster provide a particularly well-characterised model system for analysing regeneration. Although this organism has been extensively used to study this process, the cellular and molecular mechanisms underlying regeneration remain unclear. We have developed a new method to study organ regeneration under physiological conditions using the imaginal discs of Drosophila as a model system. Using this method, we have revisited different aspects of organ regeneration in Drosophila. The results presented in this report suggest that during the initial stages of disc regeneration different processes occur, including wound healing, temporary loss of markers of cell fate commitment and pattern reorganisation. These processes occur even when cell proliferation has been arrested. Our data suggests that wingless plays only a minor role during the early stages of regeneration, and its expression is down-regulated in some regions of the wing discs as a consequence of a reduction in the activity of Notch signalling.

333C

A genetic approach to enhancing tissue regeneration. Robin Harris, Iswar Hariharan. University of California, Berkeley, Berkeley, CA.

In many examples of regeneration, the capacity of a tissue to regrow following damage declines with age. Understanding the mechanisms that dictate changes in regenerative capacity is vital for developing methods to stimulate or enhance regeneration. However, the underlying cellular and genetic events that lead to such changes are unknown. I am using the larval wing primordium of *Drosophila* - a tissue that progressively loses regenerative ability during development - to investigate the genetic mechanisms that cause a tissue's regenerative response to diminish with time. I have generated a novel genetic ablation system that will be used to perform a large-scale genetic screen for genes that restore regenerative capacity in older, non-regenerating tissue. This system allows larval tissue to be ablated in a spatially and temporally controlled manner, while the extent of regeneration is assayed simply by examining adult wing tissue. Unlike previously developed ablation/regeneration systems, this system induces damage independently of the Gal4/UAS transcriptional activator, thus allowing screening to be performed using the abundant UAS-driven transgenes and purpose-made RNAi screening libraries

available in*Drosophila*. Thus, I hope to comprehensively identify genes that comprise a regeneration program, which can be manipulated to induce regenerative growth in older, non-regenerating tissue.

334A

Identifying a transcriptional program that regulates compensatory proliferation. Joy H Meserve[1], Robert J Duronio[2]. 1) Curriculum in Genetics and Molecular Biology, UNC, Chapel Hill, NC; 2) Departments of Biology and Genetics, UNC, Chapel Hill, NC.

All animals undergo damage as they develop and age, and many organisms have evolved mechanisms to respond to and repair this damage. One such mechanism is compensatory proliferation, during which cells that are dying following injury will mitogenically signal surrounding cells to increase proliferation and replace lost cells. This process is heavily utilized in damaged precursor cell populations during development and stem cells during adult life. However, many post-mitotic cells appear unable to re-enter the cell cycle and proliferate in response to damage, which hinders regeneration of adult tissues; the reasons for this inability are not well understood. Furthermore, sustained injury in adult tissues leads to cell death which may promote hyperproliferation and the development of cancer. Therefore, it is essential to understand the mechanisms underlying compensatory proliferation, particularly within post-mitotic tissues. In the eye imaginal disc of *Drosophila melanogaster*, there is a population of post-mitotic, undifferentiated cells that are able to undergo compensatory proliferation. How these cells overcome negative cell cycle regulation and re-enter the cell cycle is currently unknown. We hypothesize that a unique transcriptional program required for compensatory proliferation exists within these post-mitotic eye cells. We are currently testing this hypothesis by characterizing the transcriptional profile in these cells using fluorescence-activated cell sorting (FACS) to isolate the compensatory proliferating population and RNA-seq to identify the transcriptome. Genes that are highly expressed in these cells and not in their non-proliferating counterparts are likely to be involved in compensatory proliferation. We are also carrying out an RNAi screen to identify transcription factors required for compensatory proliferation. The results from these experiments will provide a comprehensive view of compensatory proliferation in a post-mitotic cell population.

335B

Trithorax is required for imaginal disc regeneration. Andrea Skinner, Rachel Smith-Bolton. Cell & Developmental Biology, University of Illinois Urbana-Champaign, Urbana, IL.

Drosophila melanogaster is able to regenerate lost or damaged imaginal disc tissue prior to pupariation. To identify genes critical for regeneration, we performed a dominant modifier genetic screen in which tissue was ablated from the wing imaginal disc of early third instar larvae. The animals were then screened for adult wing size as a measure of regeneration. Through this screen, we found animals heterozygous for *trithorax* (*trx*) have reduced wing regeneration. Trx is thought to control active gene expression by regulating chromatin modifications. To understand how trx is important for regeneration, we are characterizing the impaired regeneration in *trx*/+ mutants and identifying genes regulated by *trx* after tissue damage. Thus far we have shown several processes are misregulated in *trx*/+ damaged tissue. First, on the organismal level, *trx*/+ regenerating animals do not delay entry into pupariation to the same extent as wild-type regenerating animals. Second, on the cellular level, more cells are in S phase both in the regeneration blastema and at a distance from the wound, where proliferation is normally suppressed. Third, on the molecular level, JNK signaling, which is normally required for regeneration, is significantly elevated. We will present our ongoing characterization of regeneration in *trx*/+ mutants as well as our working model for how regeneration fails despite increased numbers of proliferating cells and increased JNK signaling.

336C

A novel role for cytokinesis proteins in acentrosomal spindle assembly and chromosome segregation in Drosophila oocytes. Arunika Das[1], Shital J. Shah[2], Kim S. McKim[1]. 1) Waksman Institute, Rutgers University, NJ; 2) New Jersey Medical School, Newark.

Accurate segregation of chromosomes is facilitated by the formation of a bipolar array of microtubules called the spindle. In mitotic spindle assembly, the centrosomes define the poles and organize the microtubules. In the oocytes of many animals, however, the centrosomes are absent and consequently it is poorly understood what organizes the bipolar spindle and directs the chromosomes to become attached to the microtubules, a process known as bi-orientation. Previous studies have shown that the chromosome passenger complex (CPC) is the master regulator of spindle assembly. The CPC is composed of four proteins, Incenp, Aurora B kinase, Survivin and Borealin. Unlike mitotic cells, where the CPC localizes to centromeres during metaphase, during meiotic metaphase it localizes in a ring around the chromosomes. This novel localization pattern is responsible for building a bipolar spindle and establishing bi-orientation. Our goal is to identify regulators of acentrosomal spindle assembly and CPC localization. An unbiased screen was performed based on synthetic lethal mutations with *subito*. Subito belongs to kinesin 6 family and is required for localizing the CPC to the ring in meiosis. We uncovered*tumbleweed* from this screen which is a regulator of cytokinesis like the CPC. We investigated other proteins in the cytokinesis pathway which interact with *tum* and the CPC. This study has revealed that Rho-1 and its downstream effector Sticky both regulate acentrosomal spindle assembly but do not seem to function in a similar manner. Sticky also helps to establish bi-orientation similar to the CPC. These results suggest that these proteins function in meiosis but may not act according to the pre-defined pathway. We are also testing several candidate genes like Haspin kinase, Tousled-like kinase and Shugoshin, which regulate CPC localization during mitosis. We have found that *tlk* regulates in spindle assembly and regulates CPC localization and

homolog bi-orientation.

337A

***Drosophila* tumor suppressors maintain epithelial integrity by controlling mitotic spindle orientation.** Yu-ichiro Nakajima, Emily Meyer, Matthew Gibson. Stowers Institute for Medical Research, Kansas City, MO.

During epithelial cell proliferation, planar alignment of the mitotic spindle coordinates the local process of symmetric cell cleavage with the global maintenance of polarized tissue architecture. While the disruption of planar spindle alignment is hypothesized to cause epithelial dysplasia and cancer development, the *in vivo* mechanisms regulating mitotic spindle orientation remain elusive. Here, we show that in*Drosophila* wing imaginal discs, the Actomyosin cortex and junction-localized neoplastic tumor suppressors Scribble (Scrib) and Discs Large (Dlg) play essential roles in planar spindle alignment and thus the control of epithelial integrity. During wing disc development, F-Actin is accumulated at the cortex of mitotic cells and mitotic spindles align at the level of the septate junctions. Inhibitions of cortical Actomyosin by drug perturbation or dsRNA knockdown of actin regulators (*rho-kinase/moesin*) lead to severe misorientation of the mitotic spindle. Disruptions of the septate-junction localized scaffold proteins Scrib/Dlg do not cause loss of epithelial polarity as an initial phenotype, but rather do induce misalignment of the mitotic spindle. We further show that defective alignment of the mitotic spindle correlates with basal cell extrusion and increased cell death. Blocking cell death in misaligned cells is alone sufficient to cause epithelial-to-mesenchymal transition and drive the formation of basally extruded tumor-like masses. These findings demonstrate a key role for junction-mediated spindle alignment in the maintenance of epithelial integrity, and also reveal a novel cell death-mediated tumor suppressor function inherent in the polarized architecture of epithelia.

338B

Chromosome segregation without spindle microtubules. Peter Vilmos[1], Szilard Szikora[1,2], Ferenc Jankovics[1], Ildiko Kristo[1], Laszlo Henn[1], Miklos Erdelyi[1]. 1) Dept Genetics, Biological Research Center, Szeged, Hungary; 2) Dept Biology, University of Szeged, Szeged, Hungary.

The prevailing view today is that during eukaryotic cell division chromosome segregation is carried out by spindle microtubules. However, circumstantial evidences support the idea that an actin microfilament-based spindle matrix might play direct role in chromosome movements. To get better insight into the role actin plays in chromosome segregation, we have examined the effect of the depolymerization of F-actin, Tubulin or both during mitosis by using real-time fluorescent *in vivo* microscopy in early *Drosophila melanogaster* embryos. Our data show that F-actin together with the microtubules is responsible for the compaction and alignment of the mitotic chromosomes and that it is required for the formation of the microtubule spindles. Moreover, we found that actin filaments are actively participating in chromosome segregation and that the structure marked by the spindle matrix component Chromator might be responsible for chromosome segregation observed in the absence of the mitotic spindle. Our results provide new evidences that actin filaments generate force for chromosome segregation during mitosis.

339C

Mitotic epithelial cells have a dynamic relationship with the layer. Daniel T. Bergstralh, Holly Lovegrove, Daniel St Johnston. Gurdon Inst, Univ Cambridge, Cambridge, United Kingdom.

Metaphase spindles in the follicle cell epithelium are oriented roughly parallel to the plane of the epithelium. Although prior work suggested that spindle orientation could depend on interaction between spindle poles and APC2, we show that this is not the case. Our results also suggest that apical polarity factors, including aPKC, are not restricted to the apical cortex of dividing cells, and that aPKC does not play a role in spindle orientation in follicle cells. We observe that Pins and Mud, two factors known to participate in spindle orientation in other tissues, are expressed in the ovary and co-localize along the basolateral cortex in dividing cells. Both Pins and Mud are required for orienting spindles in the FCE. We further show that exogenous expression of Inscuteable, a spindle orientation factor found in neuroblast cells, promotes dramatic reorientation of follicle cell spindles. Incorrect orientation of mitotic spindles has been implicated in tumorigenesis in mammals and epithelial disorganization in Drosophila. However, neither the loss of Pins or Mud nor the ectopic expression of Inscuteable leads to disorganization of the follicle cell monolayer. Live imaging reveals that in wild type tissue cells can divide outside the plane of the epithelium then reintegrate back into it. This process also occurs in cells with misoriented spindles. Thus reintegration serves as a robust mechanism for the preservation of a single epithelial layer.

340A

Role of Polyploid Glial Cells in Drosophila Neural Development. Laura E. Frawley, Yingdee Unhavaithaya, Terry L. Orr-Weaver. Whitehead Institute for Biomedical Research, Cambridge, MA.

Development of an organ relies on the coordinated growth among different cell types within given tissues. Our recent work (1) has established that subperineurial glia (SPG) in the Drosophila brain lobe, ventral nerve cord, and peripheral nervous system (PNS) are polyploid. Importantly, we observed that SPG ploidy must be coordinated with neuronal mass, as ablation of SPG polyploidy breached the septate junctions that form the blood-brain barrier. The increased cell size of SPG due to increased ploidy is therefore required for the integrity of the blood-brain barrier. When we increased the neuronal mass by using aurA mutants, the SPG responded by increasing ploidy and cell size, allowing the blood-brain barrier to remain intact. We have found that Notch signaling is important in controlling SPG ploidy. In addition to the SPG, we found that a

subpopulation of wrapping glia (WG) in the PNS is polyploid. We are currently investigating the function of WG polyploidy and the mechanism by which WG become polyploid.

(1) Unhavaithaya Y. and Orr-Weaver T.L. 2012. Polyploidization of glia in neural development links tissue growth to blood-brain barrier integrity. Genes Dev 26: 31-6.

341B

Activation and function of TGFβ signalling during Drosophila wing development and its interactions with the BMP pathway. Covadonga F. Hevia, Jose F. de Celis. Centro de Biología Molecular Severo Ochoa CSIC-UAM, Madrid, Spain.

The development of the Drosophila wing disc requires the activities of the BMP and TGFβ signaling pathways. BMP signaling is critical for the growth and patterning of the disc, whereas the related TGFβ pathway is mostly required for growth. The BMP and TGFβ pathways share a common co-receptor (Punt) and a nuclear effector (Med), and consequently it is likely that these pathways can interfere with each other during normal development. Here, we analyze the requirements of TGFβ signaling during wing disc development and identify possible mechanisms linking TGFβ and BMP activities. We found that the phosphorylation of Smad2, the specific transducer for TGFβ signaling, occurs in a generalized manner in the wing disc and that Smad2 influences cell division rates and cell growth. The expression in the wing disc of the four candidate TGFβ ligands (*activinβ*, *dawdle*, *maverick* and *myoglianin*) is required to obtain normal levels of TGFβ signaling. We confirm that Baboon, the specific receptor of the TGFβ pathway, can phosphorylate Mad, the specific transducer of the BMP pathway, but we find that this activation only occurs when the receptor is constitutively activated in a background of reduced expression of *Smad2*. In the presence of Smad2, the normal situation during wing disc development, high levels of activated Baboon lead to a depletion in Mad phosphorylation and to BMP loss-of-function phenotypes. Although cross- interactions between TGFβ and BMP signaling based in molecular competition for common components of the pathways seem irrelevant to determine each pathway signaling outcome in the wing disc, they could be critical in other developmental systems and in pathological conditions.

342C

Growth is coordinated during regeneration through the regulation of ecdysone by Dilp8 via nitric oxide signaling. Jacob Jaszczak, Anh Dao, Adrian Halme. Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA.

During development, coordination of organ growth produces animals of normal size and proportion. In *Drosophila*, localized imaginal disc damage initiates a regenerative response in damaged tissues and attenuates the growth of undamaged imaginal discs. The growth inhibition in undamaged tissues may function to coordinate regeneration with developmental growth. In contrast to the imaginal tissues, we show larval tissues to not experience similar growth restriction following imaginal disc damage; it is not likely that growth of undamaged imaginal discs is reduced by inhibiting global insulin signaling. Nitric oxide synthase (NOS) functions in systemic immune responses and growth inhibition. We examined NOS function during systemic growth coordination following localized imaginal disc damage, and show NOS to be necessary and sufficient for attenuating growth of undamaged imaginal discs. During localized tissue damage and regeneration, NOS activity is increased in the prothoracic gland (PG). NOS overexpression in the PG is sufficient to attenuate growth in imaginal discs and produces a substantial developmental delay. Both the growth and delay phenotypes can be suppressed by exogenous ecdysone, suggesting that NOS activity in the PG suppresses ecdysone synthesis. Previous experiments (Caceres *et al.* 2011) show that NOS activity in the PG of post-feeding larvae promotes the expression of ecdysone biosynthesis genes and ecdysone synthesis. In contrast, our experiments in earlier third-instar larvae suggest that NOS activity in the PG may have the opposite effect on ecdysone synthesis; NOS functions to decrease ecdysone signaling, thereby coordinating regenerative and developmental growth. Additionally, we show that NOS activity is increased in the PG when Dilp8 is expressed in wing imaginal discs. These results suggest that NOS activity in the PG may mediate the effects of Dilp8 and ecdysone, coordinating growth and developmental timing during regeneration.

343A

A screen to identify genes involved in tissue specific growth of the larval trachea in Drosophila. Paulo Leal, Robert Ward. Dept Molecular Biosciences, University of Kansas, Lawrence, KS.

During post-embryonic development in animals, different tissues and organs grow at different rates relative to each other, likely tied to the unique requirements of each organ's function during development and homeostasis. Differential growth occurs in spite of the fact that overall growth is tied to nutrition, which is largely regulated through the insulin signaling pathway. This suggests that there must be tissue-specific mechanisms that function in concert with or in parallel to insulin signaling to control their post-embryonic growth, although we know very little about them. One way to understand these mechanisms is through the characterization of mutations that specifically alter growth in single organs or tissues in a genetically tractable model system. The larval trachea of *Drosophila* is well suited for this study as it is a well-studied branched tubular organ required for gas exchange that grows dramatically during larval development. Embryonic tracheal development is genetically controlled to yield tubes of appropriate caliber to support gas movement throughout the newly hatched larva. Upon hatching, however, the larva begins to feed and thus organ growth is tied to nutrition. Mutations in two genes, *uninflatable (uif)* and*Matrix metalloproteinase 1 (Mmp1)* have phenotypes that include tissue specific growth reductions

within the larval trachea. To identify additional genes that regulate larval tracheal growth, we are screening two collections of late lethal mutations: 49 *P*-element induced late larval lethals obtained from the Bloomington stock center and 252 EMS induced larval/pupal lethals from the collection of 3rd chromosome late lethals generated by Dr. Bashirullah (University of Wisconsin). Preliminary screening identified 2 P-element and 4 EMS mutations that show specific larval tracheal defects, including both reduced and expanded relative tracheal sizes. We are mapping and conducting phenotypic analysis on the mutant larvae, and examining interactions between *uif*, *Mmp1*, and the isolated mutants.

344B

Two-tiered control of epithelial growth and autophagy by the insulin receptor and the Ret-like receptor, Stitcher. Fergal O'Farrell[1,2], Shenqiu Wang[2], Christos Samakovlis[2], Tor Erik Rusten[1]. 1) Dept. of Biochemistry, Institute for Cancer Research The Norwegian Radium Hospital Oslo, Norway; 2) Department of Developmental Biology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden.

Body size in Drosophila larvae, like in other animals, is controlled by nutrition. Nutrient restriction leads to catabolic responses in the majority of tissues but the Drosophila mitotic imaginal discs continue growing. The nature of these differential control mechanisms that sparing spare distinct tissues from starvation are poorly understood. Here, we reveal that the Ret-like receptor tyrosine kinase (RTK), Stitcher (Stit) is required for cell growth and proliferation through the PI3K-I/TORC1 pathway in the Drosophila wing disc. Both Stit and insulin receptor (InR) signalling activate PI3K-I and drive cellular proliferation and tissue growth. However, whereas optimal growth requires signalling from both InR and Stit, catabolic changes manifested by autophagy only occur when both signalling pathways are compromised. This was determined using RNAi, dominant negative and stit FRT mutant reagents to inactivate Stit either compartmentally or in clones in the wing followed by quantification of mitotic (BrdU/PH3/CycB), growth (TORC1 targets S6K/4E-BP), autophagic (Atg8a) and PI3K-I signalling read-outs in addition to effects upon cell numbers at larval, pupal and adult stages. This was complemented with overexpression studies in the larval fat body. The combined activities of Stit and InR in ectodermal epithelial tissues provide an RTK-mediated, two-tiered reaction threshold to varying nutritional conditions that promotes epithelial organ growth even at low levels of InR signalling.

345C

An *in vivo* RNAi screen for novel regulators of the Hippo pathway in organ size control. Carole Poon[1,2], Xiaomeng Zhang[1,2,3], Jane Lin[1,2], Samuel Manning[1,2,3], Kieran Harvey[1,2,3]. 1) Cell Growth and Proliferation Laboratory, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia; 2) Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia; 3) Department of Pathology, University of Melbourne, Parkville, Victoria, Australia.

The Salvador-Warts-Hippo (SWH) pathway is an evolutionarily conserved regulator of tissue growth that is deregulated in human cancer. Upstream SWH pathway components convey signals via a core kinase cassette to the transcription coactivator Yorkie (Yki), which controls tissue growth by modulating genes that control cell proliferation and apoptosis. Large-scale phospho-proteome studies in vivo and in vitro indicate previously uncharacterised phosphorylation sites on SWH pathway proteins, suggesting that additional kinases may influence SWH signalling. To uncover such kinases, we performed a genetic RNA interference modifier screen against the *Drosophila melanogaster* kinome. From this screen, we identified kinases that are known to be associated with SWH signalling, such as members of the JNK cascade. Furthermore, we have discovered two new SWH kinases which control tissue growth and organ size during development in the fly: Tao-1 promotes Hippo activation to restrict tissue growth, and Hipk promotes tissue growth in a Yki-dependent manner. Importantly, we have shown that the ability of Tao-1 and Hipk to regulate SWH signalling is conserved in mammalian cells. Using both fly and mammalian systems, we will continue to investigate the role of other positive screen candidates in tissue growth regulation, and determine their relationship to the SWH pathway in organ size control.

346A

Drosophila models for XPB-related cancer predisposition. Leonie M Quinn[1], Naomi C Mitchell[1], Arjun Chahal[1], Mendis Peter[1], Amandine Michaud-Cartier[1], Ross D Hannan[2]. 1) Anatomy, University of Melbourne, Melbourne, Victoria 3010, Australia; 2) Peter MacCallum Cancer Centre, St Andrews Place, East Melbourne Victoria 3002, AUSTRALIA.

Mutations that disrupt function of the DNA helicase subunit of the TFIIH transcription factor complex, XPB, have been linked with the human diseases Xeroderma pigmentosum (XP) and Cockayne syndrome (CS). XPB has roles in both DNA-repair and TFIIH-dependent transcription, however, the question of why mutations in the C-terminal domain of XPB results in cancer in some patients, but not others, remains unresolved. Here we correlate XPB/hay mutations with phenotype using a Drosophila model. We demonstrate hay mutants, which lack the conserved C-terminal domain, previously correlated with XPB-related disease, enhances cell and tissue overgrowth in a manner dependent on loss-of-function for the dmyc repressor Hfp. We provide evidence that these larval overgrowth phenotypes are associated with impaired interactions between Hfp and Hay and defective repression of dmyc transcription.

347B

The Hippo signaling pathway plays a role in homeostatic growth of soma and germ line in the D. melanogaster larval ovary. Didem P. Sarikaya, Cassandra G. Extavour. Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

The Hippo (Hpo) pathway is conserved across animals, and regulates proliferation by altering the activity of the

transcriptional coactivator Yorkie. The role of Hpo signaling during development of homogeneous cell types in various organs has been intensively investigated. However, it is not known whether Hippo may differently influence growth of distinct cell types within a single organ. The D. melanogaster larval ovary provides a useful model to study coordinated growth of different cell types during development of a single organ. The germ cells (GCs) are known to coordinate their proliferation homeostatically with the surrounding somatic intermingled cells (ICs). Some of the somatic cells anterior to the GCs differentiate into terminal filament cells (TFCs) and sort into stacks of cells (terminal filaments) that begin the process of dividing the ovary into functional units called ovarioles. The survival and proliferation of GCs, ICs, and TFCs are critical for ovariole morphogenesis and establishment of the functional adult ovary. Here we show that the Hpo pathway influences proliferation of both ICs and TFCs, and in contrast to previous reports, also plays a role in GC proliferation. Interestingly, the pathway appears to function differently in germ line and soma. While the Hpo pathway operates canonically in somatic ovarian cells, our data suggest that in the germ line yki may regulate proliferation of GCs in a hpo-independent manner. Previous studies had shown that reducing EGFR signaling from GCs to ICs reduced IC number and led to GC overproliferation, suggesting that ICs suppress GC. Surprisingly, we found that increasing IC number by abrogating Hpo pathway activity led to increased GC number. Conversely, reducing IC number via yki knockdown reduced GC number. This result contrasts with previous observations that IC reduction leads to germ cell overproliferation. Taken together, our results suggest that the Hippo pathway operates differently in distinct cell types of the ovary, and may play a role in regulating the homeostatic growth of germ line and soma.

348C

A novel mechanism for Emc transcriptional regulation of Notch-mediated proliferation in Drosophila. Carrie M. Spratford, Justin P. Kumar. Biology, Indiana University, Bloomington, IN.

During development, proliferation rates within developing tissues must be great enough to produce organs of the appropriate size and cellular complexity. Within Drosophila imaginal discs, the non-basic helix-loop-helix (HLH) protein Extramacrochaetae (Emc) is required for normal proliferation. Notch activity appears to be required for both imaginal tissue growth as well as *emc* transcription. Using the MARCM technique we have been able to establish a link between the growth defects associated with *emc* null clones and Notch-induced proliferation. Our studies demonstrate that *emc* null clones, when generated in a wild-type background, are significantly smaller when compared to neutral clones. *emc* is not required for cell viability as null clones are able to grow in a *Minute* background. We also provide evidence that *emc* is not a regulator of apoptosis as null clones fail to grow when programmed cell death is blocked via expression of p35. We will provide evidence that *emc* mediates a significant portion of Notch-induced proliferation within imaginal discs and that loss of *emc* blocks the execution of the Notch signal. The mechanism for how Emc regulates cell proliferation is poorly understood. It is currently thought that Emc functions as a competitive inhibitor and interferes with the interaction of Daughterless (Da), which itself is implicated in cell proliferation, with members of the Achaete-Scute Complex (AS-C). The resulting Da-Emc heterodimer is presumed to be unable to bind to DNA due to the missing basic domain within Emc. Here we present data to support an alternate model in which Emc interacts directly with DNA thereby interfering with ability of the Da/AS-C complex to bind and modulate target genes. We will propose a new regulatory model for Emc and its vertebrate homologs, Id1-4. Overall, the data obtained reveals that Emc may utilize several transcriptional mechanisms to affect many developmental processes including cell proliferation.

349A

Mask proteins are cofactors of Yorkie/YAP in the Hippo pathway. Barry J Thompson, Clara M Sidor. London Research Institute, Cancer Research UK, London, United Kingdom.

The Hippo signalling pathway acts via the Yorkie (Yki)/Yes-associated protein (YAP) transcriptional co-activator family to control tissue growth in both Drosophila and mammals. Yki/YAP drives tissue growth by activating target gene transcription, but how it does so remains unclear. Here we identify Mask as a novel co-factor for Yki/YAP. We show that Drosophila Mask forms a complex with Yki and its binding partner Scalloped on target gene promoters and is essential for Yki to drive transcription of target genes and tissue growth. Furthermore, the stability and sub-cellular localisation of both Mask and Yki is co-regulated in response to various stimuli. Finally, Mask proteins are functionally conserved between Drosophila and humans and are co-expressed with YAP in a wide variety of human stem/progenitor cells and tumours.

350B

Distinct replication mechanisms leading to polyploidy. Jessica R. Von Stetina[1], Noa Sher[1], George Bell[1], Shinobu Matsuura[2], Katya Ravid[2], Terry L. Orr-Weaver[1]. 1) Whitehead Inst, Dept. of Biol., MIT, Cambridge, MA; 2) Boston University Medical School, Boston MA.

Polyploidy is fundamental for the terminal differentiation of many large or highly metabolic cells in both plants and animals. In Drosophila, most differentiated larval and adult tissues increase DNA content via the endo cycle, in which repeated rounds of DNA replication take place in the absence of cell division. Mammalian placental trophoblast giant cells (TGCs) also use endocycles to become polytene. In contrast, mammalian blood megakaryocytes (MKs) polyploidize via endomitosis, mitosis without nuclear division or cytokinesis. In Drosophila endocycles replication of the genome is not uniform; differential replication leads to under-replicated domains and amplified genes. We isolated TGCs and MKs from mice and performed array Comparative Genome Hybridization to test for differential replication. Replication of the euchromatin is uniform, in striking

contrast to Drosophila tissues. Furthermore, quantitation of copy number of heterochromatic genomic regions in TGCs by qPCR revealed full replication of heterochromatin. Analysis of the transcriptome of TGCs and MKs shows profound differences in expression of cell cycle regulatory genes compared to Drosophila endocycling cells, highlighting distinct parameters for endoreplication between mice and flies.

351C

Characterization of a novel Merlin and Sip1 interaction region. Namal Abeysundara, Albert Leung, Sarah C. Hughes. University of Alberta, Edmonton, Canada.

Neurofibromatosis 2 (NF2) is a disorder characterized by the development of tumours of the central nervous system. The gene involved in NF2 encodes for the tumour suppressor protein, Merlin. Merlin is closely related to ezrin, radixin and moesin (ERM) proteins, which function together in maintaining cell integrity and coordinating cell proliferation. Even though these processes have been implicated in cancer, the specific mechanism behind Merlin function is not well understood. To investigate the mechanism(s) of Merlin activity, we analyze Merlin interacting proteins, including Sip1, the Drosophila orthologue of the Na+/H+ exchanger regulatory factor, NHERF1. Despite the biochemical evidence suggesting Merlin and NHERF1 interactions, the functional interaction between the two proteins still remains unclear. To further characterize the physical and functional interaction between Merlin and Sip1, a novel potential binding site in Merlin to the Sip1 protein was identified. A 100 amino acid region immediately downstream of the Four-point-one Ezrin-Radixin-Moesin (FERM) domain of Merlin, in addition to the FERM domain itself, was identified as being important for Sip1 binding. Within the 100 amino acid region, the substitution of two widely conserved arginine residues to the corresponding Moesin residues (R325A and R335L) resulted in reduced Sip1 binding, suggesting that these arginine residues are important for the Merlin and Sip1 interaction. To determine the functional importance of the interaction region, the over-expression of the R325A and R335L Merlin mutants were analyzed using the UAS-GAL4 system in wing imaginal discs. Immunofluorescence antibody staining of larval wing discs and adult wing size measurements provide insight into whether adhesion or proliferation is altered when Merlin and Sip1 binding is reduced. In addition, the effect of the Merlin mutants in Schneider 2 cells were analyzed using proliferation assays. Characterizing the physical and functional interaction between Merlin and Sip1 may provide insight into the mechanism behind Merlin function.

352A

Characterizing the interaction between dCAF1-p180 and the tumor suppressor Merlin. Patrick Delaney, Pam Vanderzalm, Richard Fehon. Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Merlin (Mer), the product of the Neurofibromatosis 2 tumor suppressor gene, acts upstream in the highly conserved Hippo (Hpo) tumor suppressor pathway to regulate cell proliferation and apoptosis. While Mer plays an active role in upstream Hpo signaling, the mechanism by which Mer itself is regulated remains poorly characterized. To study this regulation, a yeast 2-hybrid (Y2H) screen and subsequent functional assays were carried out to identify proteins that interact with Mer in vivo. We identified dCAF1-p180 (p180), a member of the Chromatin Assembly Factor 1 complex, as a protein that interacts with Mer. Consistent with the Y2H results, p180 can co-immunoprecipitate with wild-type and active variants of Mer when expressed in Drosophila S2 cells. p180 is primarily cytoplasmic and membrane-associated when expressed in S2 cells, but epitope-tagged p180 appears primarily nuclear in imaginal epithelial cells. RNAi depletion of p180 in wing imaginal discs results in a complex growth phenotype with increased levels of Mer. However, we observe decreased levels of the adherens junction protein Ecadherin, and other readouts of Hpo signaling, such as Expanded, are not affected by loss of p180 function. Together these results suggest that p180 affects Merlin expression but does not function directly in the Hpo pathway.

p180 plays important nuclear roles in DNA damage repair and maintenance of epigenetic memory. In contrast, Mer is thought to regulate signaling at the cell membrane due to its subcellular localization and interactions with transmembrane proteins. However, recent evidence in mammals suggests Mer may also have an important nuclear role. Given our results, we speculate that Mer may repress its own transcription via interactions with p180. Further elucidation of this interaction could reveal another layer of control for Mer and Hpo signaling, and would expand our understanding of the recently discovered class of chromatin modifying tumor suppressor genes.

353B

Hippo Activation through Homo-dimerization and Membrane Association for Growth Inhibition and Organ Size Control. Yaoting Deng[1], Yurika Matsui[2], Yifan Zhang[3], Zhi-Chun Lai[1,2,3,4]. 1) Biochemistry and Molecular Biology, Penn State University, University Park, PA; 2) Intercollege Graduate Degree Program in Cell and Developmental Biology; 3) Intercollege Graduate Degree Program in Genetics; 4) Department of Biology.

Hippo (Hpo) signaling plays a critical role in restricting tissue growth and organ size in both invertebrate and vertebrate animals. However, how the Hpo kinase is regulated during development has not been clearly understood. Using a Bimolecular Fluorescence Complementation (BiFC) assay, we have investigated the functional significance of Hpo homo-dimer formation and subcellular localization in living cells. We found that Hpo dimerization and membrane association are both critical for its activation in growth inhibition. As dimerization facilitates Hpo to access its binding partner, Hpo kinases in the homo-dimer trans-phosphorylate each other to increase their enzymatic activity. Moreover, loss- and gain-of-function studies indicate that upstream regulators, Expanded, Merlin and Kibra, play a critical role in promoting Hpo dimerization as well as association to the cell membrane. Enforced Hpo localization to the cell membrane increases Hpo dimerization and its activity. Therefore,

homo-dimerization and membrane localization are two important mechanisms for Hpo activation in growth control during animal development.

354C

Investigation of the genetic interactions between the Hippo signaling pathway and *Drosophila* C-terminal Src kinase (dCsk). Hailey J. Kwon[1,2], Indrayani Waghmare[1], Shilpi Verghese[1], Madhuri Kango-Singh[1,3,4]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) University of Dayton Honors Program, Dayton OH; 3) Center for Tissue Regeneration and Engineering at Dayton, University of Dayton, Dayton OH; 4) PreMedical Programs, University of Dayton, Dayton OH.

The Hippo signaling pathway is involved in regulating tissue size and diseases such as cancer. Hippo signaling coordinates a timely transition from cell proliferation to cellular quiescence, and ensures proper cellular differentiation. Aberrant Hippo pathway function (due to mutations or amplification of genes, epigenetic silencing, and oncogenic transformation) is often detected in human cancers and correlates with poor prognosis. The *Drosophila C-terminal Src kinase* (*dCsk*) is a genetic modifier of *warts* (*wts*), a tumor-suppressor gene in the Hippo pathway, and interacts with the Src oncogene. Reduction in Csk expression and the consequent activation of Src are frequently seen in hepatocellular and colorectal tumors. Previous studies have shown that *dCsk* regulates cell proliferation and tissue size during development. Given the similarity in the loss of function phenotype of *dCsk* and *wts*, we investigated the genetic interactions of *dCsk* with the Hippo pathway. We hypothesized that dCsk regulates growth via the Hippo pathway. To determine whether *dCsk* requires Hippo signaling to carry out its growth regulatory functions, two approaches were used. First, we tested if *dCsk* regulates the expression of transcriptional targets of Hippo signaling, e.g., *ex-lacZ, fj-lacZ, dronc1.7kb-lacZ*, and *diap1-4.3GFP*. Second, we tested genetic interactions between *dCsk* and components of the Hippo pathway in order to determine the hierarchy of gene action. Here we present our progress on establishing the genetic links between *dCsk* and the Hippo signaling pathway.

355A

Signalling pathways controlling transcription of the *myc* oncogene and cell overgrowth in *Drosophila* via Psi. Amanda Jue Er Lee[1], Nicola Cranna[1], Naomi Mitchell[1], David Levens[3], Ross Hannan[2], Leonie Quinn[1]. 1) Anatomy and Neuroscience, University of Melbourne, Parkville, VIC, Australia; 2) Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; 3) National Cancer Institute, Bethesda, Maryland, United States.

Myc proteins are critical regulators of growth and cell cycle progression during animal development. Dysregulation of Myc can result in over proliferation and malignant transformation. In addition, *Myc*transcription must rapidly respond to environmental cues, which feed into developmental signalling pathways. *In vitro* studies in mammals have demonstrated activated expression of the *c-Myc* oncogene in response to growth factors in serum, which correlates with recruitment of the single-stranded DNA binding protein FBP, but the signals promoting recruitment of FPB to activated c-Myc are currently unknown. In an effort to better understand activation and repression of *Myc* transcription in an *in vivo* signalling environment, we have developed models to study the *Drosophila* orthologs of FPB and FIR, Psi and Hfp respectively. Our work has previously shown that Hfp is also a critical *dMyc* repressor, and we will present evidence that Psi is required for activated *dMyc* transcription. In addition, we have provided the first evidence that Ras pathway activation increases the abundance of *dMyc* via transcriptional effects. Furthermore, we demonstrate that *dMyc* upregulation and cell growth observed upon activation of the Ras pathway is dependent on Psi. Together the data we will present demonstrate that Psi may provide an important link between the Ras signalling pathway, *dMyc* promoter activity, cell growth and cell cycle progression.

356B

Functional and Genetic Analysis of Compensatory Responses Induced in Tumors Caused by Loss of Scribble (apical-basal polarity). Alyssa Lesko[1,2], Shilpi Verghese[3], Indrayani Waghmare[3], Madhuri Kango-Singh[3,4,5]. 1) Department of Chemistry,University of Dayton, Dayton, OH; 2) Department of Mathematics, University of Dayton, Dayton, Oh; 3) Department of Biology, University of Dayton, Dayton, OH; 4) Pre-Medical Programs, University of Dayton, Dayton OH; 5) Center for Tissue Regeneration and Engineering at Dayton, University of Dayton, Dayton OH.

The Hippo pathway has recently been identified to regulate the proliferation and survival of cells. *scribble* (*scrib*) is a tumor suppressor gene that is involved in cell polarity. There is evidence that cell death induction in the *scri*b mutant cells is correlated to an increase in Jun N-terminal Kinase (JNK) signaling due to activation of cell competition. However, increased survival of *scrib* mutant cells (by activation of P35 or in Minute background) leads to growth of massive tumors. My project will investigate how changes in Hippo signaling are important to cell-cell interactions regulated by *scrib* in different mutant conditions. Our previous work showed that JNK and Hippo pathway interact. *scrib* mutant cells showed increased levels of phospho-JNK compared to wild type and double mutant cells. We hypothesize that this interaction determines if tumor cells survive or are eliminated. To test this, I will look at the role of JNK when it is activated and down regulated in the Hippo pathway, as well as, its interaction with*scrib*. Our aims are: 1. Test the *scrib*-JNK interactions to assess role of JNK in scrib mediated overgrowth, and 2. Test the *scrib*-Hippo interactions to delineate the signaling interactions between *scrib*mutant cells and their neighbors to promote survival and over proliferation. Our findings from these studies will be presented.

357C

Effects of the endocrine hormone ecdysone on neoplastic tumorigenesis. Thu H. Tran, Rebecca Garrett, Katherine Pfister, Adrian Halme. Cell Biology, University of Virginia School of Medicine, Charlottesville, VA.

Tumor formation is a multi-factorial process that involves contributions from genetic mutations within tumor cells as well as inputs from surrounding signals. We have begun to examine the role of endocrine hormones in regulating tumor initiation and progression. The endocrine signals ecdysone and juvenile hormone are critical coordinators of normal Drosophila developmental transitions. Using several different neoplastic tumor models, we identified a correlation between the timing of tumor formation in imaginal tissues and the increase of juvenile hormone esterase (Jhe) expression levels. Jhe expression initiates a developmentally important transition in larval hormone signaling where juvenile hormone levels drop and ecdysone signaling begins to rise. Our results suggest that ecdysone could play a role in regulating the development of neoplastic tumors. Thus, we tested the effects of disruption of the ecdysone signaling transduction on neoplastic tumors. Over-expression of dominant negative mutations of the ecdysone receptors leads to drastically reduced growth of tumors with partially restored cell polarity, suggesting that the ecdysone signaling autonomously regulates neoplastic tumorigenesis. Furthermore, we also identified the Wingless signaling pathway, an important regulator of Drosophila development and homeostasis that is regulated by the ecdysone signaling, as a potential mediator of ecdysone's effects on neoplastic tumorigenesis. Ongoing experiments are exploring the molecular mechanisms by which the hormone signal ecdysone regulates tumorigenesis.

358A

A non-transcriptional role for Hippo pathway signaling. Pam Vanderzalm, Richard Fehon. Molecular Genetics & Cell Biology, University of Chicago, Chicago, IL.

The Hippo-Salvador-Warts (HSW) tumor suppressor pathway has been well-characterized with respect to its ability to regulate growth through Yorkie-dependent transcription. Genes involved in promoting growth (cyclin E and bantam miRNA) or inhibiting cell death (diap1), as well as upstream activators of the pathway (expanded and kibra), are bone fide transcriptional outputs of the pathway.

In addition to regulating growth, the HSW pathway also regulates the polarity of epithelial cells by limiting the size of the adherens junction (AJ) and the apical domain (AD). Cells either lacking HSW function or overexpressing Yorkie have higher levels of many proteins that localize to the AD, including those involved in regulating apical polarity (such as Crumbs and aPKC). AJ proteins such as Armadillo and E-Cadherin are similarly upregulated. Many, if not all, of the components of the HSW pathway localize to the AD, and localization of HSW components may be critical for activating the pathway. For instance, tethering Mats to the membrane activates signaling through the HSW pathway.

We examined whether the apical localization of Yorkie was important for its role in controlling apical domain size. We also asked whether transcription through Yorkie was required to control AJ and AD size. By generating transgenic flies expressing a version of Yorkie that is transcriptionally-dead, we found that Yorkie, and by extension HSW signaling, controls the levels of apical polarity proteins at the membrane independent of its function in growth-regulating transcription. Consistent with this finding, mutating Ser168 to alanine, which promotes Yorkie translocation to the nucleus, diminishes Yorkie's ability to promote apical identity. Detailed characterization of the polarity phenotype and an analysis of HSW complex formation at the apical membrane will be presented.

359B

Effect of novel phosphorylation sites on the function of the tumor suppressor Merlin. Sophia Yip, Angela Effa, Sarah Hughes. University of Alberta, Edmonton, Canada.

Using *Drosophila* as a model, we are analyzing the role and mechanism of action of 4.1 family protein member Merlin in Neurofibromatosis Type II (NF2), a disorder associated with development of nervous system tumors. Merlin is a tumor suppressor that is also involved in adhesion, and its activity is deactivated by phosphorylation. Merlin can be multiply-phosphorylated, and we hypothesize that the multiple phosphorylation sites are involved in fine-tuned control of Merlin activity. Our lab has identified 14 potential novel phosphorylation sites for regulating Merlin activity. Mutations of these specific residues to non-phosphorylatable residues are hypothesized to reduce the tumor-suppressor activity of Merlin. To test this hypothesis, potential phosphorylatable serines and threonines were mutated to either a phosphor-mimic or non-phosphorylatable residue and the effects of the mutations on the location and function of Merlin were examined. As the cellular location of Merlin is related to its activity state, a pulse-chase assay was used to test the effect of the mutations on Merlin localization over time in *Drosophila* Schneider 2 cells. Mutations leading to a different localization pattern over time are likely to be potential phosphorylation sites affecting Merlin activity. Using this method, serine 371 and threonine 18 were identified as potential Merlin phosphorylation sites. To further test the functional effect of these mutations on Merlin activity, transgenic flies carrying the mutant Merlin genes were crossed to wing-specific drivers using the UAS/GAL4 system. The effect of the mutations on proliferation, adhesion and changes in morphology of adult wings and wing imaginal discs were determined. Staining of the wing imaginal discs showed differences in localization of actin and E-cadherin when Merlin^{S371D} is overexpressed, suggesting that serine 371 is a key phosphorylation site affecting Merlin function. By identifying the mechanism regulating Merlin activity, we can begin to move toward possible approaches that will allow for future treatment and diagnosis of NF2 patients.

360C

Assembly and function of centromeric chromatin in Drosophila meiosis. Nicole L. Beier[1,2], Elaine M. Dunleavy[2,3], Walter Gorgescu[4], Jonathan Tang[4], Sylvain V. Costes[4], Gary H. Karpen[1,2]. 1) Department of Molecular and Cell Biology, University of

California, Berkeley, CA; 2) Department of Genome Biology, Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA; 3) National University of Ireland, Galway, Ireland; 4) Department of Cancer and DNA Damage Responses, Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA.

Centromeres are regions of eukaryotic chromosomes essential for faithful segregation of DNA, and are defined and maintained epigenetically throughout cellular generations in most eukaryotes by the histone H3 variant CENP-A. To maintain centromeric identity, new CENP-A must be assembled to replace the two-fold dilution that occurs after S phase and chromosome segregation. Aberrant incorporation of CENP-A causes ectopic kinetochore formation and aneuploidy. Recent studies have identified the timing and regulation of CENP-A incorporation in mitosis, predominantly in cultured cells. However, the function, regulation and cell cycle timing of CENP-A assembly in meiosis in tissues are currently unknown. We investigated the timing and requirements for assembly of the CENP-A homolog CID in male meiosis in *Drosophila melanogaster*. We found that CID is assembled during prophase of meiosis I and after exit from meiosis II. Prophase I loading is conserved in females. Surprisingly, these assembly phases are progressive, occurring over a period of hours to days. We also studied the requirements for CID assembly during meiosis, and found that the assembly factor CAL1 and the inner kinetochore protein CENP-C are both required. These studies demonstrate that the cell cycle timing of CID assembly in meiosis is different from previous observations in mitotic cells, including the length of time over which assembly occurs. Future investigations will focus on the function of CID assembly in meiotic prophase and the role of meiotic cell cycle factors in regulating assembly.

361A

Mitotic chromosome phenotypes associated with a panel of Mcm10 mutants in Drosophila. Ritu Dalia, Michael Reubens, Tim W. Christensen. Biology Dept, East Carolina University, Greenville, NC.

Replication of the genome and proper formation, and packaging, of chromatin are processes essential to eukaryotic life. Maintenance of epigenetic chromatin states is essential for faithfully reproducing the transcriptional state of the cell; likewise, replication of DNA with high fidelity is crucial for accurate passage of genetic information from a cell to its progeny. Defects in DNA replication and improper regulation of the chromatin states can result in genome instability which can manifest as disease, or death of the organism. There are a plethora of factors involved in the process of DNA replication in eukaryotes, and recent studies have shed light on one of the factors called mini-chromosome maintenance 10 (Mcm10) as an essential DNA replication factor. First discovered in S. cerevisiae, Mcm10 is an abundant nuclear protein that has been implicated in the activation of the Pre-RC, interacts with members of the elongation machinery such as Polα, and has recently been shown to be required in the formation of heterochromatin in both yeast and Drosophila. Previous analysis of two Drosophila Mcm10 mutant alleles demonstrated that Mcm10 not only plays a role in DNA replication, but also has a role in heterochromatic silencing and chromosome condensation. To further investigate the roles of Mcm10 we used a collection of over 20 missense mutations generated using a Tilling approach. Mitotic index data generated shows that there is not enough evidence to show a significant mitotic delay in the mutant strains. Interestingly though, varying types of chromosomal phenotypes, such as severe condensation defects, separated sister chromatids, aneuploidy and anaphase bridge defects, were observed in these mutants suggesting that Mcm10 is involved in maintaining the genomic stability. Further evaluation of these mutants will help elucidate the biological functions of this well conserved protein as well as provide information on the domains of the protein required for its different biological functions.

362B

Redundant PREs act together to maintain *en*/*inv* gene expression. Sandip De, Judith Kassis. NICHD, NIH, BETHESDA, MD.

In Drosophila, the *engrailed* (*en*) and *invected* (*inv*) genes are required for segmentation, development of the specific cells in the nervous system, and in the posterior compartment in imaginal disks. *en* and*inv* are co-regulated genes juxtaposed in a chromatin domain marked by H3K27me3. It is well established that *en*/*inv* gene expression is very dynamic throughout development and is regulated by different DNA regulatory elements and trans-acting proteins. With the purpose of identifying the role of Polycomb group Response Elements (PREs) in setting up *en*/*inv* domain, we deleted ~1.5kb ($en^{\Delta 1.5}$) and ~24kb ($inv^{\Delta 24}$) containing the major *en* and *inv* PREs respectively. Surprisingly, both $en^{\Delta 1.5}$ and $inv^{\Delta 24}$ flies are homozygous viable and fertile. In comparison to wild type, we did not observe any significant difference in H3K27me3 accumulation within *en*/*inv* domain in either mutant. ChIP-seq analysis with anti-Pho antibody identified 6 additional potential weak PREs, present between the *en* and *tou* genes. We observe increased accumulation of Pho proteins in these weak PREs and also at a PRE present in the 5' end of *E(Pc)*, the gene next to *inv*. We believe these weak PREs act to maintain the epigenetic mark in the *en*/*inv* domain recruiting PcG proteins in the absence of the major PREs. Further research is under progress.

363C

Chromosome conformation capture and ecdysone signaling: insights into the regulation of early genes. Travis J. Bernardo, Xie Xie, Edward Dubrovsky. Fordham University, 441 East Fordham Road, Bronx, NY 10458.

The early genes are a key group of ecdysone targets that function at the top of the ecdysone signaling hierarchy. They are transcriptionally complex, encoding multiple isoforms that are activated in different tissue- and stage- specific patterns *in vivo* and exhibiting distinct temporal patterns in response to ecdysone. While the general mechanism of ecdysone-dependent transcription is well characterized, it is not understood how a pulse of ecdysone is transmitted into complicated patterns of early gene expression. We previously found that one of the early genes - *E75* - harbors multiple enhancers with functional ecdysone response elements, but it was unclear how these enhancers were involved in regulating the expression of

different *E75* isoforms. To address this question we employed the chromosome conformation capture (3C) method in S2 cells to identify interactions between the enhancers and three of the *E75* promoters. We found that the *E75A*, *E75B*, and *E75C* promoters possess pre-existing, ecdysone-responsive interactions with different enhancers and, correspondingly, each promoter exhibits distinct temporal patterns of activation by ecdysone. These observations were extended to *E74* in S2 cells and also to individual larval tissues. Our findings suggest that the distinct spatial and temporal responses to ecdysone by early genes are determined in part by local enhancers which act on different promoters in a tissue- or stage-specific manner.

364A

The SCF^{Slimb} ubiquitin ligase directly targets condensin II for degradation and functions to modulate 3D interphase chromosome spatial organization. Giovanni Bosco[1], Daniel W. Buster[2], Scott G. Daniel[2], Huy Q. Nguyen[1], Sarah L. Windler[3], Maureen Peterson[1], Meredith Roberts[2], Joy H. Meserve[2], Tom Hartl[2], Joey E. Klebba[2], David Builder[3], Gregory C. Rogers[2]. 1) Genetics & Norris Cotton Cancer Ctr, Geisel Sch Med at Dartmouth, Hanover, NH; 2) Department of Cellular and Molecular Medicine, Arizona Cancer Center, University of Arizona, Tucson, AZ 85724, USA; 3) Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA.

The SCF^{Slimb} E3 ubiquitin ligase has been previously shown to localize to the nucleus, but the role it may play in modulating chromosome structure and spatial organization is not well understood. We show that RNAi depletion of Slimb in cultured cells as well as *slimb* mutations *in vivo* lead to dramatic global changes in interphase chromosome morphology. Upon depletion of Slimb chromosomes become hypercondensed, homologs unpair and we observe defects in nuclear envelope morphology. These phenotypes are also seen in Cul-1 and SkpA RNAi, confirming that all three SCF subunits contribute to these phenotypes. Slimb RNAi in vivo and loss-of-function mutations in nurse cells as well as diploid larval tissues recapitulate all these phenotypes. Interestingly, we find that all these phenotypes are due to a failure to degrade the Cap-H2 condensin II subunit, and we show that the SCF^{Slimb} E3 ligase normally targets Cap-H2 for ubiquitination and protein degradation. Moreover, chromatin fractionation experiments reveal that Slimb itself is chromatin bound, raising the possibility that condensin II activity on chromatin may be regulated by selective ubiquitination and removal of Cap-H2. We propose a model where chromatin-tethered SCF^{Slimb} regulation of Cap-H2 protein levels is critical for maintenance of chromosome organization in interphase.

365B

Condensin II mediated interphase chromosome compaction drive changes in nuclear architecture. Julianna Bozler[1], Huy Nguyen[1], Tom Hartl[2], Christopher Bauer[2], Gregory Rogers[2], Giovanni Bosco[1]. 1) Geisel School of Medicine, Dartmouth College, Hanover, NH; 2) Molecular and Cellular Biology, University of Arizona, Tucson, AZ.

In eukaryotic cells, the nuclear membrane is an essential component of cellular organization and highly dynamic through the cell cycle. Despite its loss of structural stability during early cell replication steps, the maintenance of its structure during interphase is important for normal cell function. Given the vital role of this structure, it is not surprising that aberrant nuclear envelope morphologies are characteristic of many human diseases, such as progeria. Additionally, recent evidence suggests the nuclear membrane plays an important role in the establishment and maintenance of chromosome arrangement in the interphase nucleus. We have investigated the relationship between the nuclear envelope and the 3-dimensional organization of chromatin. We show that Drosophila condensin II provides a chromatin compaction activity in interphase cells, and this condensation force can drive distortions in nuclear architecture that include invaginations of the envelope and intra-nuclear vesicle formation. Vesicles inside the nucleus contain nuclear pore proteins, suggesting that proteins integral to nuclear membrane are force into the interior of the nucleus. We propose a model where chromatin tethers to inner nuclear envelope proteins serve as anchors that allow interphase chromosome movements to cause morphological changes of the nuclear envelope. We speculate that interphase chromatin compaction may be a normal mechanism that reorganizes nuclear architecture, while under pathological conditions, such as laminopathies, these compaction forces contribute to dramatic defects in nuclear morphology.

366C

Mis-expession of HipHop rescues cell lethality following telomere loss. Rebeccah L. Kurzhals[1], Laura Fanti[2], Sergio Piminelli[2], Yikang Rong[3], Kent Golic[4]. 1) Department of Biology, Southeast Missouri State University, Cape Girardeau, MO; 2) University of Rome, "La Sapienza", Rome, Italy; 3) National Cancer Inst., Bethesda, MD 20892; 4) University of Utah, Salt Lake City, UT 84112.

The telomere cap is a complex of proteins and nucleic acid found at chromosome ends which prevents the DNA terminus from being seen as a double strand break in need of repair. HP1, HOAP, and HipHop, among others, are critical components of this capping complex. In most cells, the absence of a single telomere cap is sufficient to trigger apoptosis. Cells that do not die are likely to experience end-to-end fusions of uncapped ends, leading to gross chromosomal rearrangements and genomic instability. The apoptotic response to telomere loss or dysfunction is mediated by the DNA damage response, primarily through Chk2 and p53. Mutation of either of the genes encoding these proteins allows for the survival and proliferation of cells that have lost a telomere. However, even in a wildtype background, a small fraction of such cells manage to evade this apoptotic response. We have developed a technique that allows for controlled loss of a single telomere during development. We wish to understand how some cells survive such telomere loss. Immunostaining for the telomere cap component HOAP revealed that in some somatic cells, non-telomeric ends can be healed by the addition of a new cap. To characterize this

process we mis-expressed genes required for telomere maintenance while simultaneously inducing telomere loss. We found that mis-expression of *HipHop*, or its paralog *ms(3)K81*, resulted in increased survival of cells that lost a telomere. However, mis-expression of *cav*, the gene encoding HOAP, or *Su(var)205*, encoding HP1, did not significantly increase cell survival, despite the fact that HipHop and HOAP have been shown to be required for each other's stability. We suggest that HipHop has the ability to seed formation of new telomeres in somatic tissue.

367A

PARP-1 marks mitotic chromatin and regulates post-mitotic transcription. Niraj Lodhi, Alexei Tulin. Epigenetics and Progenitor Cells Program, Fox Chase Cancer Center, Philadelphia, PA.

PARP-1 is an abundant nuclear protein that transfers poly(ADP)ribose residues to proteins in order to regulate DNA damage repair, chromatin remodeling and transcription. We found PARP-1 remain bound to chromatin through mitosis. However, it is not known whether its stable binding to mitotic chromatin can act as an epigenetic mark to maintain the re-establishment of gene expression state as cells exit mitosis. To explore this question, we performed ChIP-Seq to determine PARP-1 binding sites in asynchronous and mitotic cells. Additionally, we analyzed the localization of PARP-1 in these cells by confocal microscopy. ChIP-Seq data indicate that PARP-1 binds to different genes during mitosis, but we found evidence for a subset of genes bound to PARP-1 in both interphase and mitosis. Confocal data show there is a remarkable re-localization of PARP-1 in mitotic cells and it remains with chromatin during mitosis whereas other transcription factors disappear. Further, PARP-1 preferentially binds to the transcriptional start sites of genes, in both asynchronous and mitotic chromatin. Finally, we checked the transcription of genes after mitosis in PARP-1 knockdown cells or in presence of PARP-1 inhibitor. Results suggest transcription reduced by two fold in knockdown and partially reduced in PARP-1 inhibited cells. Overall results indicate that functional interaction and presence of PARP-1 in chromatin is required to re-establish post-mitotic transcription.

368B

Homeostasis of interphase chromosome length is maintained by the SCF^{Slimb} E3 Ubiquitin ligase direct targeting of the Cap-H2 subunit of condensin II. Huy Nguyen[1], Christopher Bauer[2], Maureen Peterson[1], Daniel Buster[4], Scott Daniel[2], Gregory Rogers[3,4], Giovanni Bosco[1]. 1) Geisel School of Medicine, Dartmouth College, Hanover, NH; 2) Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ; 3) Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ; 4) Arizona Cancer Center, University of Arizona, Tucson, AZ.

Although chromosomes are typically thought to undergo compaction in anticipation of mitotic segregation, it is not known whether interphase cells also require a chromosome compaction activity. Similarly, how or even if the length of interphase chromosomes is regulated is not known. Previous work from our lab has revealed that the condensin II complex functions to compact polyploid interphase chromosomes in drosophila nurse cells and salivary glands. Here, we used FISH (fluorescent in-situ hybridization) to investigate the relative contributions of condensin I, condensin II and other condensin interacting factors on chromosome length maintenance. We show that condensin II functions to regulate interphase chromosome length in Drosophila cultured Kc cells by providing an axial shortening activity. In addition, targeting of the condensin II subunit, Cap-H2, for proteasomal degradation by the F-box protein Slimb, leads to chromosome lengthening. These results show that interphase chromosome length is a regulated and dynamic feature of the interphase nucleus, and regulation of Cap-H2 protein levels is critical for chromosome length homeostasis.

369C

Condensin II inhibits heterochromatic gene silencing and facilitates transposon silencing. Maureen Peterson[1], Christopher Bauer[2], John Manak[3], Stephen Butcher[3], Giovanni Bosco[1]. 1) Genetics, Dartmouth College, Hanover, NH; 2) New York University Center for Genomics and Systems Biology; 3) University of Iowa Department of Biology.

Condensin II is a protein complex well studied for its role in mitotic chromosome condensation. However, its presence in the nucleus throughout the cell cycle indicates that condensin II may perform important functions in interphase as well. To investigate possible gene regulatory roles of condensin II, we used genomic tiling arrays to compare transcript levels in stage 10 egg chambers of wildtype and condensin II mutant flies. We found that genes located within heterochromatin are repressed in condensin II mutants, which we confirmed using qRT-PCR analysis. We also found that piRNA clusters, sequences found near heterochromatin and known to regulate transposable element transcript levels, are similarly repressed in condensin II mutants. Consistent with their role in regulation of transposons, we find that transposable element transcript levels are increased in condensin II mutants. Investigation of genomic copy number of overexpressed transposable elements by qPCR revealed that some classes of transposable elements are increased in copy number in the germline of condensin II mutants. This finding raises the possibility that transposons are actively jumping in mutant flies. We also show that localization of heterochromatin protein 1 (HP1) is perturbed in the nurse cells of condensin II mutants. We propose two possible models describing how aberrant localization of HP1 may result in hypersilencing of heterochromatic genes and, indirectly, transposon transcript levels.

370A

Interactions of HP1a, HP1b, and HP1c. Nicole C. Riddle[1,2], Tingting Gu[2], Sarah C. R. Elgin[2]. 1) Biology, The University of Alabama at Birmingham, Birmingham, AL; 2) Biology, Washington University in St. Louis, St. Louis, MO.

The heterochromatin Protein 1 (HP1) family of chromosomal proteins are involved in the formation of silent chromatin in

organisms ranging from yeast to human. Their characterizing features are two conserved domains, the chromo domain and the chromo-shadow domain, which are connected by a more variable hinge domain. In *Drosophila melanogaster*, five HP1 family proteins exist, HP1a, HP1b, HP1c, RHINO (HP1d), and HP1e, two of which - RHINO and HP1e - are germline specific. While *Su(var)205* (encoding HP1a), *HP1b*, and *HP1c* are all expressed ubiquitously, the proteins show significant differences in their localization patterns, HP1a associating mainly with heterochromatin, HP1c with euchromatin, and HP1b localizing to both on polytene chromsomes. Interestingly, chromatin immunoprecipitation experiments show that HP1a, HP1b and HP1c colocalize to a significant number of loci. These loci correspond to transcription start sites, both in euchromatin and heterochromatin. Focusing on HP1b, which is less well studied, we find that in third instar larvae, HP1b localizes to 3360 genes, 3127 in euchromatin, 233 in heterochromatin. Of these, 3231 genes are also bound by HP1c, and 472 genes are associated with HP1a, HP1b and HP1c. To determine the binding relationships between the three proteins, we analyzed mutant third instar larvae lacking either HP1a, HP1b, or HP1c. Lack of either HP1b or HP1c does not influence the binding patterns of the other family members. However, chromatin immunoprecipitation experiments suggest that lack of HP1a - while not affecting the distribution of HP1c - can disrupt proper association of HP1b with chromatin. On-going experiments explore how the presence of HP1 family members at transcription start sites influences gene expression in different genomic contexts, given that HP1b and HP1c act as transcriptional activators and HP1a is generally considered a repressor.

371B

CAP-D3, a subunit of Condensin II, regulates expression of Bithorax cluster genes. Kavitha R. Sarvepalli[1], Michelle S. Longworth[1,2]. 1) Department of Molecular Genetics, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA; 2) Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Case Western Reserve University, Cleveland, OH, USA.

Condensin I and II are essential multi-protein complexes that are required for mitotic chromatin condensation. Recent studies have indicated non-canonical roles for Condensin II in inerphase gene regulation.*Drosophila* Condensin II subunit dCAP-D3 regulates transcription of several clusters of genes. One such cluster is the Bithorax complex (BX-C), which consists of three genes, *AbdB*, *abdA* and *Ubx* that regulate anterior-posterior axis patterning. Maintenance of their expression patterns is regulated epigenetically by the Polycomb group of proteins (PcG). PcG mediate the spread of "transcription-repressive" chromatin marks across their target genes in a process that is thought to involve chromatin looping. The repressed BX-C locus is known to adopt a higher-order chromatin configuration that stabilizes its repression. However, all of the factors required for this chromatin organization have not been identified. We find that the loss of *dCap-D3* expression leads to downregulation of BX-C genes in larval tissues and in S2 cells, by qRT-PCR. Chromatin immunoprecipitation studies reveal that dCAP-D3 is recruited to the *Abd-B* promoter, suggesting that its action on BX-C genes is direct. As changes in transcription of these genes are accompanied by alterations in the chromatin configuration of the locus, we investigated the chromatin configuration of the BX-C locus in *dCap-D3* dsRNA-treated and control (T7 dsRNA-treated) S2 cells by performing chromosome conformation capture assays (3C). Results indicate that long-range interactions between distal DNA elements within the cluster are enhanced upon *dCap-D3*knockdown. This suggests that dCAP-D3 has a role in organizing the three-dimensional conformation of BX-C. We hypothesize that dCAP-D3, as part of Condensin II, regulates BX-C by altering its chromatin organization to promote transcription.

372C

The JIL-1 Kinase Does Not Phosphorylate H3S28 or Recruit 14-3-3 to Active Genes in *Drosophila*. Chao Wang, Changfu Yao, Yeran Li, Weili Cai, Jack Girton, Jørgen Johansen, Kristen M. Johansen. Biochemistry, Biophysics & Mol Biol, Iowa State University, Ames, IA.

JIL-1 is the major kinase controlling phosphorylation of H3S10 and functions to counteract heterochromatization and gene silencing (Wang et al, J Cell Sci 124:4309). However, an alternative model has been proposed in which JIL-1 is required for transcription to occur, additionally phosphorylates H3S28, and recruits 14-3-3 to active genes (Ivaldi et al, Genes Dev 21:2818; Kellner et al, Genome Res 22:1081; Karam et al, PLoS Genetics: e1000975). Since these findings are incompatible with the results of Cai et al (Development 135:2917) demonstrating robust levels of transcription in the complete absence of JIL-1 and that JIL-1 is not present at heat shock-induced polytene chromosome puffs, we reexamined JIL-1's possible role in H3S28 phosphorylation and 14-3-3 recruitment. Using two different H3S28ph antibodies we show by immunocytochemistry and immunoblotting that the H3S28ph mark is not present at detectable levels above background on polytene chromosomes at interphase but only on chromosomes at pro-, meta-, and anaphase in S2 cells and third instar larval neuroblasts. Moreover, this mitotic H3S28ph signal is also present in the *JIL-1* null mutant at undiminished levels suggesting that JIL-1 is not the mitotic H3S28ph kinase. We also demonstrate that H3S28ph is not enriched at heat shock puffs. Using two different pan-specific 14-3-3 antibodies as well as an enhancer trap 14-3-3-GFP line we show that 14-3-3, while present in salivary gland nuclei, does not localize to chromosomes but to the nuclear matrix surrounding the chromosomes. In our hands 14-3-3 is not recruited to developmental or heat shock puffs. Furthermore, using a LacI-JIL-1 targeting system to ectopic sites on polytene chromosomes we show that only H3S10ph is present and upregulated at such sites, not H3S28ph or 14-3-3. Thus, our results argue strongly against a model where JIL-1 is required for H3S28 phosphorylation and 14-3-3 recruitment at active genes.Supported by NIH grant GM62916.

373A

Mitotic telomere clustering in Drosophila melanogaster. Natalia Wesolowska, Yikang Rong. Lab of Biochemistry and Molecular Biology, National Institutes of Health, Bethesda, MD.

Telomeres are specialized structures that demarcate the ends of linear chromosomes. When their function is compromised, natural DNA ends can be improperly identified as broken ends and subjected to repair, resulting in chromosomal fusions and genomic instability. As obligatory chromosomal landmarks, telomeres can also serve to organize the genome. In yeast, telomeres cluster at the nuclear periphery, potentially to sequester the ends from the rest of the genome. To bring some insight into telomere organization in higher order organisms, we investigated the situation in interphase nuclei of Drosophila embryo. The syncytial blastoderm stage when nuclear divisions are still synchronized and take place at the surface of the embryo, presents a perfect experimental setting for imaging of a population of nuclei. To follow telomeres in vivo, we used a fluorescently labeled telomere protein HOAP. The 16 telomeres assemble into 4-6 fluorescent foci per nucleus. Furthermore, this organization appears to be present in other somatic tissues in the fly. In light of the findings from yeast, our results suggest that clustering may be a feature conserved through evolution. We made several testable predictions as to the rules governing telomere clustering and investigated them in embryos using fluorescence in situ hybridization to visualize telomeres. First, by inspecting anomalous embryos that develop without the paternal chromosome subset, we found that clustering is not mediated by associations between homologs. Second, using a fly with a novel telomere sequence at one of its ends, we showed that DNA sequence homology is irrelevant to clustering. Third, by marking the two ends of chromosome 3 with an exogenous sequence tag, we determined that clustering is not simply the association of telomeres of the same chromosome. Having ruled out these possibilities, we focus on a model where clustering is a protein-mediated process. So far, we found that telomere proteins do not play a major role in clustering. Through further mutant analysis we hope to bring insight to this mode of nuclear organization.

374B

Dosage compensation of the X chromosome and inverse effect on the autosomes in RNASeq analysis of triple X metafemales compared to normal females. James A. Birchler[1], Lin Sun[1], Adam Johnson[1], Jilong Li[2], Jianlin Cheng[2]. 1) Division Biological Sci; 2) Department of Computer Sci, Univ Missouri, Columbia, MO.

An RNAseq experiment was conducted to examine global gene expression in larval metafemales, normal females and normal males. Triplicate biological replicates were used to determine the number of sequencing reads per gene in each genotype. Then, a ratio distribution analysis was conducted using bins of 0.05. The distribution of the X chromosome for metafemales compared to normal females was largely centered around a ratio of 1.0 representing dosage compensation in metafemales as first noted by Stern (1960). A minor peak was centered near 1.5 representing a subset of genes that exhibited a dosage effect of the X chromosome. For the autosomes, the major peak was centered near 0.67 representing an inverse dosage effect compared to normal females. A minor peak was present near 1.0 representing no change. Another minor peak centered near 0.44, which is the inverse of an inverse effect, which has previously been found in segmental trisomic experiments in flies. Phenotypic validation was conducted by examining the eye color intensity of a mini-white reporter on the X chromosome and the autosomes. One copy of the X linked reporter had the weakest eye color in metafemales; one copy in normal females showed more color and one copy males exhibited the strongest intensity. This continuum illustrates that each gene copy on the X has the lowest expression in metafemales, increases in normal females and is greatest in males conforming to an inverse relationship with the dosage of the X chromosome. The autosomal reporter showed the lowest expression in metafemales, was greater in females and, as is commonly known, the greatest expression was found in males. These results are consistent with an inverse dosage component to dosage compensation.

375C

Targeting the MSL complex counteracts the effect of increased histone acetylation and does not induce dosage compensation. Lin Sun[1], Harvey Fernandez[1], Jilong Li[2], Jianlin Cheng[2], James Birchler[1]. 1) Biological Sci Div; 2) Department of Computer Sci, Univ Missouri,Columbia, MO.

In order to study the effect of histone modification produced by the histone acetyltransferase, MOF, a GAL4 DNA binding domain fusion was produced. Reporters were constructed that contained the GAL4 target sequences (UAS) preceding the mini-white reporter. In females there is a strong up-regulation of targeted mini-white insertions. Immunocytochemistry and ChIP demonstrated that the reporter had increased H4Lys16Ac thus showing a correlation between histone modification and gene expression. However, in males, the targeting of GAL4 MOF showed a reduced expression with all X and autosomal reporters. Interestingly, the autosomal reporter has the components of the MSL complex brought to the targeted reporter. For comparison, a GAL4-MSL2 fusion construct was made. When targeted to UAS-mini-white reporters, immunocytochemistry and ChIP showed that the components of the MSL complex were brought to the reporters and were effective in modifying H4Lys16. Using both molecular and phenotypic assays, there was no evidence that gene expression of the reporters was detectably changed suggesting that the MSL complex does not mediate dosage compensation. This hypothesis was further examined by conducting a global gene expression analysis of ectopically expressed MSL2 in adult females compared to normal females. Triplicate biological replicates were subjected to RNAseq and the average sequencing reads per gene were determined. The distribution of expression ratios of X chromosomal genes showed a major peak surrounding a value of 1.0 rather than 2.0 expected if compensation were induced. Phenotypic and northern validation using mini-white reporters on the X and the autosomes showed no increase in expression of the X reporter in females and a slight reduction of the autosomal reporter. The collective results are consistent with the hypothesis that the MSL complex overrides the effect of histone

acetylation and is not the primary determinant of dosage compensation.

376A

Sex-specific heterochromatin: How does chromatin become male? Manasi Apte, Victoria Meller. Dept. of Biological Sciences, Wayne State University, Detroit, MI.

Almost ~30% of the *Drosophila* genome is heterochromatic. Although relatively gene-poor, heterochromatic regions of *Drosophila* contain over 500 predicted genes. While heterochromatin is generally not considered to display sexual dimorphism, we have observed male-specific heterochromatic gene regulation. Interestingly, *roX* RNAs, critical components of the Male Specific Lethal (MSL) complex involved in dosage compensation, are required for full expression of heterochromatic genes at the autosomes. The MSL complex modifies X-chromatin to equalize the ratio of gene expression between the sex chromosomes and autosomes. We observed that the sex-specific role of *roX* RNAs in dosage compensation is genetically distinguishable from their role in regulating heterochromatic genes. Loss of *roX*RNAs results in down-regulation of the autosomal heterochromatic genes in males but not in females. Heterochromatic insertions that display position effect variegation (PEV) show de-repression in *roX*mutant males but not in females. We hypothesize that heterochromatin is different in male and female flies. These differences are expected to be under genetic control. To test genes in the conventional somatic sex-determination pathway for a role in establishing the heterochromatic sex, we are performing a targeted screen. The screen exploits a PEV reporter that is de-repressed in males, but not in females, upon loss of *roX* genes. PEV is examined in XX pseudo-males created by mutation of Sex-Lethal (*Sxl*), transformer2 (*tra2*) and other members of the canonical sex determination pathway. If PEV is sensitive to the loss of *roX* RNA, we conclude that heterochromatin has been masculinized. Our preliminary findings suggest that neither *tra2* nor *Sxl* regulate sex of the heterochromatin. Further studies will focus on possible role of numerator elements and chromosome pairing status as possible signals that establish heterochromatic sex.

377B

Localization of Mini-chromosome Maintenance Protein 10. Nicholas W. Faulkner, Tim W. Christensen. East Carolina University, Greenville, NC.

In order for organisms to maintain homeostasis, it is vital for DNA to replicate with high fidelity. Failure to do so will leave uncorrected mistakes, which have the capacity to cause lethal mutations, cancerous growth, or disease. Mini-chromosome maintenance protein 10 (Mcm10), a key component of replication initiation, was first identified in *Saccharomyces cerevisiae,* is highly conserved across species, and is postulated to form a homohexameric ring. Structurally, Mcm10 contains an N-terminal self-binding site, DNA and Polα binding sites on the internal domain, and DNA and Polα binding sites on the C-terminal domain. Moreover, Mcm10 has been shown to associate with pre-initiation and elongation complexes. Recent studies however, have demonstrated that Mcm10 may have alternative functions including chromatin remodeling, as hypomophic mutants suppress position effect variegation. The role for Mcm10 in heterochromatin formation may be through its interaction with heterochromatin protein 1. Despite two decades of research, the exact role of Mcm10 remains elusive, likely due to Mcm10's multiple cellular roles. To further understand these possible roles, it is of interest to investigate the localization of Mcm10, both spatially and temporally. To achieve this, a combination of approaches will be taken including; immunofluorescence, live-cell imaging, and western blots. Localization studies of Mcm10 will be carried out in both wild-type and multiple Mcm10 mutant backgrounds, of which chromatin defects, lethality, or sterility are displayed. Furthermore, localization will be studied in endoreplicating tissues, meiotic tissues, and cell lines in an effort to clarify Mcm10's possible roles. These localization studies will likely help in understanding the wide variety of Mcm10 mutant phenotypes and, in combination with future co-localization experiments, will allow the assignment of these phenotypes to interactions with specific proteins. Taken together, knowledge of the roles and mechanisms of Mcm10 make it an attractive therapeutic target for cancer and replication associated disease.

378C

Developmental time-course of gene inactivation caused by position effect. Aleksei Shatskikh, Sergey Lavrov, Vladimir Gvozdev. Department of Molecular Genetics of Cell, Institute of Molecular Genetics of Russian Academy of Sciences, Moscow, Russian Federation.

In(2)A4 is a chromosomal rearrangement with breakpoints in 39A region and pericentromeric heterochromatin of chromosome 2L. This inversion causes position effect variegation in euchromatic region that is adjacent to the breakpoint. In In(2)A4, expression of genes is perturbed at distances of approx. 40-50 kb starting from eu-heterochromatin boundary. In adults no continuous spreading of inactivation is observed and many genes escape inactivation. We suggested that earlier in ontogenesis some genes within the affected region are prone to inactivation. Expression of affected genes was measured in larvae and pupae. We detected paradoxical effect of PEV-induced activation of some genes in larvae. Interestingly, at different stages of development heterochromatin may disturb the expression of the same gene in the opposite direction. The results of time-course mRNA level measurements suggest that heterochromatin mostly affects expression of a target gene at the stage of its transcriptional activation in ontogenesis. These data may give a clue to mechanisms of interaction between heterochromatinization and transcriptional machinery.

379A

Enhancer-associated H3K4 mono-methylation by Trithorax-related. Hans-Martin Herz[1], Man Mohan[1], Alexander S.

Garruss[1], Kaiwei Liang[1], Yoh-hei Takahashi[1], Kristen Mickey[1], Olaf Voets[2], C. Peter Verrijzer[2], Ali Shilatifard[1]. 1) Shilatifard lab, Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Biochemistry and Center for Biomedical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands.

Mono-methylation of histone H3 on lysine 4 (H3K4me1) and acetylation of histone H3 on lysine 27 (H3K27ac) are histone modifications that are highly enriched over the body of actively transcribed genes and on enhancers. Although in yeast all H3K4 methylation patterns including H3K4me1 are implemented by Set1/COMPASS (complex of proteins associated with Set1), there are three classes of COMPASS-like complexes in *Drosophila* that could carry out H3K4me1 on enhancers: dSet1, Trithorax, and Trithorax-related (Trr). Here, we report that Trr, the *Drosophila* homolog of the mammalian Mll3/Mll4 COMPASS-like complexes, can function as a major H3K4 mono-methyltransferase on enhancers in vivo. Loss of Trr results in a global decrease of H3K4me1 and H3K27ac levels in various tissues. Assays with the *cut* wing margin enhancer imply a functional role for Trr in enhancer-mediated processes. A genome-wide analysis demonstrates that Trr is required to maintain the H3K4me1 and H3K27ac chromatin signature that resembles the histone modification patterns described for enhancers. Since the Trr complex is distinguished by bearing a unique subunit, the H3K27 demethylase, dUTX, we propose a model in which the H3K4 mono-methyltransferase, Trr, and the H3K27 demethylase, dUTX, cooperate to regulate the transition from inactive/poised to active enhancers.

380B

Role of nucleosome modification, composition, and position in specification of replication origins. Neha P. Paranjape, Jun Liu, Brian R. Calvi. Department of Biology, Indiana University, Bloomington, IN.

Metazoans initiate DNA replication from many sites in the genome called origins. Origins are binding sites for a pre-Replicative Complex (pre-RC) of proteins that is then activated in S phase to initiate replication. It is currently unclear, however, how genomic loci are selected to be pre-RC binding sites and active origins. Metazoan replication origins lack a DNA consensus sequence and show remarkable developmental plasticity. We use developmental gene amplification- a specialized replication program in the *Drosophila* ovary - as a model system to study how origins are specified in development. Amplification involves re-replication from origins at six specific loci in follicle cells late in *Drosophila* oogenesis, which results in an increase in the DNA copy number of genes required for rapid eggshell synthesis. We have found that hyperacetylation of nucleosomes on multiple lysines contributes to activation of amplicon origins. Moreover, the level of acetylation at the six amplicon loci correlates with their different levels of amplification. Genomic location analysis from a number of labs has revealed a correlation between pre-RC binding sites, nucleosome depleted regions (NDRs) and enrichment for histone variants H3.3 and H2Av. Using MNase-seq and ChIP-qPCR, we have found that amplicon origins are also NDRs and enriched for the nucleosome variants H3.3 and H2Av. At the well-defined amplicon DAFC-66D, NDRs and H3.3/H2Av correspond to regions that are essential for origin function. Analysis of H3.3 mutant strains indicated, however, that H3.3 is not required for origin activity. Moreover, our data suggest that, although essential origin elements are NDRs, this nucleosome depletion is not sufficient to specify origin location or timing. We will also describe an unbiased genetic screen to identify new attributes of the epigenome that influence origin function. Thus, the amplicon model system provides a unique opportunity to discover how different chromatin features contribute to differential origin activity in development.

381C

Investigations of Drosophila Suppressor of Hairy-wing zinc-finger mutants identify distinct subclasses of genomic binding sites. Ryan M. Baxley[1], Michael W. Klein[2], Ashley G. Fell[2], Joel A. Morales-Rosado[2], James D. Bullard[2], Pamela K. Geyer[1,2]. 1) Molecular & Cellular Biology Program, University of Iowa; 2) Biochemistry Department, University of Iowa, Iowa City, IA.

Suppressor of Hairy-wing [Su(Hw)] is a twelve zinc finger (ZnF) DNA binding protein that localizes to ~3,000 genomic regions. While its role in *gypsy* insulator function is well characterized, its essential function in oogenesis is poorly understood. Our recent investigations demonstrate that loss of Su(Hw) alters transcription of many target genes in the ovary. These findings imply that Su(Hw) is a multi-functional transcription factor, capable of conferring insulator, repressor and activator effects when bound at distinct target sites. The features that contribute to the diverse regulatory functions of Su(Hw) binding sites (SBSs) are unknown. To understand these processes, we performed an EMS mutagenic screen and identified two new *su(Hw)* mutations that genetically separate Su(Hw) functions. One mutant retains *gypsy* insulator activity and not female fertility, while a second mutant retains female fertility and not *gypsy* insulator function. Interestingly, each of these alleles encodes a protein that disrupts a single ZnF, suggesting that the ZnF domain contributes to Su(Hw) regulation. To test this prediction, analyses of genome-wide occupancy of the Su(Hw) ZnF mutants were completed. These studies revealed that the functionally separate ZnF mutants occupy different sequence subclasses of SBSs that show enrichment for different co-factors. These observations suggest that DNA sequence may define the regulatory output of an SBS. These predictions are being tested through functional analyses. Together, our studies provide insights into how multiple regulatory roles are executed by a single DNA binding transcription factor.

382A

Diversity in function: How a polydactyl zinc finger protein confers multiple functional outputs. James D Bullard[1], Ryan M Baxley[2], Jake M Traxler[1], Bianca N Mason[1], Pamela K Geyer[1,2]. 1) Biochemistry Department, University Of Iowa; 2) Molecular & Cellular Biology Program, University of Iowa, Iowa City, IA.

Zinc fingers (ZnFs) represent the most common DNA binding domain in metazoan transcription factors, with these proteins often carrying arrays of five or more ZnFs. It is unclear how individual ZnFs contribute to the function of such polydactyl transcription factors. While classically considered DNA binding motifs, ZnFs also direct protein-protein and RNA interactions, raising the possibility that ZnFs make regulatory contributions in addition to DNA association. To understand the role of individual ZnFs in a polydactyl DNA binding domain, we are studying Suppressor of Hairy-wing [Su(Hw)], a twelve ZnF DNA binding protein. This multifunctional transcription factor is required for *gypsy* insulator function and gene regulation in the ovary. Sequence comparisons demonstrate that the Su(Hw) ZnF domain is highly conserved, with each ZnF displaying 55% to 96% identity over 40 million years of evolution. We defined the in vitro DNA binding properties of bacterially produced full-length Su(Hw) and mutants that carry a disruption of a single ZnF. We found that eight of the twelve ZnFs contribute to binding, with four being essential. While the essential ZnFs are among the most conserved, the degree of conservation does not always correlate with a requirement for DNA binding. Interestingly, these studies uncovered distinct binding modes for Su(Hw), suggesting that ZnFs usage at genomic binding sites may impact the conformation of Su(Hw) and contribute to differential effects on transcriptional regulation. This postulate is being tested through in vivo studies of the ZnF mutants. To date, we have confirmed that the ZnFs essential for in vitro DNA binding are required for in vivo Su(Hw) function, as these mutants fail to rescue *su(Hw)^{null}* phenotypes. Investigations of the other ZnF mutants are underway. Together, these data will provide insights into how polydactyl transcription factors utilize different combinations of ZnFs to carry out multiple functions.

383B

Structure-function analysis of Argonaute2 in chromatin insulator activity. Madoka Chinen, Elissa Lei. Laboratory of Cellular and Developmental Biology, NIDDK, Bethesda, MD.

Chromatin insulators are DNA-protein complexes distributed throughout the genome that can act as barriers to prevent spreading of repressive chromatin and interfere with enhancer-promoter interaction by promoting alternative chromatin loop formation. We described a role for Argonaute2 (AGO2), a canonical member of the siRNA pathway, in CTCF/CP190-dependent *Fab-8* insulator activity, which prevents inappropriate enhancer interactions with the Hox gene *Abd-B* promoter. AGO2 is important for promoting or stabilizing chromatin loop formation at the *Abd-B* locus. Genome-wide localization analysis demonstrated that AGO2 localizes extensively throughout euchromatin including the *Fab-8* element as well as many promoters. Interestingly, an AGO2 RNAi-catalytic mutant does not show defects in insulator activity, indicating that insulator-related activity of AGO2 is independent of catalytic activity. Since the AGO2 RNAi-catalytic mutant can bind to RNA, it is unclear whether RNA is involved in AGO2-related insulator function. *Drosophila* AGO2 contains 4 domains including the GRR, DUF, PAZ, and PIWI domains, the latter 3 of which are conserved to humans. The PAZ domain binds nucleic acid, and the PIWI domain has RNase-H like nuclease activity. The function of the GRR and DUF domains are not well understood; the GRR is specific to *Drosophila* and is not required for RNAi activity. Here, we seek to define which domains are important for AGO2 nuclear localization and insulator function. As a first step, we generated transgenic AGO2 point mutants, RNA-binding mutant and truncation mutants, which are expressed under control of the genomic *AGO2* promoter. We are currently examining whether these mutants are functional for enhancer blocking activity at *Fab-8* in the AGO2 null mutant background. The minimal AGO2 domain required for chromatin binding will be determined by chromatin immunoprecipitation and immunostaining salivary gland polytene chromosomes expressing AGO2 truncation mutants. Current progress of our AGO2 mutant analysis will be reported.

384C

The *even skipped* insulator Homie blocks Polycomb response element-mediated repression of the adjacent gene *TER94*. Miki Fujioka, James B Jaynes. Dept Biochem. & Mol. Biol, Thomas Jefferson Univ, Philadelphia, PA.

Previously, we identified a boundary region between *even skipped (eve)* and *TER94* that has enhancer blocking activity, and promotes both long range enhancer-promoter communication and P-element homing. We call it Homie, for hom ing insulator of eve. Here, we show that one function of Homie in a native context is to prevent repression of *TER94* by a Polycomb-response element (PRE) that is near the 3' end of the *eve* locus. When Homie is deleted, the normal ubiquitous expression of *TER94* is repressed in both ovaries and embryos by the *eve* PRE. That is, *TER94* is repressed when Homie is deleted, and expression returns when the PRE is also deleted. In embryos, the *eve* locus was shown by others to be a sharply delineated Polycomb (Pc) domain: both Pc binding and the associated histone modification H3K27me3 are seen throughout the *eve* locus, but not nearby in *TER94*. Consistent with Homie blocking the spread of PRE-dependent chromatin, we see spreading of the *eve* Pc domain into the *TER94* region when Homie is deleted (or replaced by λ DNA), as evidenced by an increase in H3K27me3. Other known PREs substitute for the *eve* PRE to repress *TER94* in the absence of Homie. Furthermore, most known insulators are capable of blocking PRE-dependent repression in this context. These studies reveal a novel function of both PREs and insulators during oogenesis and embryogenesis. When Homie is deleted, ubiquitous *TER94* expression in embryos is "replaced" by expression in an *eve* pattern, suggesting that Homie also blocks the positive action of *eve* enhancers on the *TER94*promoter. Intriguingly, when Homie is deleted, the *eve* enhancers that are located 3' of the *eve* transcription unit (between the eve promoter and Homie) show reduced activity, suggesting that Homie also facilitates the action of the 3' *eve* enhancers on its own promoter, while blocking their action on *TER94*.

385A

Characterization of an RNA Binding Protein Involved in Chromatin Insulation. Matthew R. King, Ryan K. Dale, Elissa P Lei. National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

Chromatin insulators are DNA-protein complexes defined by the ability to prevent enhancer-promoter interaction or the spread of silent chromatin, functions termed enhancer-blocking and barrier activity, respectively. Previous work suggests that the gypsy insulator complex interacts with RNA, though none of its three core proteins contain an RNA-binding motif. To identify gypsy insulator associated transcripts, we carried out sequential RNA immunoprecipitation followed by high throughput sequencing (RIP-seq) of core gypsy proteins Centrosomal Protein 190 (CP190) and Suppressor of Hairy wing (Su(Hw)). In order to test the functional significance of their interaction, null or putative loss-of-function alleles of the genes encoding six of the most highly enriched transcripts were tested for enhancer-blocking activity. We also tested a null allele of CIP3, which encodes an RNA-binding protein known to bind one of the transcripts. Of these candidates, only CIP3 null mutants showed a negative effect on enhancer-blocking. We are currently testing the capacity of CIP3 to modulate gypsy barrier activity using a quantitative luciferase-based barrier assay. We found that CIP3 interacts with gypsy insulator proteins and at least a subset of gypsy associated transcripts. The three core gypsy proteins co-immunoprecipitate with CIP3 from embryonic nuclear extracts. Additionally, anti-CIP3 RIP of nuclear extracts followed by qRT-PCR revealed that many of the transcripts enriched in Su(Hw)/CP190 RIP-seq are also present in CIP3 RIP. Immunostaining of salivary gland polytene chromosomes of CIP3 null mutants showed that loss of CIP3 does not affect localization of gypsy insulator proteins to chromatin. Furthermore, double staining of wildtype polytenes for CIP3 and Su(Hw) shows only limited overlap of both proteins. However, CIP3 extensively colocalizes with Shep and Rm62, two RNA-binding proteins known to negatively affect gypsy insulator function. These data demonstrate a novel role for an RNA-binding protein in the regulation of gypsy insulator activity.

386B

Genome-wide localization of exosome components to active promoters and chromatin insulators. Su Jun Lim, Patrick Boyle, Madoka Chinen, Ryan Dale, Elissa Lei. Laboratory of Cellular and Developmental Biology, NIDDK, NIH, Bethesda, MD.

Chromatin insulators are functionally conserved DNA-protein complexes situated throughout the genome that organize independent transcriptional domains. Previous work implicated RNA as an important cofactor in chromatin insulator activity, although the precise mechanisms are not yet understood. Here we identify the exosome, the highly conserved major cellular 3' to 5' RNA degradation machinery, as a physical interactor of CP190-dependent chromatin insulator complexes. Genome-wide profiling of exosome by ChIP-seq in two different embryonic cell lines reveals extensive and specific overlap with the CP190, BEAF-32, and CTCF insulator proteins. Colocalization occurs mainly at promoters but also boundary elements such as scs, scs', Mcp, and Fab-8. Surprisingly, exosome associates primarily with promoters but not gene bodies of active genes, arguing against simple cotranscriptional recruitment to RNA substrates. Similar to insulator proteins, exosome is also significantly enriched at divergently transcribed promoters. Directed ChIP of exosome in cell lines depleted of insulator proteins shows that CTCF is required specifically for exosome association at Mcp and Fab-8 but not other sites, suggesting that alternate mechanisms must also contribute to exosome chromatin recruitment. Taken together, our results reveal a novel positive relationship between exosome and chromatin insulators throughout the genome.

387C

Tissue-specific regulation of chromatin insulator function mediated by an RNA-binding protein. Leah H. Matzat, Ryan K. Dale, Nellie Moshkovich, Elissa P. Lei. Laboratory of Cellular and Developmental Biology, NIDDK, Bethesda, MD.

Chromatin insulators organize the genome into distinct transcriptional domains and contribute to cell type-specific chromatin organization. However, factors regulating tissue-specific insulator function have not yet been discovered. In this study, we identify the RNA recognition motif-containing protein, Shep, as the first known tissue-specific regulator of insulator function in any organism. Shep is a direct interactor of two individual components of the *gypsy* insulator complex, Su(Hw) and Mod(mdg4)2.2. Mutation of *shep* improves *gypsy*-dependent enhancer blocking in a $mod(mdg4)^{u1}$ null genetic background, indicating a role as a negative regulator of insulator activity. Furthermore, both strong loss of function or overexpression of *shep* results in synthetic lethality with $mod(mdg4)^{u1}$; however, synthetic lethality is not observed by overexpression of an RNA-binding point mutant. These data suggest that both *shep* dosage as well as RNA-binding are important for insulator activity.

Unlike ubiquitously expressed core *gypsy* insulator proteins, Shep is highly expressed in the central nervous system (CNS) with lower expression in other tissues. We developed a novel, quantitative tissue-specific barrier assay to demonstrate that Shep functions as a negative regulator of insulator activity in the CNS but not in muscle tissue. Additionally, mutation of *shep* alters insulator complex nuclear localization in the CNS but has no effect in other tissues. Consistent with negative regulatory activity, ChIP-seq analysis of Shep in a CNS-derived cell line indicates substantial genome-wide colocalization with a single *gypsy* insulator component but limited overlap with intact insulator complexes. Taken together, these data reveal a novel, tissue-specific mode of regulation of a chromatin insulator.

388A

Mechanisms of transcriptional regulation by a Drosophila insulator protein. Alexey A. Soshnev, Pamela K. Geyer. Molec & Cellular Biol, Univ Iowa, Iowa City, IA.

Insulators are DNA elements that bind protein complexes and constrain the action of enhancers and silencers. The Drosophila genome encodes several insulator proteins, including the model insulator protein, Suppressor of Hairy-wing

[Su(Hw)]. This zinc finger (ZnF) DNA binding protein binds the *gypsy* retrotransposon, as well as over 3000 constitutively occupied endogenous non-*gypsy* sites. At the*gypsy* insulator, Su(Hw) recruits Centrosomal Protein of 190 kD (CP190) and Modifier of *mdg4* 67.2 kD isoform (Mod67.2), two partner proteins required for gypsy insulator function. Surprisingly, CP190 and Mod67.2 localize to fewer than a third of endogenous Su(Hw) binding sites (SBSs), and their loss of function phenotypes are distinct from loss of Su(Hw). These observations imply that the regulatory roles of Su(Hw) extend beyond formation of endogenous insulators. To gain a better understanding of the Su(Hw) function, we defined transcriptional requirements for this protein in the ovary, as this is the only tissue where Su(Hw) function is essential. Our studies revealed that Su(Hw) is a direct transcriptional regulator, with the majority of Su(Hw) target genes upregulated upon Su(Hw) loss. Most of these upregulated genes display enriched expression in the central nervous system, suggesting that Su(Hw) is a repressor of neuronal genes in non-neuronal tissues. Several findings are consistent with this prediction. First, Su(Hw) does not accumulate in post-mitotic neurons. Second, *su(Hw)* mutants are temperature sensitive, a phenotype consistent with upregulation of neuronal Su(Hw) target genes. Third, ectopic expression of Su(Hw) is associated with developmental defects and cell death. Based on these data, we propose that Su(Hw) may represent a functional homologue of the vertebrate RE1-Silencing Transcription Factor (REST), a ZnF transcription factor that acts as a repressor of neuronal genes in non-neuronal tissues. Investigations into the repressor function of Su(Hw) will provide insights into how this protein achieves multiple transcriptional regulatory roles.

389B

Environmentally induced rDNA instability as a driver of epigenetic variation. John Aldrich, Keith Maggert. Department of Biology, Texas A&M University, College Station, TX.

An organism's patterns of gene expression are responsive to environmental input. Often, this influence is not limited to short-term regulatory changes, but can persist through multiple cell divisions and can, in some cases, be transmitted to offspring. It is typically assumed that such "epigenetic" changes are mediated by chromatin modifications in the form of histone or DNA modifications or expression of regulatory RNAs. However, the mechanisms through which epigenetic changes are established at specific promoters, are maintained through mitoses and meioses, and affect an organism's phenotypes remain unclear. In this work, we show that alterations to diet affect the expression of the ribosomal RNA genes which in turn results in DNA damage and loss of rDNA. These induced genomic changes have all the hallmarks of epigenetics since they are inducible, heritable, and consequential. We show that mutations in silencing factors result in nucleolar fragmentation and rDNA copy number reduction. Furthermore, we find that drugs that alter rDNA expression suppress these effects. This induced variation is stable throughout development, and correlates with altered heterochromatic silencing and gene expression. Dietary perturbation in adults alters the rDNA of offspring, providing a clear mechanism for transgenerational inheritance of dietary effects in flies.

390C

Analysis of Sex combs reduced HOX gene cis-regulatory elements. Monica T. Cooper, James A. Kennison. Program on Genomics of Differentiation, NIH, Bethesda, MD.

The Drosophila Hox gene, Sex combs reduced (Scr), is required for patterning the first thoracic segment. The Scr transcription unit spans 35 kb, with at least 35 kb of upstream cis-regulatory sequences. We are testing Scr genomic fragments to identify Polycomb Group response elements (PREs). We are also making targeted knock-out deletions of the PREs in the endogenous gene.

391A

Epigenetic regulation in *Drosophila melanogaster* via DNA methylation- a systems biology approach. Deepti D. Deobagkar, Chitra Pannikar. Department of Zoology, University of Pune, Pune, India,411007.

Drosophila melanogaster is a very useful model system to investigate the genotype-phenotype correlations using systems biology approach. Although DNA cytosine methylation is known to be present in Drosophila, the molecular genetic mechanisms regulating methylation machinery and it's components have not yet been unraveled. The physiological role of this important epigenetic modulation remains poorly understood in Drosophila. By utilising high throughput approaches we have investigated the nonCpG methylation in the fruit fly Drosophila. We demonstrate the modulation of methylation levels by employing methylation modulators and several environmental, developmental and epigenetic regulatory factors.In order to study the genome wide methylome, we have developed and utilised a novel method of methylation detection which utilizes a cDNA microarray based approach using anti 5methyl cytosine antibody. This has resulted in elucidation of genome wide methylation map. Gene ontology, involvement of miRNA, epigenetic regulatory proteins in these epigenetic interactions were analysed. This information of methylome and its modulation is utilised to define interactomes and pathways involved in regulatory networks. This method and data generated help us to establish a network of genome wide methylome in any given condition in a biological system. Pathway analysis of these genes revealed statistically significant enrichment of known functions such as DNA binding proteins, signal transduction cascades,etc . The involvement of miRNA in chromatin remodeling and establishing methylation patterns have also been studied in detail. We also demonstrate the utility of methylation modulators in evaluating methylation machinary. The link between processes involved in regulation of alterations in gene expression profiles, protein - protein interaction networks and chromatin structure have been established. This analysis provides important insight into the molecular genetic pathways which govern the process of establishing epigenetic imprints

which have not yet been unraveled.

392B

Intercalary heterochromatin regions in salivary gland polytene chromosomes of *Drosophila melanogaster* tend to have conserved gene order across the genus *Drosophila*. Tatiana D. Kolesnikova, Natalya G. Andreyenkova, Elena S. Belyaeva, Fedor P. Goncharov, Tatyana Yu. Zykova, Lidiya V. Boldyreva, Galina V. Pokholkova, Igor F. Zhimulev. Institute of Molecular and Cellular Biology , Russian Academy of Sciences, Novosibirsk, Russian Federation.

About 240 specific regions are identified on *D. melanogaster* polytene chromosomes which are replicated at the very end of the S-phase. They have a repressive chromatine state, low gene density, long intergenic distances and are enriched in tissue specific genes. In polytene chromosomes, about a quarter of these regions have no enough time to complete replication, as a result, underreplication zones, represented by less DNA copy number, appear. We studied 60 chromosome regions that demonstrate more pronounced underreplication. Having compared location of these regions on a molecular map and syntenic blocks found earlier for *Drosophila* species by von Grotthuss et al., 2010, we have shown that across the genus *Drosophila* these regions tend to have conserved gene order. It makes us to propose existence of evolutionary mechanisms directed to maintain the integrity of these regions.

393C

Gene Environment Interactions - Implications for Epigenesis. Yoav Soen. Biological chemistry, Weizmann Institute of Science, Rehovot, Israel.

Studies of gene regulation often focus on defined genetic programs, ignoring the ability of the environment to promote multiple genotype-to-phenotype transformations and the potential of epigenetics to influence multiple generations of non-exposed offspring. We investigate epigenetic implications of gene-environment interactions using a synthetic drug/anti-drug system which allows us to confront the development of the fly, D. melanogaster, with artificial distributions of toxic stress that are not expected to occur during fly development. Survival of the flies in this system depends of their ability to modify their development. We found that under a wide range of toxic scenarios, the challenge modifies the otherwise robust patterns of development, resulting in changes in gene expression as well as in the rate of larval development and adult morphology (in some of the cases). We show that part of this response is enabled by suppression of Polycomb group genes (PcG), which leads to de-repression of developmental regulators and their expression in new domains, hence the change in developmental patterns. Remarkably, some of the developmental alterations were non-genetically inherited by subsequent generations of unchallenged offspring suggesting that the challenge also modifies the germline of the flies. This was indeed confirmed by analysis of maternal RNA in eggs of challenged versus unchallenged flies. These results reveal a process of epigenesis by which stressful, non-familiar environment suppresses the Polycomb system and induces developmental modifications that persist across generations through non-Mendelian mechanisms.

394A

Wash interacts with Lamin and affects nuclear organization. Jeffrey M. Verboon, Hector Rincon, Tim Werwie, Tobias Ragoczy, Dave Scalzo, Steven Erikson, Jeff Delrow, Mark Groudine, Susan Parkhurst. Fred Hutchinson Cancer Research Center 1100 Fairview Ave. N., Seattle, WA 98109.

The Wiskott-Aldrich Syndrome (WAS) family proteins have been shown to promote the formation of branched actin in the cytoplasm by activating the Arp2/3 complex. However, there is a growing body of work suggesting that actin and actin nucleation factors may also have a role in the nucleus. We find that Wash, a WAS family member, is present in the nucleus and associates with specific chromosome regions. Futhermore, wash mutants have an altered nuclear morphology, where the normally smooth, spherical nuclear envelope is puckered and amorphous. Interestingly, we find that Wash directly binds to B-type Lamin, a nuclear intermediate filament that lines the inside of the nuclear envelope. Recently, Lamin has been shown to play a role in gene repression as specific chromosomal regions associate with Lamin at the nuclear envelope and these Lamin Associated Domains (LADs) correspond with transcriptionally inactive genome regions. We performed chromatin profiling for Wash and Lamin in Kc cells and find that ~85% of the chromosome regions that Wash associates with overlap with LADs. Lamin chromatin profiling in wash knockdown cells results in a significant loss of LADs indicating that Wash is necessary for the proper formation of LADs. We also find that general nuclear architecture is impaired in wash knockdown cells as we see a loss of the repressive marker HP1, nucleolar staining by fibrillarin, and Cajal bodies by coilin, as well as disruption of chromosome territories by FISH. Our results suggest that the proper tethering of genomic regions to the nuclear envelope by Wash and Lamin may not only be important for maintaining repressive LAD domains but may also function to help organize the nucleus. Currently, we are purifying Wash nuclear complexes to gain a better understanding of how Wash may be performing its nuclear functions.

395B

The telomeric retrotransposons in Drosophila are activated and replicated at the G1/S boundary. Liang Zhang, Yikang Rong. National Cancer Institute, Bethesda, MD.

In place of telomerase, Drosophila species have tamed a group of retrotransposons that transpose exclusively and repeatedly to chromosome ends to buffer the loss of chromosome end sequences during DNA replication. To better understand how the retrotransposons co-operate with the host cellular machinery to accomplish the end-elongation function, we characterized

Het-A, the most abundant telomeric element in Drosophila melanogaster. The single open reading frame of HeT-A encodes a 110kD protein (ORF1p). We showed that in a narrow window during the cell cycle, Het-A sense transcript and ORF1p forms RNP complexes that are targeted to telomeres. Furthermore, Verrochio, a protein homologous to conserved Stn1 protein with a potential role in the maintenance of telomeric single stranded overhangs, is a key regulator of this end-targeting process. Using cytological markers for different phases of the cell cycle in Orf1p co-immunolocalization experiments, we revealed that Het-A RNPs are only present during late G1 to early S phase. By analyzing the behavior of a single telomere that is marked with a lacO array, we discovered that HeT-A RNP is often associated with a telomere undergoing DNA replication. Our results have served as the first evidence that Drosophila telomeres are likely among the first genomic regions replicated during the S phase, and these findings have implications for the underlying mechanism that leads to the exclusive targeting of these retro-elements to the chromosome ends.

396C

De novo establishment of Polycomb-mediated repression. Jumana S AlHaj Abed, Judith Benes, Richard Jones. Dept. Of Biology, Southern Methodist University, Dallas, TX.

Polycomb group proteins (PcG) are conserved epigenetic regulators that control target genes by taking over repression from gene-specific transcription factors. Once PcG-mediated repression is established, it is maintained through many cell divisions. Most studies have focused on the activities of PcG proteins during the maintenance phase. Much less is known about the mechanisms and molecular events by which PcG proteins initially recognize the repressed state of a gene and lead to the establishment of PcG-mediated silencing. The challenge to understanding PcG silencing mechanisms in vivo is the difficulty of acquiring a homogeneous population of cells in which all cells are exhibiting PcG-mediated repression of a particular gene. This study focuses on understanding the molecular events that lead to the initiation of PcG-mediated repression of *giant* (*gt*). Maternal-effect mutations are used to generate embryos in which *gt* is uniformly repressed. Chromatin immunoprecipitiation (ChIP) assays are being used to examine the distribution of PcG proteins and other transcription factors at *gt*. By perfroming ChIP assays on a time course of embryos from nuclear cleavage stages, and throught cellular blastoderm, it is possible to track the events at *gt* as PcG proteins take control of repression from maternal Hunchback (Hb). In addition, transgenic reporter lines have been generated in order to map the location of Polycomb Response Elements (PREs) within the upstream regulatory region of *gt*.

397A

Function of the bxd ncRNA. Ana Borges, Welcome Bender. bcmp, harvard medical school, boston, MA.

In Drosophila, the production of a ncRNA transcribed through the bxd regulatory region in the BX-C has been suggested as a mechanism for the regulation of Ubx expression. Using recombineering we were able to reconstruct a 45kb segment of the BX-C region bearing the bxd promoter/enhancer in a inverted fashion. This segment was patched back into a fly deleted for the same region though RMCE integration. Flies carrying the modified segment were analyzed for "in situ" RNA assays: no detection of bxd ncRNA was seen and a loss of the Ubx repression was noticeable in early development. However the flies did not show any homeotic phenotype. Our result suggests that the lack of transcription from the bxd promoter might have an affect on setting the state of the bxd PRE for the activation of Ubx.

398B

Analysis of two closely-linked *engrailed* Polycomb Response Elements: similarities and differences. J Lesley Brown, Judith Kassis. NICHD, NIH, Bethesda, MD.

In Drosophila, Polycomb group response elements (PREs) play an essential role in gene regulation by the Polycomb group (PcG) repressor proteins. They are required for the recruitment of and for the maintenance of repression by the PcG proteins. Here we compare and contrast different characteristics of two closely linked yet separable PREs of the Drosophila engrailed (en) gene, PRE1 and PRE2. We define a binding site for an as yet unidentified protein that binds to PRE2. We find that PRE1 and PRE2 have different requirements for the number of binding sites for the DNA binding PcG protein pleiohomeotic (pho). PRE1 requires two Pho binding sites whereas PRE2 requires only one. In addition, for full function, PRE1 requires an AT rich region not seen in PRE2. These two PREs behave differently in an embryonic maintenance assay when inserted at an identical location in the genome. Such differences in PRE activity may be important for regulation of engrailed.

399C

Drosophila taranis is an important mediator of Polycomb mediated transcriptional silencing. Pranabananda Dutta, Willis Li. Medicine, University of California, San Diego, La Jolla, CA.

The Polycomb group (PcGs) proteins are implicated in epigenetic transcriptional repression in development, stem cell maintenance and tumorigenesis. The molecular mechanism by which PcGs silence target loci is not fully understood. Here we show that Drosophila taranis (tara) is required for positioning Pc to its target genes. Embryos lacking tara exhibit partial homeotic transformation in the cuticular segments, a phenotype associated with Pc mutants. Consistent with the homeotic transformation, tara loss of function results in misexpression of homeotic gene Ultrabithorax (Ubx) and in reduced Pc recruitment on polytene chromosomes. Hence, we show that taranis modulates the spatialexpression pattern of Polycomb target genes during Drosophila development.

400A

Polycomb group gene *E(z)* prevents germline-to-soma conversion in Drosophila adult testes. Suk Ho Eun, Xin Chen. Dept Biol, Johns Hopkins Univ, Baltimore, MD.

In many metazoans, germ cells are separated from somatic lineages early in development. However, little is known about the mechanisms that maintain germline versus somatic cell fate throughout life. Here we show that a key Polycomb group (PcG) component, Enhancer of Zeste [E(z)] H3K27me3-specific methyltransferase, is required to maintain germ cell identity in *Drosophila* adult testes. We find excessive early-stage somatic gonadal cells in *E(z)* mutant testes, which originate from both over-proliferative cyst stem cells and a potential germline-to-soma cell fate conversion. Lineage-specific markers reveal cells with both somatic and germline identities in *E(z)* mutant testes, suggesting an intermediate state between germline and somatic lineages. Using complementary lineage-tracing experiments in *E(z)* mutant testes, we demonstrate that some excessive early-stage somatic gonadal cells are derived from early-stage germ cells, including germline stem cells. Furthermore, we find that knocking down *E(z)* specifically in somatic cells causes this germline-to-soma conversion. Thus, our results demonstrate that one role of the somatic gonad is to maintain germline identity. Because mammalian male germ cells have a unique reprogramming potential, our discoveries will bring new insight to the application of germ cells in stem-cell-based regenerative medicine.

401B

Binding profile comparison of Tritrorax-like across Drosophila species. Lijia Ma, Nicolas Negre, Matt Slattery, Rebecca Spokony, Sasha Ostapenko, Ryan Ptashkin, Jennifer Zieba, Kevin White. Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL.

PcG proteins and the counterpart TrxG proteins are key regulators to govern gene repress and active after the first wave of early embryonic repressors begin to disappear. Histone marks, like H3K27me3 and H3K4me3 have been proved to involve in PcG/TrxG regulation. However, it is still unclear how PcG/TrxG proteins were recruited to polycomb response elements (PREs) and work with histone marks then further regulate target genes activity. Here we identified binding profiles of Trithorax-like (Trl) in early embryo and White Prepapue in four Drosophila species (Drosophila melanogaster, Drosophila simulans, Drosophila yakuba and Drosophila pseudoobscura). The wide distribution of Trl peaks is consistent with its recruiting function, in which TrxG proteins function as antagonist to PcG proteins as well as general transcription activators. We observed the binding events of Trl are generally conserved across species, and the pair-wise conservations of non-melanogaster peaks relative to Dmel decrease along their evolutionary distance increasing. The conservation rates also drop with the distance between peaks and transcription starting sites indicating TSS-proxy binding events are more functionally essential. There is no significant difference in nucleotide divergence and motif quality between conserved peaks and species-unique peaks, but chromatin structures and co-factor availability might contribute to binding events gain or loss.

402C

Identification and characterization of DNA binding proteins necessary for epigenetic silencing by Polycomb group proteins. Payal Ray, Judith A Kassis. Eunice Kennedy Shriver National Institutes of Child Health and Human Development, NIH, Bethesda, MD.

Polycomb group proteins (PcG) are a class of transcriptional regulators thought to mediate epigenetic inheritance of a repressed transcriptional state. PcG proteins play an important role in Drosophila and have been shown to have similar functions in vertebrates. PcG proteins act through specific DNA sequences known as PcG response elements (PREs]. PREs range from several hundred to a few thousand base pairs and often can be subdivided into smaller fragments with similar activities. PREs are made up of binding sites for multiple proteins. Our goal is to determine the identity of all the DNA binding proteins that bind to the *engrailed* PREs. The *engrailed* gene contains a PRE that has been studied extensively by our group. Previous studies have shown that a minimal 139 bp region contains binding sites for Pho (Pleiohomeotic), Spps (Sp1-like factor for Pairing Sensitive-silencing) GAF (GAGA Factor) and two unknown factors. We aim to identify these unknown proteins that bind to this region and characterize them. To this end, we performed a pull-down using a biotin-tagged oligonucleotide containing the binding site in parallel with an oligonucleotide containing a mutated site. The pull-down samples were analyzed by mass spectrometry and we identified a few candidates that potentially bind to the 139bp fragment. Currently, we are validating these candidate genes by biochemical and genetic approaches and will be presenting the results.

403A

The EGFR/MAPK pathway is a target of developmental ethanol exposure in *Drosophila*. Rachael L. French, Peter Luu, David Do, Nicole Delgado. Biological Sciences, San Jose State University, San Jose, CA.

Alcohol exposure during development causes a variety of abnormalities in a broad range of taxa, from mammals to insects. In humans, prenatal alcohol exposure leads to an array of complications, from growth deficiency and birth defects to mental retardation and behavioral abnormalities, collectively described as fetal alcohol spectrum disorder (FASD) or fetal alcohol syndrome (FAS).

Using our previously established fly model of FASD, we have found that at least some of ethanol's deleterious effects can be modulated by mutation of genes in the Epidermal Growth Factor Receptor (EGFR) signal transduction pathway. In addition, we have found that ethanol exposure during development can reverse the lethality associated with ubiquitous overexpression of the EGFR pathway, demonstrating that ethanol exposure reduces signaling through this pathway during development.

Finally, we have preliminary data indicating that flies reared in ethanol do not develop normal preference for ethanol-containing food as adults, and that this response is further blunted by mutation of *dsor*, the *Drosophila* homolog of MAP Kinase Kinase (MAPKK). These data indicate that the EGFR pathway is a target of ethanol exposure during development.

We will present the above data, including analysis of the expression and activity of the EGFR pathway and MAP Kinase (MAPK) in ethanol-exposed larvae and microarray results indicating that genes in at least two MAPK pathways, the EGFR pathway and the Jun Kinase (JNK) pathway, are downregulated as a result of developmental ethanol exposure. Future research will focus on understanding how ethanol-induced changes in EGFR signaling lead to changes in growth and behavior and identification of neuronal targets of ethanol during neurobehavioral development.

404B

TSPO/PBR, a component of mPTP, modulates ethanol-related behaviors in Drosophila. Ran Lin, Douglas Wallace. Children's Hospital of Philadelphia Research Institute, Philadelphia, PA.

The translocator protein 18kDa (TSPO), formerly named peripheral benzodiazepine receptor (PBR), is a putative component of mitochondrial permeability transition pore (mPTP). As the binding site of benzodiazepine, a psychoactive drug that induces tolerance and addiction, TSPO is hypothesized as an essential factor involved in addiction of benzodiazepine and other abusive substances. By pharmacological and genetic inactivation, we analyzed the function of dTSPO in Drosophila, concentrating on mPTP and behavioral responses to the most commonly used abusive substance, ethanol. Inactivation of dTSPO by ligands (PK11195 and Ro5-4864), P-element insertion in the genomic region, and transgenic expression of dsRNA all inhibited mPTP opening, based on recording for swelling in isolated mitochondria from adult flies. In living cells of larval brain, mPTP opening was also shown to be attenuated by PK11195, or in dTSPO-/- flies, based on Cobalt/Calcein quenching assay. Thus dTSPO is required for mPTP opening in flies. To monitor the sensitivity to ethanol, we measured the time to sedation in flies exposed to ethanol vapor. The dTSPO-/- flies were more sensitive, while neuronal-specific dTSPO knock-down flies were more resistant, than control flies. After 6 hours of first exposure to ethanol, flies exhibited resistance if exposed to ethanol again, indicating the formation of tolerance. However, dTSPO-/- flies were not able to form tolerance in this condition, while neuronal-specific dTSPO knock-down flies were more tolerant than control. Moreover, neither dTSPO-/- nor neuronal-specific dTSPO knock-down Drosophila performed strong preference to ethanol-containing food over regular food in two-choice feeding assay, as control flies did. Taken together, dTSPO is an important component of mPTP in Drosophila, and modulates multiple ethanol-related behaviors in tissue-specific manner.

405C

The Role of Oxidative Stress in a *Drosophila* Model of Fetal Alcohol Syndrome. Theresa A. Logan-Garbisch[1], Kiara Y. Amaro-Rivera[1,2], Audrey A. Ford[1], David Do[1,3], Hilal J. Jarar[1], Melissa K. Ruiz[1], Omar Fateen[1], Rachael French[1]. 1) Biological Sciences, San José State University, San José, CA; 2) Industrial Biotechnology Department, University of Puerto Rico-Mayagüez, Yaguez, Mayagüez, Puerto Rico; 3) Computer Science, San José State University, San José, CA.

Fetal alcohol syndrome (FAS) is a spectrum disorder affecting individuals exposed to ethanol during gestation and often results in developmental delays and decreased survival rates; it is also the leading cause of non-genetic mental retardation. Previous studies have shown *Drosophila melanogaster* larvae exposed to ethanol-treated food model these phenotypes. We hypothesize that ethanol and oxidative stress are acting in the same pathway and therefore predict that 1) oxidative stress will phenocopy FAS symptoms; 2) increased oxidative stress will increase sensitivity to ethanol exposure; 3) alleviated oxidative stress will ameliorate the phenotypes, and 4) threshold doses of ethanol and peroxide will act synergistically. Both pharmacological and genetic manipulations were utilized to induce or alleviate oxidative stress. To date, developmental exposure to hydrogen peroxide has been found to phenocopy the delay and decreased survival phenotypes. Transgenic constructs which pan-neuronally upregulate the antioxidant enzyme superoxide dismutase (sod) resulted in increased resistance to ethanol-induced lethality. Correspondingly, pan-neuronal downregulation of sod resulted in increased lethality. Moreover, flies exposed to combined threshold doses of peroxide and ethanol show increased developmental delays and decreased survival when compared to conditional controls. In addition, microarray data indicate that some markers of oxidative stress are significantly altered in larvae reared in ethanol relative to unexposed larvae. Collectively, these data implicate oxidative stress in ethanol-induced phenotypes, specifically in relationship to the decreased survival and developmental delay. Future work includes looking for biochemical markers of oxidative stress in ethanol-reared larvae via western blot as well as immunohistochemistry assays.

406A

Psi regulates dmyc transcription via modulation of RNA Polymerase II. Nicola J. Cranna[1], Amanda Lee[1], Naomi Mitchell[1], Ross Hannan[2], Leonie Quinn[1]. 1) Anatomy and Neuroscience, University of Melbourne, Melbourne, VIC, Australia; 2) Peter MacCallum Cancer Centre, Melbourne, VIC, Australia.

Two single stranded DNA binding proteins have been implicated in gene specific control of RNA Polymerase II (Pol II) pausing at the c-MYC oncogene transcriptional start site through *in vitro* mammalian studies; FBP1 and FIR. These studies suggest that FBP1 may be required for the activation of *c-myc* transcription and show that FIR acts antagonistically as a repressor. The *Drosophila* FIR homolog, Hfp binds to the *Drosophila myc* (*dmyc*) promoter and is required for repression of transcription. In mammals there are 3 FBP family members which bind overlapping targets, leading to difficulty in dissecting the role of FBP1. Psi is the sole *Drosophila* ortholog of the mammalian FBP family. In support of these *in vitro* studies we have

evidence that Psi is required for regulation of *dmyc* transcription, through the control of RNA Polymerase II. qPCR on Psi RNAi knockdown animals demonstrates a significant reduction in dmyc mRNA levels suggesting Psi is required for the activation of transcription. In line with a transcriptional role, ChIP experiments indicate Psi is directly bound to the *dmyc* promoter, suggesting an important role in the control of *dmyc* transcription. To determine how Psi may regulate *dmyc*transcription, ChIP revealed enrichment of initiated RNA pol II (Ser 5) around the *dmyc* transcription start site and the Psi RNAi results in a significant reduction in RNA pol II Ser 5 accumulation. Combined data suggests Psi is normally required to stimulate the activation of RNA pol II and for regulation dmyc transcription *in vivo*.These data demonstrate that Psi, the ortholog of FBP1, is required for controlling myc transcription. Psi is required for the activation of RNA pol II and is necessary for controlling the level of enrichment of the *dmyc* repressor Hfp, suggesting Psi might modulate Pol II activity to control*dmyc* transcription via Hfp. Our future studies are aimed towards elucidating the mechanism by which Psi controls Hfp enrichment and/or Pol II activity.

407B

Genomic and epigenetic changes occurring during carcinogenesis: A fly perspective. Delphine Fagegaltier[1], Mary-Lee Dequeant[2], Gregory Hannon[1], Norbert Perrimon[2], Amanda Simcox[3], STARR Consortium. 1) CSHL - HHMI, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) HHMI - Department of Genetics Harvard Medical School Boston, MA; 3) Department of Molecular Genetics Ohio State University Biological Sciences Columbus, OH.

Despite tremendous efforts in various organisms, the questions of how cancer cells initially become transformed, which pathways are involved in reaching a transformed state, and whether each cell takes the same route to reach such a state remain poorly understood. To answer these questions we are using the Drosophila model to study the basic mechanisms by which genomes coordinate their genetic and epigenetic responses towards a transformed state activated by specific oncogenes or tumor-suppressors. This STARR consortium project has three major aims : **i)** generating Drosophila cell lines with cancer-relevant genotypes; **ii)** profiling the transcriptional and epigenetic changes that occur during the establishment of these cell lines; **iii)** addressing whether cells remain addicted to the presence of the initiating oncogene or loss of tumor suppressor and establish which factors are required for the cells to proliferate and maintain a transformed state. We have established various cell lines expressing an oncogene or depleted for a tumor-suppressor. A pilot array experiment on primary cell lines derived from ras oncogene expressing embryos suggests that cells undergo major epigenetic changes via the Polycomb Group of proteins before reaching a transformed state. To further confirm the role of these proteins during transformation, we have compared the transcriptomes of a larger set of transformed cell lines using RNA-Seq. By dissecting the progressive transcriptional changes generated during transformation, these studies shed light to general mechanisms and pathways leading to tumorigenesis and reveal changes specific of each oncogenic molecule studied.

408C

Regulation of E-cadherin expression by Poly(ADP-ribosyl)ation during Development and Tumorigenesis. Yingbiao Ji, Alexei Tulin. Cancer Biology Program, Fox Chase Cancer Ctr, Philadelphia, PA.

Metastatic prostate cancer is a leading cause of cancer death due to resistance to the androgen deprivation therapy in the male population in USA. E-cadherin expression induces an epithelial-mesenchymal transition within tumor cells to promote prostate cancer metastasis. We have found that Drosophila HnRNP A1(Hrp38) binds to the 5'UTR of E-cadherin mRNA to control its translation likely by an IRES (Internal Ribosome Entry Site)-mediated process. Hrp38 loss-of-function causes oocyte mislocalization and loss of GSC self-renewal ability due to decreased E-cahderin expression. In contrast, the accumulation of poly(ADP-ribose) in the progenitor cells disrupts the interaction of Hrp38 with the 5'UTR of E-cadherin mRNA, decreasing E-cadherin expression. Therefore, hnRNP poly(ADP-ribosyl)ation regulates E-cadherin translation during Drosophila oogenesis. We are exploring if poly(ADP-ribose) also controls E-cadherin expression levels through the same mechanism during tumor metastasis. Our preliminary data demonstrates that the prostate cancer cell lines overexpress PARP1, a pattern that is associated with significantly reduced Parg and E-cadherin expression compared to the wild-type prostate cell lines. This result suggests that regulation of E-cadherin expression by poly(ADP-ribosyl)ation may be conserved between Drosophila and mammals.

409A

Cell type-specific, BMP-dependent regulation of growth and migration by the ecdysone receptor in secondary cells of the male accessory gland. Aaron Leiblich[1,2], Michael Williams[1], Luke Marsden[2], Carina Gandy[1], Laura Corrigan[1], Shih-Jung Fan[1], Freddie Hamdy[2], Clive Wilson[1]. 1) Deparment of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom; 2) Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom.

The steroid hormone ecdysone plays several critical roles during development but its functions in adults are less well characterised. We previously showed that secondary cells, a subclass of secretory cells in the male accessory gland, grow selectively as males age. A subset of these cells delaminates apically in multiply-mated males and can be transferred to females upon mating. These processes are normally promoted by BMP signalling. Recent work in our lab has shown that secondary cells also secrete exosomes that can fuse to sperm in females, indicating a surprising parallel with the mammalian prostate, an organ whose growth and secretion is critically regulated by steroid hormone signalling through the androgen receptor (AR) in normal and tumorigenic cells. We now demonstrate that the ecdysone receptor isoform, EcR-B1, which shares structural similarities with the AR, is specifically expressed in secondary cells, where it promotes cell growth and suppresses BMP-

dependent delamination. Remarkably, EcR activity is controlled in a novel cell-type-specific manner by BMP signalling via an interaction involving the N-terminal AF1 domain of the EcR protein. BMP signalling regulates EcR protein levels and the nucleocytoplasmic distribution of the receptor. Our data reveal that, as in mammals, steroid receptor and BMP signalling plays a sex- and cell-type-specific role in controlling the growth and secretory activity of cells in Drosophila, providing a new in vivo model to investigate the importance of the interplay between these two pathways in the male reproductive system.

410B

Novel functions of the Drosophila Mps1 homologue, altered disjunction (ald), regulating epithelial integrity. Beatriz Perez San Juan, Antonio Baonza Cuenca. Developmental Biology, CBMSO, Madrid, Madrid, Spain.

Mps1 (Mono-polar spindle 1) is an evolutionary conserved serine treonine kinase that regulates normal mitotic progression and the spindle checkpoint in response to stress. Changes in Mps1 protein levels have been related with the development of cancer in human. This tumor-promoting function it's been attributed to the chromosomal instability observed when the activity of this kinase is modified. However, other functions of this kinase involved in tumor progression still remain unknown. We have analyzed the function of the Drosophila melanogaster Mps1 homologue, altered disjunction (ald). Our data indicate that the alteration of the activity of ald causes the lost of the adherents junctions components and the disruption of the apico-basal polarity domains, compromising the epithelial integrity. These cells undergo a pseudo epithelial to mesenchimal transition (EMT) and acquire cell motility and invasiveness. Ald mediates part of these effects regulating the activity of Rho1 and the Myosin II light chain (sqh). These results uncovered novel functions for this kinase that can help to understand its contribution to the development of tumors.

411C

Alcohol and cancer: dietary alcohol enhances tissue overgrowth upon loss of Hippo Pathway signaling. Cathie M. Pfleger[1], Anoj Ilanges[1,2], Maryam Jahanshahi[1]. 1) Dept Oncological Sci, Mount Sinai Sch Med, New York, NY; 2) Yale University, New Haven, CT.

Alcohol consumption is a significant risk factor in cancers of organs that contact alcohol and in the liver where alcohol is metabolized. Interestingly, a link is also reported in breast cancer. Despite strong epidemiological links, the role of alcohol in cancer is not understood. *Drosophila* models have been established to explore the role of alcohol in other disease contexts including models of fetal alcohol syndrome and alcohol addiction. *Drosophila* can also model cancer-relevant phenotypes such as tissue overgrowth, making it an ideal system to elucidate the relationship between alcohol and cancer. We report here that screening *Drosophila* overgrowth models for response to dietary ethanol identified interactions with the Hippo tumor suppressor. The Hippo Pathway is a bona-fide tumor suppressor pathway highly conserved from flies to mammals that acts as a master regulatory pathway to restrict growth and proliferation and to promote apoptosis. Of note, loss of Hippo signaling is implicated in a range of cancers that overlaps strikingly with the spectrum of alcohol-mediated cancers including digestive tract, liver, and breast cancers. A host of upstream factors activate the core cassette of Hippo signaling via Hippo (Hpo). Activated Hpo kinase phosphorylates and activates downstream kinase Warts (Wts). Wts phosphorylates and inhibits transcriptional co-activator Yorkie (Yki), a potent oncogene. We report that in multiple tissues, including the eye and wing, alcohol enhanced tissue overgrowth upon loss of multiple Hippo Pathway tumor suppressor components. Surprisingly, alcohol did not enhance overgrowth due to over-expressing Yki. Consistent with this, mammalian cells exposed to alcohol showed phosphorylation of Wts homolog Lats1 but not of the Yki homolog YAP. Our studies reveal a novel, highly conserved interaction between alcohol and the Hippo Pathway and may implicate a YAP-independent role for Hippo Pathway tumor suppression in alcohol-mediated cancers.

412A

A troponin-t mutation initiates cardiomyopathy due to impaired contractile inhibition in *Drosophila melanogaster*. Anthony Cammarato[1], Meera Cozhimuttam Viswanathan[1], Gaurav Kaushik[2], Adam J. Engler[2], William Lehman[3]. 1) Johns Hopkins University, Baltimore, MD; 2) University of California, San Diego, San Diego, CA; 3) Boston University School of Medicine, Boston, MA.

Muscle contraction results from a series of orchestrated molecular events that involve transient interactions between myosin-containing thick and actin-containing thin filaments. Regulation of striated muscle contraction is primarily achieved by Ca^{2+}-dependent modulation of myosin crossbridge cycling on actin by the thin filament (TF) troponin-tropomyosin complex. Alterations in various subunits of the complex trigger contractile dysregulation and myopathy. For example, point mutations located over a span of ten amino acids (130-39) of human cardiac troponin T (cTnT) are associated with distinct cardiomyopathic responses. The *Drosophila* up^{101} (E88K) mutation localizes to the end of this well-conserved region of TnT. Here, using multiple image-based approaches we define the consequences of the lesion on the fly cardiac tube. Direct immersion DIC optics, high-speed video imaging and motion analysis resolved a phenotype reminiscent of human restrictive cardiomyopathy in up^{101} hearts. Relative to controls, end-diastolic and end-systolic dimensions and percent fractional shortening were significantly reduced. Furthermore systolic intervals were significantly prolonged. This suggests TF dysregulation initiates excessive periods of force production and diastolic dysfunction. Electron microscopy and three-dimensional reconstruction of TFs revealed the vast majority of Ca^{+2}-free mutant TFs exhibited tropomyosin in a position distal to known myosin binding sites where it is unlikely to prevent crossbridge formation. Finally, atomic force microscopy and nanoindentation identified elevated up^{101} cardiomyoctye stiffness in the absence of Ca^{2+} that was attenuated via

incubation with a myosin-specific inhibitor. This is consistent with unregulated active forces contributing to incomplete relaxation. Thus, as found in humans with distinct cTnT mutations, *up*^{101} TnT likely promotes TF dysinhibition and consequently restrictive cardiac remodeling.

413B

Pygopus Is Required for Age-dependent Maintenance of Heart Function Independent of Canonical Wnt Signaling. Karen Ocorr[1], Min Tang[2], Wuzhou Yuan[2], Xiushan Wu[2], Rolf Bodmer[1]. 1) Dept Neuroscience & Aging, Sanford-Burnham Medical Research Institute, La Jolla, CA; 2) The Center for Heart Development, College of Life Science, Hunan Normal University, Changsha Hunan Province, P.R. 410081.

Age-dependent decline in cardiac function has been demonstrated for both flies and humans. Although important for cardiac development and differentiation, the role of Wnt signaling components in the adult myocardium or with age is unclear. Of these components, *pygopus* (*pygo*) was originally identified as a nuclear adapter, along with β-catenin, that promotes TCF-dependent Wnt target gene transcription, but its role in maintaining adult cardiac performance is unknown. In this study, we show that Pygo is prominently expressed in the adult myocardial cells, and that *pygo* function is strongly required for cardiac performance and myocardial integrity, unlike other canonical Wnt pathway components tested. Cardiac-specific knockdown of *pygo* in the adult heart results in increased arrhythmias, reduced contractility (systolic dysfunction) and myofibrillar disorganization. In contrast, cardiac-specific disruption of Wnt signaling components β-catenin/*armadillo* and TCF/*pangolin* results in relatively weak heart defects compared to *pygo* loss-of-function. *pygo* also failed to exhibit a significant genetic interaction with these canonical Wnt components, as well as with TCF target Ubx and with mediator complex genes associated with canonical Wnt signaling, suggesting that *pygo* function in the adult heart does not require canonical Wnt signaling. Taken together, our studies suggest a novel role for *pygo* that is critical for adult heart function and structural integrity, but unexpectedly this role is likely independent of canonical Wnt signaling.

414C

New cellular functions for the Lowe Syndrome phosphoinositide phosphatase dOCRL in diverse *Drosophila* tissues. Sarah A Biber, Abdulmuhsen Ali, Avital Rodal. Biology Department, Brandeis University, Waltham, MA.

Lowe syndrome is an X-linked disorder caused by mutations in OCRL (Oculocerebrorenal Syndrome of Lowe), a phosphatidylinositol phosphatase with previously identified roles in endocytic trafficking, phosphoinositide metabolism, cytokinesis, and cilium formation and function. However it is not understood how defects in the OCRL enzyme result in the debilitating neurological, kidney and eye symptoms that are prevalent in Lowe syndrome. We have taken advantage of the high conservation of OCRL between humans and insects to model Lowe syndrome in *Drosophila melanogaster*. Here we have generated a *Drosophila* OCRL (dOCRL) null mutant with numerous deficiencies at both the cellular and tissue levels. Loss of dOCRL causes lethality in larval stages. Third instar larvae lacking dOCRL present with large melanotic masses and neuromuscular junction defects. Consistent with mammalian studies, our data indicates that dOCRL localizes to early endosomes and partially co-localizes with the PH domain-containing protein dSes/CG12393. In addition, we have uncovered a potential new role for dOCRL in nuclei. dOCRL localizes to S2 cell nuclei as well as to larval brain, salivary gland and garland cell nuclei. PI(4,5)P2, a preferred substrate of dOCRL, is known to localize to nuclei and to participate in chromatin remodeling and regulation of specific transcripts. dOCRL shuttles between the cytoplasm and the nucleus, and its nuclear localization appears to be negatively regulated by dSes and positively regulated by a non-canonical NLS sequence. Our findings suggest that dOCRL may perform multiple cellular functions in both the cytoplasm and nucleus. *Drosophila* is proving to be a useful model for gaining new insights into the complex mechanisms underlying the pathology of Lowe syndrome in diverse tissues.

415A

The role of Cad99C, the Drosophila Usher Syndrome Protocadherin, in light-induced eye degeneration and apical membrane dynamics. Se-Yeon Chung, Deborah Andrew. Dept Cell Biol, Johns Hopkins Univ, Baltimore, MD.

Usher Syndrome (USH) is the most frequent cause of hereditary deaf-blindness in humans. The gene products of ten USH disease genes have been identified so far, most of which are highly conserved from flies to humans. Cad99C, the Drosophila orthologue of human Usher cadherin PCDH15, is strongly expressed in embryonic tubular organs, including the salivary gland and trachea, where the apical membranes undergo dynamic changes during tube morphogenesis. Cad99C localizes to the apical domains suggesting a role in apical membrane dynamics. Our studies on Cad99C in the embryonic salivary gland revealed that Cad99C functions to regulate lumenal dimensions. Confocal and TEM analysis revealed that overexpression of Cad99C causes a dramatic increase in apical membrane at the expense of other cellular membrane domains. We also show that the intracellular domain of Cad99C is necessary for its apical targeting and that Cad99C mislocalization to the basolateral membrane results in a change in epithelial cell morphology from columnar to spherical, suggesting that Cad99C may promote cell-matrix interactions over cell-cell interactions. By learning how the USH genes function at the cellular and molecular level during the formation of relatively simple Drosophila tissues, we expect to gain additional key insight into how the USH genes function in human development and disease.

416B

Quantitative Gene Expression Analysis of Drosophila melanogaster in a Fetal Alcohol Spectrum Disorder Model. David

Do, Theresa Logan, Peter Luu, Omar Fateen, Brianna Hagen, Janet Lafler, Luke Lajoie, Melissa Ruiz, Clare Wadsworth, Audrey Ford, Schehrbano Khan, Hilal Jarrar, Elizabeth Benn-Hirsch, Rachael French. Biological Sciences, San Jose State University, San Jose, CA.

The purpose of our research is to elaborate upon an existing model for Fetal Alcohol Spectrum Disorder (FASD) using Drosophila melanogaster as a genetic model organism. Preliminary research has shown that Drosophila raised on ethanol-treated food exhibit physical and behavioral defects commonly associated with FASD in humans. This has led to a plethora of divergent research that is attempting to implicate the various biochemical pathways responsible for regulating these phenotypes. In order to determine the target genes for further investigation, our lab used Affymetrix GeneChip microarrays in order to conduct pangenomic expression analysis on extracted RNA samples. Thorough analysis of the microarray data shows strong evidence for altered expression levels for the genes that regulate oxidative stress; cell growth, proliferation, and differentiation; insulin signaling; lipid metabolism; olfaction; and responses to environmental toxins. We hypothesize that the aforementioned pathways are involved in mediating the physical and behavioral phenotypes of ethanol-reared flies. Along with microarray data, we intend to conduct a series of qPCR experiments on our various RNA samples as a means to concretely affirm the altered expression levels of key gene constructs as a result of ethanol exposure during development. We are currently waiting for the results of said experiment and the data accrued will serve to expand the established model of FASD.

417C

A Step Closer to Understanding Social Behavior: Social Interactions and Dopamine in *Drosophila melanogaster*. Robert W. Fernandez[1], Adesanya A Akinleye[1], Marat Nurilov[1], Zulekha Rouzyi[1], Anne F Simon[1,2]. 1) School of Arts And Sciences, Department of Biology, The City Univ New York, York College, Jamaica, NY; 2) York College and The Graduate Center, The City University of New York.

Background: Autistic individuals typically have difficulty with social interactions, including being socially avoidant, indifferent, and awkward, but the underlying causes are not well understood. In addition, there are known variation in the level of dopamine (DA) and serotonin in autistic individuals. We hypothesize that modulating the levels of DA in *Drosophila melanogaster* will modify its social behavior.

Methods: We manipulated the expression of the vesicular monoamine transporter (VMAT), through its overexpression (UAS-cDNA) or loss of function (UAS-RNAi) in DArgic cells (Gal4-Th). We also feed drugs known to increase (L-DOPA) or decrease (3-IT) DA content in the adult. We contrasted two different social behavior assays to test the flies' response to others: measure of closest neighbor in the Resource Independent Local Enhancement assay (RILE), and innate avoidance of stressed individuals.

Results: Our data indicates that there is a negative correlation between copies of VMAT in DArgic cells and social space. These results were mimicked in the pharmacology experiments. However, no effect of manipulating VMAT was found in response to stressed flies.

Conclusion: Flies with increased DA lost a sense of personal boundary and came closer to each other. Flies with decreased DA signaling were socially avoidant since they were responding to stressful social signals, but avoided their neighbor in a stable group. This data indicates that we can use behavioral paradigms in the fruit fly as assays to examine the underlying mechanism of asocial behavior such as what is seen in autism.

418A

Drug Rescue of Repetitive Grooming Behaviors in Drosophila Fragile X Mental Retardation Mutants. Catalina Florez[1], Matthew Whitmill[1], Melissa Kepke[1], Linda Restifo[2], William Conner[1]. 1) Department of Biology, Wake Forest University, Winston-Salem, NC 27106; 2) Department of Neuroscience and Neurology, and Center for Insect Science, University of Arizona, Tucson, AZ 86721.

Fragile X Syndrome (FXS) is a condition that strongly increases the prevalence of autism. The cause for FXS is a single gene mutation in the fragile-x-mental retardation 1 gene that leads to the loss of functional fragile-x-mental retardation protein (FMRP), which is an important regulator of postsynaptic protein synthesis. Drosophila melanogaster have proven to be very significant in FXS research, as the dFMR1 gene is the fruit fly ortholog of the human FMR1 gene. Previous studies have shown that compounds such as 6 methyl-2-(phenylethynyl) pyridine hydrochloride (MPEP) inhibit glutamate receptor activation, reducing the overexpression of postsynatpic proteins thought to be responsible for many FXS symptoms. In this study, genetically altered mutants (dFMR1) and wild type (EX16) Drosophila were administered 10 µg of the drug MPEP in their larval diet. The effects of the drug on the grooming behavior of the Drosophila were examined through video recording and scoring the individual behavior of individual flies. It has been observed that Drosophila have a specific repertoire of grooming behaviors, and that the display of these behaviors is different in dFMR1 mutants than wildtype animals. This study investigates these differences in an effort to quantify the effects of MPEP on FXS symptom expression.

419B

Fragile X Mental Retardation Protein Regulates Trans-Synaptic Signaling. Samuel H. Friedman, Neil Dani, Kendal Broadie. Department of Biological Sciences, Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN 37212 USA.

Fragile X Syndrome (FXS), the most common inherited determinant of intellectual disability and autism spectrum disorders,

is caused by the loss of the fragile x mental retardation 1 (fmr1) gene product (FMRP), an RNA-binding translational repressor. Screens for upregulated neuronal proteins in Drosophila fmr1 (dfmr1) null mutants reveal strong elevation of two synaptic heparan sulfate proteoglycans (HSPG); GPI-anchored glypican Dally-like (Dlp) and transmembrane Syndecan (Sdc). Our recent work has shown that Dlp and Sdc act as co-receptors that regulate both extracellular ligand abundance and intracellular signal transduction in trans-synaptic pathways driving synaptogenesis. Consistently, dfmr1 null synapses exhibit altered WNT signaling, with changes in both Wingless (Wg) ligand abundance and downstream Frizzled-2 (Fz2) receptor C-terminal nuclear import. Similarly, a parallel anterograde signaling ligand, Jelly Belly (Jeb), together with downstream ERK phosphorylation (dpERK), is altered at dfmr1 null synapses. In contrast, the retrograde BMP ligand Glass Bottom Boat (Gbb) and downstream signaling via transcription factor MAD phosphorylation (pMAD) is not affected, revealing the mechanism to be selective for anterograde pathways. These dysregulations in trans-synaptic signaling pose exciting new insights into the synaptogenesis and functional phenotypes correlated with the loss of FMRP in FXS.

420C

Kismet-dependent regulation of glutamate receptors at the Drosophila Neuromuscular Junction. Rupa Ghosh[1], Srikar Vegesna[1], Hong Boa[3], Bing Zhang[3], Faith Liebl[2], Daniel Marenda[1]. 1) Drexel University, Philadelphia, PA; 2) Univ of Southern Illinois at Edwardsville, IL; 3) Univ of Oklahoma, OK.

CHARGE syndrome (CS) is a developmental disorder with a birth incidence of 1:8000-12,000 worldwide. CS affects multiple organ systems such as the eye, ear, heart, facial nerve, nose, CNS and the reproductive system. Additionally, 90% of CS patients exhibit hypotonia & motor co-ordination defects. Two-thirds of the disorder are caused due to haploinsufficiency of the Chromodomain DNA Helicase Binding Protein (CHD7). CHD7 is an epigenetic transcription factor and its Drosophila homolog is Kismet. We reduced kis function in a tissue-specific manner by RNAi using the GAL4-UAS system. We analyzed behavior by a ubiquitous and motorneuron - specific reduction of Kismet protein. This led to a “Held-out-wing” phenotype and reduced larval and adult locomotion and motor co-ordination. From microarray data, reduced Kismet protein showed a significant upregulation of glutamate biosynthesis genes and downregulation of glutamate receptor subunits. Drosophila neuromuscular junction (NMJ) synapses are glutamatergic in nature. Glutamatergic synapses in Drosophila conduct fast synaptic transmission, which form the basis of locomotion. The analysis of NMJ morphology in 3rd instar larvae with ubiquitous reduction of Kismet protein led to pre-synaptic changes that are most likely compensatory in nature, such as increased branching of the NMJ synapse. We also found a significant reduction of GluRIIC mRNA and relative fluorescent intensity. Further, our electrophysiology results suggested decreased synaptic transmission of the NMJ synapse of muscle 6/7. Taken together, our data is the first to identify GluRs as a downstream target of kismet function. It also indicates that Kismet affects the pre-synaptic component of the NMJ and effects on the post-synaptic side are more likely secondary to the pre-synaptic defects. This study will help better understand the role of Kismet during development and may be extended to the function of CHD7 in humans and CS.

421A

Low Doses of Iron-Oxide Nanoparticles have a Detrimental Effect on Reproduction and Development. Benjamin W. Henderson[1], Rami R. Ajjuri[1], Sarah Boyd[1], Gavin Daigle[1], Yuping Bao[2], Janis M. O'Donnell[1]. 1) Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35401; 2) Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL.

Nanoparticle applications are becoming increasingly used in the biomedical fields, with applications ranging from target drug delivery systems to medical imaging technologies. Toxicity analysis in cell culture has been the principle means of determining their safety. With rapidly increased use, there is need for in-depth toxicological analysis of nanoparticles in whole organisms to determine whether their use has deleterious side effects. We have developed toxicological assays to assess the biological consequences of nanoparticle exposure in Drosophila melanogaster. The current study focuses on the effects of transient exposure to polyacrylic acid-coated iron oxide nanoparticles. Larvae were fed on yeast paste containing varying concentrations of nanoparticles for 24hrs. Concentrations of 10-100 µg/ml had no discernable effect on larval survival or development to the adult stage. However, we noted slight elevations in larval lethality at concentrations below 10 µg/ml. Subsequently, we exposed larvae to nanoparticle concentrations below 10 ug/mL and then assayed the effects on development to pupation, eclosion rates, fertility of males and females that had been dosed as larvae, and the fertility rates of their progeny. We detected a narrow concentration window for elevated larval toxicity. Pupation rates of survivors were nominal. However, both male and female survivors that had ingested nanoparticles within the toxicity window had a significant long-term effects resulting in diminished fertility. Moreover, the surviving progeny of treated females had elevated sterility. Higher nanoparticle concentrations appear to induce a protective innate immune response. We hypothesize that transient exposure to concentrations within the toxicity window are insufficient to induce this response, but is sufficient to cause cellular damage.

422B

Genes *Cam* and *nAchRα-30D* suppress mutant dystrophin phenotype in *Drosophila melanogaster*. Natalia Holub, Ruslana Mykula, Yaroslava Chernyk. Departament of Genetics and Biotechnology, Ivan Franko National University, lviv, Ukraine.

Muscular dystrophies - a group of genetic diseases that are classified as incurable and are accompanied by a gradual

degradation of skeletal and cardiac muscles. At the basis of their development are disturbances in the structure and functioning of the dystrophin-glycoprotein complex (DGC), which connects actin cytoskeleton to extracellular matrix and stabilizes sarcolemma during contraction of muscles.Drosophila melanogaster is a good model for studying a new approach to treatment muscular dystrophy with using genes-modifiers. It has got homologues of all components of the DGC. The aim of work study was to examine the influence of genes *nAchR-30D* and *Cam* (involved in the functioning of muscle and cytoskeleton) as a possible genes-modifiers of mutant dystrophin phenotype in strain *Dys Df//TM6,Tb*. Genome of this strain contains deletion (170 kb) of dystrophin gene and adjacent to dystrophyn genes. Mutants are characterized by defective thorax muscular structure, decreased indexes of physical activity (IPA) and life span. Offsprings F_1 containing an supplementary copy of gene-modifier and mutant dystrophin gene were analysed after these variables. In all crossings we observed restore of thorax muscle structure at 56%-69% comparing to 0,11% in dystrophy mutants. In climbing-test was shown increasing of IPA in hybrids *nAchR-30D//DysDf* in 2-4 times and in hybrids *Dys Df//Cam* in 3-5 times compared to *Dys Df//TM6Tb*. Also, genes *nAchR-30D* and *Cam* caused increasing of average life span indexes on 90-143% and maximum life span on 62%. We can conclude that supplementary copies of genes *Cam* and *nAchR-30D* have a suppressive effect on expression of dystrophin mutant phenotype.

423C

ACSL4 inhibits synapse growth by attenuating BMP signaling via endocytic recycling of its receptors. Yan Huang, Zhihua Liu, Qifu Wang, Yong Q. Zhang. Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China.

Mutations of acyl-CoA synthetase long-chain family member 4 (ACSL4), an enzyme that converts long chain fatty acids to acyl-CoAs, result in non-syndromic X-linked mental retardation (MRX). Using the Drosophila neuromuscular junction (NMJ) as a model, we found that the Drosophila homolog dAcsl inhibits synaptic growth by attenuating bone morphogenetic protein (BMP) signaling, a major growth-promoting pathway at NMJ synapses. Specifically, dAcsl mutants exhibited NMJ overgrowth that was suppressed by reducing the doses of the BMP pathway components. There was an increased level of activated BMP receptor Thickveins (Tkv) and phosphorylated Mad, the effector of the BMP signaling in NMJ terminals. Furthermore, the receptor Tkv accumulated in early endosomes but reduced in recycling endosomes, together with the expression pattern of Rab11-positive recycling endosomes altered in dAcsl synapses. This study reveals a novel mechanism whereby dAcsl restrains BMP signaling at NMJ synapses by facilitating Rab11-dependent endosomal recycling of BMP receptors and offers new insight into the pathogenesis of ACSL4-related MRX.

424A

Effects of Perfluoroocatanoic Acid (PFOA) on growth and development in the fruit fly, Drosophila *melanogaster*. AnnJosette Ramirez, Kristin Johndreau, Amber K. Weiner, Ashley Parker, Kara Bennett, Caroline Rachfalski, Sheryl Smith. Biology, Arcadia University, Glenside, PA.

Perfluorooctanoic Acid (PFOA) is a synthetic compound that is used in the manufacture of water-repellent products such as nonstick cookware, household cleaners, furniture and carpet treatments, clothing, and food packaging containers. Human exposure to PFOA has been addressed in a number of studies including one report that PFOA serum levels for adults living in the US were in the range of 4-5ng/mL, with even higher levels reported for children. *In vivo* studies using vertebrate and invertebrate model systems suggest that PFOA affects endocrine signaling that results in reproductive abnormalities. We investigated the effects of PFOA in *Drosophila melanogaster* using three concentrations (5mM, 0.5 mM, and 0.05 mM) orally administered through feeding, beginning at the first instar larval stage. At 5 mM PFOA concentration, growth was affected, resulting in larvae that were approximately one half the size of the untreated control larvae. Interestingly, lower concentrations of PFOA (0.5 mM, and 0.05 mM) produced larvae that were slightly larger in size than non-treated control larvae. Although the mechanisms underlying PFOA-induced size defects are poorly understood, a mutation in the *Tor* gene ($Tor^{\Delta P}$) have produced similar effects to those observed for the 5 mM treatment. We therefore tested the effects of PFOA in this mutant and found that PFOA-induced growth defects were slightly modulated in this background, suggesting that PFOA exerts its effects, in part, through the Target of rapamycin (Tor) signaling pathway. We are currently carrying out gene expression studies to further elucidate the mechanism(s) underlying PFOA toxicity in *Drosophila*.

425B

Immunity Defects in the *Drosophila* Model of Fragile X Syndrome. Elizabeth Stone, Mimi Shirasu-Hiza. Columbia University, New York City, NY.

Fragile X Syndrome (FXS) is the most common monogenic cause of intellectual disability and autistic behaviors. In FXS, the silencing of FMR1, the gene that encodes the translational regulator FMRP, causes altered neuronal signaling and firing, leading to defects in learning, memory, behavior, and circadian regulation. Patients with FXS also exhibit changes in immune system parameters. The *Drosophila*homolog of FMR1, *dfmr1*, is highly conserved, and *dfmr1* mutants have neuronal and behavioral defects similar to those seen in vertebrates. The immune system function of *dfmr1* mutants has not yet been examined. We find that *dfmr1* mutants are highly resistant to certain bacterial pathogens. We are examining specific immune mechanisms that may be responsible for this phenotype. Because FXS and autistic patients appear to have abnormal immune system function, this work may have implications for therapeutic interventions.

426C

Novel Web-based, High-throughput Drosophila Computational Tool used to Investigate the role of UBE3A in Autism Spectrum Disorders. Ryan Turner[1], Rami R. Ajjuri[2], Larry Reiter[3], Janis M. O'Donnell[2]. 1) Computer-Based Honors Program, University of Alabama, Tuscaloosa, AL; 2) Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama; 3) Department of Neurology, University of Tennessee Health Science Center, Memphis, Tennessee.

Autism Spectrum Disorders (ASDs) affect 1 in 88 American children. This cluster of related disorders includes behavioral and developmental defects. Current evidence indicates strong genetic components. Among these are duplications or deletions in human UBE3A. In humans, UBE3A encodes E3 ubiquitin-protein ligase which functions in the degradation of specific proteins via the ubiquitin-proteosome pathway. However, the protein is also predicted to be a transcriptional co-activator for other genes, a feature that complicates genetic analysis of these disorders. Dube3a, the Drosophila homolog, has been used by our lab to model the molecular basis for neurodysfunction as seen in human ASD. To define networks of genes that respond to changes in UBE3a expression, whole genome expression profiling was conducted, comparing the wild-type expression profile to Dube3a null mutant, an over-expressed wild-type transgene, and a transgenic mutant gene with a loss of the ligase function but retention of the co-activator function. We report here the expression profile analysis conducted to identify the following: 1) genes altered in patterns correlating with the over-expression and loss-of-function lines; 2) potential transcription factor binding site (TFBS) clusters of these genes to detect prospective transcriptional co-activators; 3) gene ontology subsets with relevant neuronal function. These candidate genes were then queried for human homologs. A high-throughput computational program was created to access multiple online databases and facilitate this analysis. Additionally, behavioral assays have been conducted to model aberrant behaviors and will be employed to validate candidate genes resulting from the microarray analysis. Together, these analyses will provide potential target genes involved in various domains of Autism Spectrum Disorders.

427A

Effects of Bisphenol A exposure on growth and onset of metamorphosis in *Drosophila melanogaster*. Amber K Weiner, Ashley Parker, AnnJosette Ramirez, Kara Bennett, Kristin Johndreau, Caroline Rachfalski, Sheryl Smith. Biology, Arcadia University, Glenside, PA.

Bisphenol A (BPA) is a high production volume chemical used in the manufacture of polycarbonate plastics, epoxy resins, food packaging, thermal paper, dental composites and sealants and other products. Human exposure to BPA through dietary and non-dietary sources has been well-documented. In numerous vertebrate studies, BPA has been reported to act as a teratogen as well as an endocrine disruptor. Conversely, BPA studies in a variety of invertebrate models suggest that BPA exerts its effects primarily through endocrine disruption, where alterations in fecundity, sex ratio, onset of sexual maturity and other effects have been reported. The mechanisms underlying these effects are incompletely understood and a substantial number of studies report a variable concentration-dependent toxicity. We investigated the effects of BPA exposure in *Drosophila melanogaster* and observed a significant increase in larval size with an administered exposure of 0.1 mg/L. We further found that exposure to BPA at concentrations of 10 mg/L and 0.1 mg/L resulted in an earlier onset of metamorphosis than non-treated control larvae. Treatment with 1 mg/L BPA had no effect on the onset of pupariation. Body size in Drosophila is determined by growth rate and length of time to reach metamorphosis. These processes are governed, in part, through the effects of the insulin-signaling pathway and the ecdysone signaling pathway. We examined the expression of *Ecdysone receptor (EcR)* and *broad (Br)*, two ecdysone-responsive genes critical for the processes of molting and metamorphosis, and found that these genes are expressed earlier in development, at 48 hours, in BPA-treated larvae versus non-treated larvae, where the expression of *EcR* and *Br* was observed at 72 hours of development. These findings suggest that BPA exerts its effects through endocrine disruption in *Drosophila*. A gene expression analysis is currently underway to elucidate possible mechanisms underlying variable dosage effects.

428B

Protein expression profiling of genes implicated in cognitive disorders. Monika Zuberova[1], Korinna Kochinke[2], Pavel Mejstrik[1], Annette Schenck[2], Pavel Tomancak[1]. 1) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 2) Radboud University Medical Centre, Department of Human Genetics, Nijmegen, Netherlands.

Cognitive disorders are, due to their high frequency and unavoidable lifelong dependence on external help, a major medical and socio-economic problem world-wide. Particularly for the large amounts of disorders characterized by intellectual disability, full cures are not possible and the search for effective treatment is unlikely to be successful until the pathology of these disorders is better understood. Currently, mutation in more than 300 human genes are thought to cause cognitive disorders. These genes provide an exciting molecular window into fundamental neuroscience and translational research (from "bedside to the bench and back"). To characterize their function, a large international project (FP7 Gencodys) has been launched in 2010. Fast accumulating evidence indicates that their protein products are functionally not very diverse and that they are involved in several molecular pathways regulating neural development and synaptic plasticity. Taking part in this project, we are creating a systematic library of Drosophila strains bearing fluorescently tagged (and thus easily trackable) Drosophila homologs of these human cognitive disorders genes. Combining this novel reporter toolbox with modern microscopy techniques and specialized computer vision approaches, we are profiling their protein expression in Drosophila nervous tissue, throughout development. We expect that the resulting high resolution 3D and 4D atlases will significantly contribute to our understanding of their function and thus to our understanding of common processes driving the

development and function of the nervous system.

429C

The Use of a Drosophila Laminin A Mutant as a Model for Gestational Diabetes. Joana M. Hubickey, Lauren Perkins- Ross, Laura K. Reed. University of Alabama , Tuscaloosa , AL.

Mutations in the Laminin A (LanA) gene show significant metabolic effects on Drosophila melanogaster adults; these include changes in TAG storage and body weight. Since these phenotypes correlate to the development of diabetes, this finding led us to our present study. We aim to model gestational diabetes in Drosophila using a previously implicated LanA mutant. In humans, gestational diabetes is characterized by high blood glucose and triglyceride levels in the mothers, as well as the mothers giving birth to larger babies. Therefore, we measured the following phenotypes in the flies; total glucose concentration, total triglyceride concentration, egg volume, and pupae weight of the Drosophila LanA mutant, 1389B, in comparison to its wildtype counterpart Canton S (CSB). The results showed that the mutant flies had significantly higher glucose concentrations, and lay larger eggs than the wildtype, which correlates to what is seen in humans. However, the mutant had significantly lower lipid concentrations, and pupae weight than the wildtype. A second aspect of the experiment was the effect of dietary perturbations on the phenotypes. The specialty diets consisted of 6 sugar, 12% sugar, and 1.5% fat. The 6% sugar and 1.5% fat diet caused the most variance in glucose concentration, lipid concentration, and pupae weight in the mutant fly from the wildtype. Moreover, all three specialty diets caused significant variation in egg volume in the mutant while the egg volume of the wildtype remained stable. Additionally, we found that age of the mother dramatically affects egg volume in the mutant. 15 day old mothers laid significantly smaller eggs than the wildtype, while 30 day old mothers laid significantly larger eggs. These findings support the continued explanation of this model for gestational diabetes.

430A

Functional characterization of ACN9 in Drosophila mitochondria. Wendou Yu[1], Daniel K. Bricker[1], James E. Cox[2], Dennis R. Winge[3], Jared Rutter[3], Carl S. Thummel[1]. 1) Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT; 2) Metabolomics Core Research Facility, University of Utah School of Medicine, Salt Lake City, UT; 3) Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT.

Mitochondria have a wide range of cellular functions, including metabolism, signal transduction and cell death. Consistent with these roles, mitochondrial dysfunction is central to many human diseases, including neurodegenerative disorders, type 2 diabetes, and cancer. Accordingly, extensive efforts have focused on functional analysis of the mitochondrial proteome. In spite of this work, however, about one fifth of the nuclear-encoded mitochondrial proteins remain largely uncharacterized. Among these are many proteins that are conserved through evolution, from yeast to humans. We are characterizing these proteins with the goal of gaining new insights into mitochondrial physiology and function. Here we describe our work on a mitochondrial intermembrane space protein ACN9, which is required for efficient succinate dehydrogenase activity in yeast. Drosophila mutants lacking ACN9 are sensitive to a variety of stresses, including starvation and exposure to paraquot or ethanol. Metabolomic analysis shows that ACN9 mutants accumulate succinate and have decreased fumarate and malate, consistent with a defect in succinate dehydrogenase activity. In addition, we see reduced levels of metabolites that are involved in gluconeogenesis, including phosphoenolpyruvate and 3-phosphoglycerate. Interestingly, homocysteine accumulates in ACN9 mutants when compared to controls under normal conditions. Elevated homocysteine is associated with exercise or alcohol consumption in humans as well as cardiovascular and neurodegenerative diseases. This is consistent with genomewide studies that have associated ACN9 polymorphisms with racehorse performance and alcohol dependence in humans. Current efforts are focused on defining the physiological and biochemical functions of ACN9 in flies and yeast.

431B

The Role of SOD1 in a *Drosophila* Model of Spinocerebellar Ataxia 3 (Machado-Joseph's Disease). Christopher Acquafredda, John Warrick. University of Richmond, Richmond, VA.

Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph Disease (MJD), is a dominantly inherited human ataxia caused by an unstable CAG repeat on human chromosome 14q32.1. Research has been done that shows the overexpression of Superoxidative Dismutase 1 (SOD1), which naturally reduces free radicals in the cytoplasm, increases the lifespan of flies up to 40%, suggesting SOD1 and its effects on oxidative stress are important factors in aging and lifespan determination. Based on this, we propose that adding additional copies of SOD1 into the genome of the Drosophila by the use of GAL4/UAS system with a driver targeted to the photoreceptor, that the progression of MJD will be slowed and may show a rescued phenotype. Also, down regulation of SOD1 will be detrimental to fly aging and will cause a quicker progression of MJD due to an increase in ROS. We assessed the degeneration using semi thin plastic sections of fly eyes and light microscopy. Our data suggest influencing the expression of SOD1 in fly eyes with neurodegeneration caused by MJD had little effect. Other work in our lab altering SOD2 levels showed different results. SOD2 is expressed in the mitochondria. This suggests a specific link between mitochondrial and cytoplasmic SOD levels and neurodegeneration.

432C

Catecholamines-up modulates alpha-synuclein- induced neurotoxicity in a Parkinson's disease model. Rami R Ajjuri, Faiza Ferdousy, Janis M. O'Donnell. Department of Biology, University of Alabama, Box 870344, Tuscaloosa, AL, 35487-0344.

While alpha-synuclein has been widely studied in respect to Parkinson's disease (PD) pathology, the exact mechanisms

underlying its role in neurotoxicity have not yet been elucidated. As dopamine neurons in the substantia nigra are preferentially damaged during early stages of PD, much research has been devoted to the role of alpha-synuclein in relation to the production and homeostasis of dopamine, a vital neurotransmitter. Catsup, or Catecholamines-up, plays a critical role in negatively regulating GTP cyclohydrolase and tyrosine hydrolase, key components of the dopamine biosynthesis pathway, and has been shown to modify neurotoxicity in a paraquat-induced PD model in Drosophila. We report that expression of wildtype human alpha-synuclein and human alpha-synuclein mutant (A30P) result in the reduction of tyrosine hydroxylase activity as well as a reduction of dopamine levels. We also observe an increase in dopamine turnover, indicating dysfunction of vesicular packaging. When expressed in the Catsup loss-of-function mutant background, however, both dopamine levels and turnover in wild type alpha-synuclein and A30P mutant transgenic flies are rescued. Similarly, dopamine neurons in Catsup mutants expressing wild type or the A30P mutant forms of alpha-synuclein were significantly protected when compared with those expressing alpha-synuclein wt or the A30P mutant form alone. These results indicate a potentially important role for Catsup in modulating the detrimental effects of alpha-synuclein expression in Parkinson's disease pathology.

433A

***Drosophila* Tau is Required for Proper Maintenance and Survival of Neurons.** Bonnie J. Bolkan, Doris Kretzschmar. CROET, L606, Oregon Hlth & Sci Univ, Portland, OR.

Tau is a neuronal Microtubule Associated Protein (MAP) involved in both microtubule association and stabilization. Aggregation of Tau is one of the pathologies associated with Alzheimer's Disease and neurodegenerative primary-tauopathies. Despite its important role in disease the molecular mechanism of Tau mediated toxicity is not well understood. Transgenic *Drosophila* lines have been used as a model for human Tau toxicity for over a decade. Here we address the role of the endogenous dTau by focusing on the phenotypes resulting from loss of dTau.

While dTau expression appears to be pan-neuronal its expression is highest in the eye and photoreceptor neuron projections. This expression is required for maintenance of these neurons as the eye appears to develop normally even in GMR dTAU^{RNAi} flies, however within days after eclosion the eye begins to significantly degenerate. Transmission Electron Micrographs show very few intact rhabdomeres by 3 days post eclosion. Pan-neuronal knowndowns results in high levels of larval lethality and the flies that survive to adulthood show significant vaculolization in the central brain by 36 hr post eclosion but this degeneration is not progressive.

These data support the loss of function models in human tauopathies. We, therefore, wanted to look at the effects of human (h) and bovine (b) Tau, both of which are toxic in *Drosophila*, on dTau. Immunohistochemistry of larval eye discs expressing bTau or hTau driven by GMR show a dramatic decrease in dTau and a change in dTau staining patterns. This decrease in dTau levels was confirmed in Westerns. Furthermore, microtubule assays show that the vast majority of hTau is phosphorylated and cytosolic yet very little dTau is still bound to microtubules. We therefore propose that the toxicity of bTau and hTau is caused by the removal of functional Tau from microtubules.

434B

The role of SOD2 and autophagy in a *Drosophila* model of Machado-Joseph Disease. Natalie M. Clark, John M. Warrick. Department of Biology, University of Richmond, Richmond, VA.

Spinocerebellar ataxia 3 (SCA3), also known as Machado-Joseph Disease (MJD), is an autosomal dominant neurodegenerative disorder caused by an expanded polyglutamine repeat in the ataxin-3 (ATX3) protein. Research has suggested that MJD potentially increases the amount of reactive oxidative species within the body, accelerating the cell aging process and increasing neural death. It is hypothesized that the increase of naturally occurring antioxidant gene products such as Superoxide Dismutase 2 (SOD2) could decrease the severity of this disease and serve as a possible treatment. UAS-ATX 3 alleles of mutant and normal MJD as well as UAS-SOD2 were expressed in the fly eye using the *gmr*Gal4 driver. Fly heads were fixed and embedded in epon blocks. Semi-thin sections of fly retinas were evaluated using light microscopy. We found flies expressing both MJD and increased levels of SOD2 had greater eye degeneration and faster progression of disease than flies with MJD and endogenous SOD2 levels. Other research has implicated superoxide in the autophagy pathway, and autophagy has been suggested to reduce the degeneration caused by MJD by removing aggregates. We propose that the increase in SOD2 levels interfered with the autophagy pathway causing the increase in degeneration. To test this hypothesis, flies were aged and their heads were frozen in OCT. 12 micron-thick frozen sections were taken using a cryostat, and the sections were stained with antibodies to ATX3 and Autophagy Protein 12 (ATG12). Viewing the sections using confocal fluorescence microscopy revealed that flies with MJD have strong expression of ATG12 protein co-aggregated with ATX3 in the nucleus at 2 and 7 days, while flies expressing normal ATX3 do not show significant expression of ATG12. Likewise, flies with MJD and increased SOD2 have co-aggregation at 2 days and 7 days, yet the staining appears weaker. Since ATG12 is not able to function in these nuclear aggregates, these results suggest a decrease in autophagy in MJD due to SOD2 overexpression.

435C

TDP-43 neurotoxicity due to loss-of-function in Map205-dependent steroid receptor-mediated gene program switching in Drosophila. Bart Dermaut[1,2], Lies Vanden Broeck[2], Marina Naval Sanchez[3], Yoshitsugu Adachi[4], Danielle Diaper[4], Pierre Dourlen[1], Julien Chapuis[1], Gernot Kleinberger[5], Marc Gistelinck[2], Christine Van Broeckhoven[5], Jean-Charles Lambert[1], Frank Hirth[4], Stein Aerts[3], Patrick Callaerts[2]. 1) Inserm U744, Institut Pasteur de Lille, University of Lille 2, Lille, France; 2) Laboratory of Behavioral and Developmental Genetics, VIB Center for the Biology of Disease, University of Leuven, Belgium; 3)

Laboratory of Computational Biology, Center of Human Genetics, University of Leuven, Belgium; 4) MRC Centre for Neurodegeneration Research, King's College London, Department of Neuroscience, Institute of Psychiatry, London, UK; 5) Department of Molecular Genetics, Neurodegenerative Brain Diseases Group, VIB, Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerpen, Belgium.

TDP-43 proteinopathy is strongly implicated in the pathogenesis of amyotrophic lateral sclerosis and related neurodegenerative disorders. Whether TDP- 43 neurotoxicity is caused by a novel toxic gain-of-function of the aggregates or by a loss of its normal function is unknown. We increased and decreased expression of TDP-43 (dTDP-43) in the Drosophila central nervous system and identified an important role for dTDP-43 in the survival of CCAP/bursicon neurons at the pupal-adult transition. While upregulation of dTDP-43 induced neuronal ubiquitin- and dTDP-43-positive inclusions, both up- and downregulated dTDP-43 resulted in neuronal apoptosis and highly similar transcriptome alterations in late metamorphosis. Gene network analysis and genetic validation showed that both up- and downregulated dTDP-43 directly and dramatically increased the expression of the neuronal microtubule associated protein Map205 resulting in cytoplasmic accumulations of the ecdysteroid receptor (EcR) and a failure to switch EcR-dependent gene programs from a pupal to adult pattern. We propose that dTDP-43 neurotoxicity is caused by a loss of its normal function in EcR-dependent gene program switching.

436A

Photoreceptor cell death triggered by rhodopsin aggregation requires immunity signaling and transcriptional activation through NF-kB. Patrick J. Dolph, Ron Kinser, Yashodhan Chinchore. Biological Sciences, Dartmouth College, Hanover, NH.

Retinitis pigmentosa (RP) is a common retinal disease characterized by an age-related progressive loss of vision. Specific forms of RP are typified by the abnormal localization of the light receptor rhodopsin to cytoplasmic compartments. We have been able to phenocopy this form of RP in Drosophila. We have identified mutations that induce retinal degeneration and are characterized by the massive internalization of rhodopsin via receptor-mediated endocytosis. This internalized rhodopsin is not degraded in the endolysosomal system but instead accumulates in the late endosome where it forms insoluble aggregates. Genetic analysis has revealed that this mislocalized aggregated rhodopsin does not trigger cell death through any of the classical apoptotic pathways. Instead, we found that cell death requires components of the innate immunity pathway eventually leading to the activation of NF-kB transcription factors. Interestingly, mutations affecting both the Toll signaling pathway and the Imd pathway rescue retinal degeneration in our model and two different NF-kB orthologues, Relish and Dorsal, are also both required. In addition, we have show that expression of an activated form of Relish or the induction of the Toll pathway in photoreceptors and other cell types triggers cell death, suggesting that these protective pathways may induce apoptosis in specific cell types. These results define a new role for innate immunity signaling and NF-kB transcription factors in cell death induction.

437B

The role of Swiss cheese, the *Drosophila* homologue of Neuropathy target esterase, in glia development. Sudeshna Dutta, Janis McFerrin, Bruce Patton, Doris Kretzschmar. CROET, Oregon Health and Science University, Portland, OR.

Neuropathy target estarase (NTE), a molecular target of organophosphates (OP) found in pesticides and nerve gases, is an important factor in an induced delayed neuropathy (OPIDN) and an inherited spastic paraplegia in humans. OPIDN is characterized by axonal degeneration mainly of motoneurons. Similarly, loss of the *Drosophila* homologue of NTE, Swiss Cheese (SWS) causes progressive neurodegeneration and also glial degeneration in flies and we have previously shown a cell autonomous requirement of SWS in both cell types in the adult brain of *Drosophila*. Using cell type specific down regulation of SWS, we can now specifically address its requirement in glia. Our recent findings demonstrate that only one type of glia, the ensheathing glia is affected by SWS down-regulation. We are also investigating what functional domains of SWS are required in glia, by using point mutations in the kinase domain and esterase domain of SWS in these glia specific knock-downs. Similar to flies, our findings in mice also demonstrate the presence of SWS/NTE in glia cells in the sciatic nerve, suggesting a conserved role of SWS in glia in higher vertebrates. NTE is expressed in high levels in nonmyelinating Schwann Cell (SC) and lower levels in myelinating SC. These studies, using both *Drosophila* and mouse models, will help us to understand the importance of the SWS protein in glia, its role in axonal-glial interaction and its pathogenic function in inherited spastic paraplegia and OPIDN in humans.

438C

A novel rationally designed chaperone that blocks amyloid beta neurotoxicity. Shailaja Emani[1,2], Swati Khare[1], Alfonso Martin-Pena[1], Yan Zhang[1], Pedro Fernandez-Funez[1], Diego Rincon-Limas[1]. 1) Neurology, University of Florida, Gainesville, FL; 2) HHMI-UF Science for Life.

Alzheimer's disease (AD) is an incurable disorder characterized by memory loss, brain neurodegeneration, and an abundance of extracellular amyloid deposits composed of misfolded Amyloid-β42 (Aβ42) peptide. Since Aβ42 oligomers are the neurotoxic agents driving AD pathology, targeting these toxic assemblies with chaperones that enhance protein folding capacity may have therapeutic effects. In this regard, the heat shock chaperone Hsp70 is a promising candidate due to its potent anti-misfolding activity. Unfortunately, this normally intracellular chaperone exists extracellularly at very low concentration and thus its activity has never been tested in this context. To address this, we engineered a new secreted version of Hsp70 (secHsp70) using dedicated software and capitalized on our fly model of AD-like pathology to test its

protective activity (Casas-Tinto, HMG 2011). Strikingly, we found that secHsp70 exerts a robust protection against extracellular Aβ42 deposition and toxicity in photoreceptor neurons. This dramatic effect requires the presence of Hsp70 in the extracellular space as neither overexpression of WT cytosolic Hsp70 nor the ER-bound chaperone BiP suppressed Aβ42 toxicity. Remarkably, secHsp70 exerts its protection without the cochaperone Hsp40. We also confirmed the secretion of secHsp70 by looking at its distribution and confirming that it does not rescue the toxicity of Ataxin3-78Q, an intracellular amyloid implicated in Spinocerebellar ataxia type 3. Finally, secHsp70 does not affect total Aβ42 levels, suggesting that its protection is mediated by regulating Aβ42 misfolding, aggregation, and/or interaction with cellular targets. In summary, our new secHsp70 chaperone has an extraordinary ability to block Aβ42 insults. Thus, enhancing protein-folding capacity in the extracellular space could represent a new therapeutic strategy for many serious extracellular amyloidoses such as AD, prion diseases, and type II diabetes.

439A

Discovery of *SAD*, a novel gene required for axonal integrity in ageing, by an unbiased genetic screen using the *Drosophila* wing as a model. Yanshan Fang, Xiuyin Teng, Yongqing Zhu, Nancy Bonini. HHMI, Dept. of Biology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Axon degeneration is a prominent feature of spinal cord injury and neurodegenerative diseases. Studies of the Wld^S mouse indicate that axon degeneration is an active process, however, the underlying mechanisms remain elusive. To identify novel components controlling axonal integrity, it is desirable to perform unbiased, large-scale screening.

Drosophila is an exceptional model system for the study of human diseases. We thus developed a model of nerve injury using the *Drosophila* wing, which is translucent, allowing us to highlight the axons using fluorescent proteins and to monitor axonal changes in response to traumatic injury and ageing in *live* flies. Using this model, we conducted a genetic screen in a Wld^S-sensitized background.

Among the initial candidates, we found a novel mutant of a functionally unknown gene. This mutant not only diminishes Wld^S protection, but also displays striking age-dependent spontaneous axon degeneration on its own. For this phenotype, we named it: *Spontaneous Axon Degeneration (SAD)*. Further examination reveals massive vacuoles in the brain of aged *SAD* flies, a hallmark of progressive neurodegeneration in the CNS. Moreover, the lifespan of the *SAD* mutant is significantly shortened. In addition, aged *SAD* flies have elevated sensitivity to heat and physical stress, although their climbing capability is normal.

We are defining the nature and function of *SAD*. Protein feature analysis suggests that SAD is involved in chromatin remodeling. Ongoing experiments include generating SAD transgenic flies to confirm its neural effects and making anti-SAD antibody to define its expression patterns. By such study, we hope to reveal the molecular mechanism of SAD in age-associated maintenance of the nervous system, which will provide important foundation for new therapeutic targets of neurodegeneration.

440B

Anti-Aβ miniantibodies suppress Aβ42 neurotoxicity in flies. Pedro Fernandez-Funez[1,2,3], Swati Khare[1], Krishanu Mathur[1], Alfonso Martin-Peña[1], Diego Rincon-Limas[1,2]. 1) Dept Neurology; 2) Dept Neuroscience; 3) Genetics Institute and Center for Translational Research on Neurodegenerative Diseases; University of Florida, Gainesville, FL.

Alzheimer's disease (AD) is an incurable neurodegenerative disorder characterized by irreversible cognitive decline. Soluble assemblies of the Amyloid-β (Aβ42) peptide are the leading neurotoxic agents in AD pathogenesis; thus, strategies that target Aβ42 are likely to slow or revert the disease. Passive immunotherapy is a promising approach in AD; sadly, intravenous delivery of a full size anti-Aβ antibody failed to improve cognition recently in human clinical trials. There is still hope, though, that single chain variable fragments (scFv) may demonstrate better efficacy due to their higher penetration in the brain. We want to use Drosophila as a platform to test the in vivo activity of new anti-Aβ42 scFvs. As proof-of-principle, we generated flies expressing scFv-Aβ9 and -213, two scFvs known to bind Aβ42 and reduce plaque formation in mice. Since the AD mice do not exhibit significant neuronal loss, we examined the neuroprotective effect of these scFvs in our Drosophila model expressing human Aβ42. We found that both scFv-Aβ9 and -213 partially suppressed the Aβ42 phenotype, although scFv-Aβ9 worked better. Interestingly, co-expression of both scFv-Aβ9 and -213 resulted in even better suppression of Aβ42 neurotoxicity, suggesting a synergistic activity from binding to the two epitopes. Expression of each scFvs significantly reduced the number of apoptotic cells induced by Aβ42. But the combination of both miniantibodies potently inhibited Casp3 activation to levels comparable to normal eyes. We also found that the scFvs do not affect the levels of total Aβ42 by Western blot or the accumulation of amyloid fibers by thioflavin-S staining. Thus, the scFvs mediate their protective activity by preventing the interaction of Aβ42 with cellular targets, not by promoting Aβ42 degradation. These results demonstrate that secreted scFvs work well in flies and, hence, can be used to screen for highly effective scFvs or combinations thereof against Aβ42.

441C

Repeat Associated Non-AUG initiated Translation mediates neurodegeneration in a Drosophila models of Fragile X-associated Tremor Ataxia Syndrome. Michelle A. Frazer[1], Fang He[2], Peter K. Todd[2]. 1) Cellular & Molecular Biology, University of Michigan, Ann Arbor, MI; 2) Neurology, University of Michigan, Ann Arbor, MI.

Fragile X-associated Tremor Ataxia Syndrome (FXTAS) is a neurodegenerative disease that results from a CGG repeat expansion in the 5'UTR of FMR1. Pathogenesis in FXTAS is thought to involve a dominant RNA gain of function mechanism,

whereby the CGG repeat mRNA binds to and sequesters specific RNA binding proteins. However, our group has recently discovered that the repeats are also capable of eliciting aberrant translation initiation in the 5'UTR in the absence of an AUG start codon (RAN translation), leading to the production of a polyglycine-containing protein that forms ubiquitinated aggregates in cells and animal models, as occurs in patients. A critical question that emerges from this work is whether this polyglycine protein contributes directly to toxicity, or whether the neurodegeneration is mediated strictly via RNA toxic mechanisms. To investigate this question, we created strains of drosophila that decouple the potential RNA and protein mediated toxic processes. This was achieved by placing the CGG repeat in either the 5'UTR or 3'UTR of a heterologous gene, eGFP. Placement in the 3'UTR precludes RAN translation. To enhance the protein mediated toxic effects, we have inserted an AUG start codon 5' to the repeat, which leads to increased production of the polyglycine protein. As previously reported, expression of a $(CGG)_{100}$ repeat in the 5'UTR of eGFP leads to a modest rough eye phenotype with isolated oomatidial expression and a decrease in viability with ubiquitous expression compared to control flies. In lines where the CGG repeat is in the 3'UTR of eGFP, there is very little overt oomatidial degeneration and no effect on viability. In contrast, flies with an ATG codon inserted 5' to the repeat, exhibit a dramatic degenerative eye phenotype and further reduced viability compared to flies lacking this ATG. These studies support a model where aberrant translation of a polyglycine protein in FXTAS contributes significantly to disease pathogenesis.

442A

Effects of Nicotine and Indole-3-carbinol on Rotenone-induced Drosophila model of Parkinson's disease. Cassie K. Huang, Jessie Rottersman, S. Tariq Ahmad. Biology, Colby College, Waterville, ME.

Parkinson's disease (PD) is a neurodegenerative disorder primarily affecting the dopaminergic neurons in the nigrostriatal pathway resulting in debilitating motor impairment in both familial and sporadic cases. Chronic exposure to the pesticide rotenone also selectively degenerates dopaminergic neurons and causes locomotor impairment and early mortality in a Drosophila model of chemically-induced PD. Nicotine, a nicotinic acetylcholine receptor agonist, produces stimulant effects on animals. It is widely consumed by humans, and substantial losses in nicotinergic receptors have been found postmortem in Parkinson's disease. Previous research has shown positive results using nicotine to treat rotenone toxicity in vitro. Indole-3-carbinol (I3C) is found naturally in many cruciferous vegetables such as brussel sprouts, kale, and broccoli. It is thought to have antioxidant effects and has been targeted as a possible cancer treatment after a study showed I3C dose-related decreases in tumor susceptibility. This study investigated the effects of nicotine and indole-3-carbinol on early mortality in a rotenone-induced PD model. We show that treatment with 10 uM nicotine and 1mM indole-3 carbinol-supplemented food improve the early mortality in flies. The recovery of rotenone-induced locomotor deficits by nicotine and indole-3-carbinol is currently being explored. Furthermore, future studies will explore the antioxidant effects of these two drugs through a superoxide dismutase (SOD) assay.

443B

A large-scale RNAi screen to identify novel modifiers of polyglutamine toxicity in *Drosophila*. Sara Imarisio[1], Ashley R. Winslow[2], Benjamin R. Underwood[3], Wun Lam[1], Evangelia K. Ttofi[2], Viktor I. Korolchuk[4], Jörg Gsponer[5], M. Madan Babu[6], David C. Rubinsztein[2]. 1) Department of Genetics, University of Cambridge, Cambridge , UK; 2) Department of Medical Genetics, University of Cambridge, Cambridge Institute for Medical Research,Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0XY, UK; 3) Norfolk and Suffolk Huntington's Disease Service, Mental Health Team, Newmarket Hospital, Newmarket, Suffolk CB8 7JG, UK; 4) Institute for Ageing and Health, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne, NE4 5PL, UK; 5) Centre for High-Throughput Biology, The University of British Columbia, 2125 East Mall, Vancouver, BC V6T 1Z4, Canada; 6) Medical Research Council (MRC) Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK.

Polyglutamine (PolyQ) diseases are a family of neurodegenerative disorders caused by an expanded CAG repeat in the target gene. Mutant proteins form toxic intracellular aggregates, associated with cell death. To date there is no cure or treatment that delays the progression of degeneration. With the aim to identify genes and pathways affecting polyQ toxicity, we analysed the loss-of-function of almost half of the Drosophila genome in a line carrying 48 glutamines, which has a severe eye phenotype. We identified 174 suppressors and 748 enhancers that we extensively validated, i.e. using an independent RNAi library to minimise the possibility of off-target effects, and confirmed 76% of the hits. Moreover,being aggregate formation a hallmarks of polyQ toxicity, we checked whether the suppressors reduced the number of aggregates in a line expressing expanded huntingtin in the eye, finding that 70% of them significantly reduced the number of inclusions. To get a better understanding of the biological processes affected by our modifiers, we used a bioinformatic approach to categorise gene ontologies that were over-represented amongst modifiers, such as members of proteolysis, transcription regulation and apoptosis. Thus, our screen results a valuable resource to study polyQ diseases, highlighting novel genes and processes regulating toxicity.

444C

FUS/TLS mutations disrupt axonal transport, synaptic development, and synaptic function: a screen for genetic modifiers. James B. Machamer[1], Thomas Lloyd[1,2]. 1) Dept of Neurology, JHMI, Baltimore, MD; 2) Dept of Neuroscience, JHMI, Baltimore, MD.

FUS is an RNA binding protein that has been implicated in the pathogenesis of both familial and sporadic Amyotrophic Lateral Sclerosis (ALS). FUS regulates RNA metabolism in both the nucleus and in the cytoplasm, and the majority of ALS-causing mutations lie within the nuclear localization sequence (NLS) of FUS. These mutations result in the formation of

cytoplasmic aggregates and loss of protein function in the nucleus. However, it remains unclear whether FUS-mediated ALS is due to a gain of toxic function or a loss of FUS protein, and little is known about the mechanism leading to neuronal dysfunction. In this study, we analyze FUS overexpression in larvae to investigate the earliest changes in motoneuron function. We first investigated the effect of mutant FUS on axonal transport and found decreased processivity of cargo transport. We next measured the level and localization of essential synaptic proteins in the larval neuromuscular junction (NMJ) and find that FUS expression in the motoneurons reduces the number of presynaptic active zones and postsynaptic levels of Discs large (DLG), a scaffolding protein that localizes glutamate receptors to the active zone. Consequently, we find reduced amplitude of synaptic transmission at the NMJ due to decreased quantal content. Interestingly, overexpression of mutant forms of the Drosophila homolog of FUS, Cabeza (Caz) also disrupt normal synaptic transmission, but as a result of reduced quantal size. Finally, we screened for modifiers of FUS-mediated rough eye and motoneuron phenotypes using 2nd chromosome deficiencies followed by RNAi and identified multiple splicesome subunits, suggesting that FUS-mediated alterations in RNA splicing underlie neuronal toxicity. Thus, in this fly model of ALS, we find significant early changes in mutant FUS-expressing motoneurons including disruption of axonal transport, synapse development, and synapse function. Furthermore, the identification of splicesomal proteins as genetic modifiers suggests that these changes may be a consequence of disrupted nuclear RNA processing.

445A

Effects of nicotine on motor deficits and lifespan when given on different treatment days in a Parkinson's disease model. Mukul Mallick[1], Lori M. Buhlman[1], Gerald B. Call[2]. 1) Biomedical Sciences, Midwestern University, Glendale, AZ; 2) Dept. of Pharmacology, Midwestern University, Glendale, AZ.

In the US, Parkinson's disease (PD) affects about 13 out of 100,000 and is the second leading neuromotor degenerative disease (Van Den Eeden et al., 2002). *Drosophila melanogaster* with *parkin* loss-of-function mutation exhibit similar pathology to patients with familial PD such as motor deficits, mitochondrial pathology and decreased lifespan, which makes it especially viable as a model for familial PD compared to other *parkin* loss-of-function models, which do not exhibit these symptoms. Motor deficits may stem from mitochondrial pathology, which leads to indirect flight muscle degeneration (Greene et al., 2003). Epidemiological studies suggest a delay in the onset of PD in tobacco smokers (Hernan et al., 2001; Grandinetti et al., 1994; Rajput et al., 1987) and that nicotine has neuroprotective effects in models of sporadic PD (reviewed in Quik et al., 2009). Previous data in our lab suggests that nicotine rescues motor, viability and loss of olfaction symptoms in *+/park^{25} D. melanogaster* when given at day one post eclosion. This study was initiated to determine if nicotine can rescue symptoms when administered at later days by assessing climbing and flight assays on wild-type and *+/park^{25} D. melanogaster*when exposed to nicotine at later days post eclosion. Initial results indicate that treatment with nicotine at 3 days post eclosion improves climbing and flight. Further details and a complete time course for nicotine administration will be presented at the meeting. These results will clarify whether nicotine can be an effective treatment for familial PD when given to patients after they first start experiencing symptoms such as loss of olfaction.

446B

Role of Transcriptional Co activator CREB Binding Protein in Amyloid Beta-42 (Aβ42) mediated neurodegeneration. Gregory F. Mancini, Meghana Tare, Amit Singh. Biology, University of Dayton, Dayton, OH.

Alzheimer's disease (hereafter AD), a common progressive neurodegenerative disorder in the aging population, has no early detection tests or proper cure. AD results in gradual decline of cognitive functions of learning and memory due to neurodegeneration in central and peripheral nervous system. My project focuses on understanding role of transcriptional co-activator CREB binding protein (hereafter, CBP) in preventing neurodegeneration caused by Aβ42 plaques in the Drosophila eye. CBP binds to a variety of transcription factors and components of several signal transduction pathways. It has been observed in high throughput approaches that CBP levels are reduced in cells undergoing cell death due to stress. Therefore, we propose to test if CBP can serve as a neuroprotective agent, and can prevent neurodegeneration seen in AD using Drosophila eye model.

447C

Study of the Regulation of Aggregates Formation by ALS associated SOD1 Mutations Using Drosophila. Michael Mccarthy[1,2,3,4], Dongsheng Chen[1,2,4], Zhihua Zhen[1,2,4], Antonio Tito[1,2,3,4], Zhen Xu[1,2,4], Yanning Rui[1,2,4], Sheng Zhang[1,2,3,4]. 1) Center for Degenerative and Metabolic Disorders, Houston, TX; 2) Institute of Molecular Medicine, 1825 Pressler St., Houston, TX; 3) GSBS, Houston, TX; 4) UT-Houston, Houston, Texas.

The presence of protein aggregates is a common pathological feature of most neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS). While it is still controversial whether or not aggregates is deleterious or protective, understanding the cellular pathways that regulate aggregates formation will help elucidate the pathogenic mechanisms underlying these devastating diseases and facilitate the search for their effective treatment. ALS, also known as Lou Gehrig's disease, predominantly affects motor neurons. Mutations in Superoxide Dismutase 1 (SOD1), which are linked to about 20% of familial ALS (fALS), are believed to lead to a gain of toxicity. Among the common fALS mutations, neither A4V nor G93A interferes with SOD1's normal cellular function, while G85R does have an effect and also is aggressive at forming aggregates. To identify regulators of aggregates formation associated with SOD1 mutations, I plan to establish both cell- and animal-based models for ALS in Drosophila. To facilitate convenient visualization of aggregates, I will co-express mutant SOD1 (A4V, G85R,

or G93A) labeled with eGFP fluorescent tag together with wildtype (wt) SOD1 labeled with mCherry tag, the latter serves as an internal control for the level of protein expression and for confirming the mutant SOD1-specific aggregates formation. To date, I have engineered all the planned SOD1 tagging constructs both for copper-inducible expression in cultured Drosophila cells and for establishing transgenic fly lines. I am characterizing the established SOD1 transgenic fly lines for potential formation of aggregates and neuronal toxicity. Establishment of these new ALS models will help uncover the molecular networks that control SOD1-associated aggregates formation, potentially providing targets for effective therapeutic intervention.

448A

Determining the tissue basis of nicotine rescue in the Drosophila Parkinson's Disease model. David O. Meyer[1], Lori M. Buhlman[1], Gerald B. Call[2]. 1) Dept. of Biomedical Sciences, Midwestern University, Glendale, AZ; 2) Dept. of Pharmacology, Midwestern University, Glendale, AZ.

The Drosophila Parkinson's Disease (PD) model based on homozygous loss of function of the *parkin* gene has been shown to have both flight muscle degeneration and dopaminergic neuronal loss in the brain. Our previous data also indicates that flies heterozygous for the *park[25]* null allele also experience motor function defects, olfaction loss and decreased lifespan. Interestingly, administration of nicotine to these flies in their food improved or rescued all of the observed deficits. This study was initiated to determine the mechanism of this rescue by histological and genetic methods. The first method consists of histologically analyzing the indirect flight muscle and dopaminergic neurons in the brain to determine if the morphology or numbers of these tissues are affected by nicotine treatment. Initial results indicate that nicotine does not affect neuron numbers in 20-day-old *park[25]* heterozygotes, nicotine = 12.62 neurons/cluster (n=37) vs. no nicotine = 12.92 neurons/cluster (n=26). Further analysis, including muscle morphology will be presented at the meeting. In addition to histological analysis, a genetic mechanism using RNAi to knock down Parkin in a tissue specific manner will help us determine the site of nicotine rescue. We are currently determining if using *Actin-Gal4* to drive expression of *UAS-park RNAi* can phenocopy the *park[25]* flies. These experiments are underway and will be presented at the meeting. These results will help identify where nicotine is rescuing the phenotypes observed in this model in an effort to help understand PD better.

449B

Drosophila eye model to identify genetic modifiers of Aβ42 mediated neurodegeneration. Michael T. Moran, Oorvashi Roy Puli, Meghana Tare, Amit Singh. University of Dayton, Biology Department, 300 College Park Dayton, OH 45469.

The neurodegeneration that results from Alzheimer's disease (AD) is caused by the improper cleavage of APP to form the polypeptide amyloid beta 42 (Aβ42). Being hydrophobic, Aβ42 clumps together forming plaques which in turn accumulate around the neurons of the brain causing many cellular disturbances and, eventually, neuronal death. The characteristically slow degeneration of neurons in AD has been accredited to this accumulation of Aβ42 in the brain. However, the exact mechanisms of how and why this accumulation happens are not yet fully understood. Using the Aβ42 misexpression model where we misexpress Aβ42 in the differentiating neurons of the eye using GMR-Gal4 driver, we carried out a screen to look for downstream modifiers of the neurodegenerative phenotype of Aβ42 accumulation. Here we present the results of the screen and further characterizations of genetic interactions of two genetic modifiers and their role in Aβ42 mediated neurodegeneration in the Drosophila eye.

450C

Investigating interactions of TDP-43 with the insulin pathway in a *Drosophila* model of amyotrophic lateral sclerosis. Andrés A Morera[1], Taylor Podolsky[1], Alyssa Coyne[2], Archi Joardar[1], Daniela Zarnescu[1,3]. 1) Department of Molecular and Cell Biology, University of Arizona, Tucson, AZ; 2) Department of Neuroscience, University of Arizona, Tucson, AZ; 3) Department of Neurology, University of Arizona, Tucson, AZ.

ALS is a devastating adult-onset neurodegenerative disease that causes progressive muscle atrophy due to degeneration of upper and lower motor neurons, leading to paralysis and death within 2 - 5 years of onset of clinical symptoms. TDP-43, a ubiquitously expressed RNA binding protein, was found to be a common component of intraneuronal inclusions in motor neurons of ALS cases, as well as in other neurodegenerative diseases. We have developed a Drosophila model of ALS based on TDP-43, which recapitulates many features of the human disease, including locomotor defects, neuromuscular junction defects, motor neuron degeneration, and increased mortality. Using a combination of genetic and drug screening approaches we found that TDP-43 overexpression in motor neurons impacts the insulin/Pi3K/Akt/TOR signaling cassette. This led us to hypothesize that insulin signaling is dysregulated in our ALS model based on TDP-43. Current experiments are aimed at identifying and characterizing components of the insulin/PI3K/Akt/TOR signaling cassette that may mediate TDP-43's neurotoxicity. To accomplish this, we use the Gal4-UAS system to manipulate expression of insulin pathway components in the context of TDP-43 overexpression in various Drosophila tissues including photoreceptors, motor neurons or glia. By observing the effects of these manipulations on the TDP-43 neurodegenerative phenotype, we can better understand the physiological role of TDP-43 and dissect the mechanisms by which TDP-43 and components of the insulin pathway may lead to neurotoxicity in ALS and other neurodegenerative diseases.

451A

Neurodegeneration in mitochondrial Complex III deficiency involves necrotic cell death. Francesco Napoletano, Diane Lebrun, Gilles Chatelain, Bertrand Mollereau. LBMC-Laboratory of molecular cell biology, ENS Ecole Normale Superieure de

Lyon, Lyon, France.

Defects in mitochondrial respiratory chain (RC) are linked to many neurodegenerative disorders, and specifically to rare mitochondrial diseases, such as Complex III (CIII) deficiency. Symptoms of CIII deficiency include encephalopathy, optic atrophy and muscle weakness. Genetic defects preventing the incorporation of the Rieske iron sulfur protein (RISP) in the mitochondrial CIII lead to CIII deficiency. Loss of RISP function has been shown to trigger oxidative-stress dependent neurodegeneration in mice, however the underlying molecular mechanisms are unknown. Degenerating neurons often exhibit apoptotic (caspase dependent) and necrotic (caspase independent) hallmarks. While a lot is known on apoptosis, much less is understood on necrotic pathways and their regulation. In addition, the distinct contribution of these forms of cell death to neurodegeneration is still unclear.

Using the *Drosophila RISP* mutant, we have established a genetic model of necrosis to dissect the pathways of necrotic cell death and their role in the pathogenesis of disorders due to RC defects. *RISP*mutant photoreceptor neurons showed progressive degeneration with necrotic morphology, and no apparent developmental defect. In situ analysis of caspase activity suggests that necrotic pathways are predominant in our model. Through genetic and biochemical approaches, we have identified candidate pathways of necrosis in the *RISP* mutant. We are currently dissecting these pathways, and analyzing their interaction with apoptosis and with potential neuroprotective mechanisms, such as autophagy and oxidative stress response.

Clarifying the multiplicity of cell death mechanisms will provide potential therapeutic strategies for effective cytoprotection in human diseases due to mitochondrial dysfunction.

452B

Drosophila cd Mutant of the Kynurenine Pathway as a Model for Dementia-Like Disorders. Ekatherina Nikitina, Yulia Dolgaya, Nadiya Utesheva, Elena Savvateeva-Popova. Dept Neurogenetics, Pavlov Inst Physiology, St Petersburg, Russian Federation.

Neurodegenerative diseases, accompanied by cognitive disturbances, i.e gradual memory loss (dementia), are characterized by late onset, relentless progression, and finally death. Molecular-genetic studies of the human genome have emphasized the evolutionary conservation of homologous genes from different organisms. Drosophila mutants with phenotypes similar to neurodegenerative diseases accompanied by dementia might help to unravel the etiology of these polygenic disorders. A large number of neurodegenerative diseases are known to share a common pathological feature of abnormal brain deposits. It results from the alterations in the functioning of heat shock/chaperone machinery. Moreover, neurodegenerative disorders are characterized by altered content of the intermediates of the kynurenine pathway of trypthophan metabolism (KPTM). We developed Drosophila mutant model which reproduces main symptoms of neurodegenerative diseases. Mutant cardinal (cd, excess of 3-hydroxykynurenine, 3-HOK, the generator of oxidative stress) can serve as model for dementia since it is characterized by age-dependent memory loss, synaptic pathology, apoptosis under heat shock. Here, we tested the effects of heat shock (HS) on the main disease manifestations - impairments in learning/memory and HSP70 intracellular localization. For this, we used a 30-min HS given at the stage of formation of the central complex implicated in learning and memory. Having no effect on wild type flies (CS), HS in cd mutants lead to a drastic 4-fold and 10-fold decrease in long-term memory retention tested 2 and 8 days after training. Using confocal microscopy we demonstrated a decrease in anti- HSP70 staining in cd cells in comparison to wild type under normal and stress conditions. Without heat shock HSP70 was determined on nuclear surface both in cd and CS. In both stocks HS treatment resulted in HSP70 relocation into the nuclei. Found differences can result from an accumulation of 3-HOK in cd mutant. Therefore, the cd mutant may be regarded as an appropriate model for dementia-like disorders.

453C

Glial involvement in neuronal synaptic bouton formation implicates *pak3* and *draper* function. Emily F. Ozdowski, Nina T. Sherwood. Dept Biol, Duke Univ, Durham, NC.

Neurons require cytoskeletal regulators, such as the microtubule-severing protein Spastin, to produce proper axonal branching and functional synaptic connections. When Spastin function is compromised in humans, the motor neuron disease Hereditary Spastic Paraplegia (HSP) results. This disorder is characterized by degeneration of long axons within the corticospinal tracts and ultimately loss of mobility in the lower limbs. Similarly, when *spastin* function is lost in Drosophila, neuronal signaling at the larval neuromuscular junction (NMJ) is diminished, and adult flies are not able to walk normally, jump, or fly. In*spastin* null mutants, larval axons form unique grape-like bunches of small synaptic boutons at the NMJ, and microtubules are missing from the distal tips. These aberrant structures are useful in searching for regulators of *spastin* function in neurons, and we discovered that the actin regulator, *p21-activated kinase 3* (*pak3*) is a bypass suppressor of the *spastin* phenotype. *pak3* hypomorphic mutations have little effect on wild-type NMJ synapses but strongly suppress the bunched bouton morphology of *spastin* null mutants. In addition, neuronal overexpression of *pak3* results in numerous actin-rich filopodial projections, as observed in cell culture. However, we found that Pak3 is expressed primarily in glia, and glial-specific reduction of *pak3* also suppresses *spastin* bunches. Glia have previously been linked to synaptic bouton number and synaptic debris clearance via Draper receptor function. We found that both the *draper* null mutation and glial-specific *draper* knockdown by RNAi suppress *spastin* bunches, suggesting that *draper* and *pak3* work in a similar pathway. We are currently examining mutations in Draper ligands and effectors for suppression of *spastin* null bouton bunches. We are also imaging the physical interactions between glia and neurons during normal development compared to these mutants. Understanding the mechanism by which glia influence synaptic bouton formation could ultimately instruct us on potential

methods for disease amelioration in humans.

454A

Identification of protective prion protein residues with flies: insights into the dog PrP-N158D substitution. Diego E. Rincon Limas[1], Jonatan Sanchez-Garcia[1], Yan Zhang[1], Joaquin Castilla[2], Pedro Fernandez-Funez[1]. 1) Dept Neurology, University of Florida, Gainesville, FL; 2) CIC bioGUNE, Bizkaia, Spain.

The central event in the pathogenesis of all forms of prion disease involves a conversion of the host-encoded cellular prion protein PrPc to its pathogenic conformer PrPsc. However, the molecular mechanisms that regulate this conformational conversion are mostly unknown. A clue to understanding the structure and conformational dynamics of PrP has come from the dog, a rare mammal resistant to prion diseases. A comparative study identified a charged amino acid (PrPD158) in dog PrP that is not conserved in other mammals susceptible to prion diseases (PrPN158). Unfortunately, little is known about how this residue affects PrP structure. We hypothesized that altering the charge of the loop connecting Helix 1 and the first beta-sheet could affect the stability of the globular domain and, thus, the toxicity of PrP. To determine the stabilizing effect of Asp158, we compared transgenic flies expressing wild type (MoPrP) and mutant (MoPrP-N158D) mouse PrP. We first observed that the MoPrP-N158D protein is more stable than MoPrP since significantly lower levels of mRNA lead to comparable levels of protein, suggesting that MoPrP is actively degraded in flies. We have shown before that MoPrP accumulates disease-specific PrP isoforms by immunoprecipitation with the 15B3 conformational antibody. However, flies expressing MoPrP-N158D do not accumulate 15B3-specifc conformations, indicating its higher conformational stability. Finally, whereas expression of MoPrP in motor neurons induced aggressive locomotor dysfunction in climbing assays, flies expressing MoPrP-N158D were similar to control flies, supporting the lack of toxicity. These results demonstrate that Asp158 exerts a key stabilizing activity on PrP and prevents formation of disease-specific PrP isoforms. Altogether, our data indicate that residue PrPN158 might be a target for anti-prion therapies and that Drosophila is an ideal system to genetically dissect fundamental, unknown aspects of PrP-associated pathology.

455B

The influence of up-regulating basket in a Drosophila model of Machado-Joseph Disease. Catherine Romberger, John Warrick. University of Richmond, Department of Biology, Richmond, VA.

Machado-Joseph Disease/ Spinocerebellar Ataxia 3 (MJD/SCA3) is a dominantly inherited, neurodegenerative disease caused by an expansion of a naturally occurring glutamine repeat in the coding region of the Ataxin-3 (ATX3) protein. The mutant expanded glutamine ATX3 formes aggregates within the nucleus of cells. These aggregates are thought to impede cellular function and lead to toxicity. The basket (bsk) gene is the homologue of the human c-Jun N-terminal kinase (JNK), which is involved in autophagy and, when stimulated by stress, removes old proteins from the cell. Research suggests that JNK has a role in other neurodegenerative diseases including Huntington's disease, which is in the same family of diseases as MJD. We hypothesized that the up-regulation of the bsk pathway may increase the removal of these aggregates, decreasing the severity of neurodegeneration. In order to test this hypothesis, UAS-ATX 3 alleles of mutant and normal MJD as well as UAS-BSK were expressed in the fly eye using a Gal4 driver. In order to determine the level of degeneration, fly heads were fixed and embedded in epon blocks and semi-thin sections of retinas were evaluated using light microscopy. In order to determine the amount of aggregates present, flies were aged and frozen sections were stained with antibodies to ATX3. The sections were viewed using confocal fluorescence microscopy. Our results suggest that the co expression of bsk influences the amount of degeneration of the photoreceptors and the number of aggregates.

456C

Polar substitutions in helix 3 produce toxic, transmembrane isoforms of the Prion protein. Jonatan Sanchez-Garcia[1], Daniela Arbelaez[1], Kurt Jensen[1], Diego Rincon-Limas[1,3], Pedro Fernandez-Funez[1,2,3]. 1) Department of Neurology, Univ of Florida, Gainesville, FL; 2) Department of Neuroscience, Center for Movement Disorders and Neurorestoration, University of Florida, Gainesville, FL 32611, USA; 3) Genetics Institute and Center for Translational Research on Neurodegenerative Diseases, University of Florida, Gainesville, FL 32611, USA.

Prion diseases encompass a diverse group of neurodegenerative conditions characterized by vacuolar degeneration and accumulation of misfolded conformers of the Prion protein (PrP). Although transmission of these disorders are mediated by the protease-resistant scrapie conformation (PrPSc), other PrP isoforms mediate neurodegeneration. To better understand how PrP misfolding leads to neurotoxicity, we introduced polar substitutions in two conserved methionines in helix 3, M205 and M212, in mouse PrP. In vitro studies revealed that these two residues controlled the stability of the globular domain, while oxidation of these Met was proposed to promote PrP conversion in humans and mice. To study the consequence of M205S and M205,212S on PrP biogenesis, folding, and pathogenesis in vivo, we expressed these mutants in Drosophila. We found that, unlike PrP-WT, M205S and M205,212S underwent hyperglycosylation, intracellular accumulation, and widespread conformational changes due to the lack oxidative folding. Surprisingly, PrP-M205S and PrP-M205,212S accumulated as C-terminal transmembrane (Ctm), a topology that had only been described for mutations in the signal peptide and the transmembrane domain and it is linked to prion disease. Finally, PrP-M205,212S not localized in the lipid rafts altering localization of syntaxin and neuroglian in the lipid rafts. These mislocalizations induce abnormal development of axonal projections in the brain and indicate PrP-M205,212S neurodevelopmental toxicity. These results identify the lack of oxidative folding as a key factor in the formation of Ctm PrP, a mechanism that may be relevant in the pathogenesis of several inherited

forms of prion diseases.

457A

Drosophila mth mutant resists paraquat induced Parkinson's like symptoms. Arvind K. Shukla[1*], Prakash Pragya[1], M.Z. Abdin[2], Debapratim Kar Chowdhuri[1]. 1) Embryotoxicology, Indian Institute of Toxicology Research, Lucknow, Uttar Pradesh, India; 2) Department of Biotechnology, Jamia Hamdard, Hamdard Nagar, New Delhi 110 062.

Parkinson's disease (PD) is the second most common neurodegenerative disorder which involves degeneration of dopaminergic neurons. A well-known herbicide, paraquat (PQ), has been considered as one of the important risk factor for PD. Considering the well-characterized association between PQ and PD occurrence, there is a need for amelioration of PQ toxicity against PD. In recent years, the application of G protein coupled receptor (GPCR) in neurodegeneration has been suggested. Earlier, one of the important GPCRs in Drosophila, methuselah (mth), has been shown to provide increased survival to the organism against dietary PQ. Thus, the present study was aimed to examine the role of mth against PQ induced PD like symptoms using Drosophila as a model organism. Significant resistivity of mth mutant (mth1) flies against PQ induced oxidative stress in comparison to its parental control w1118 was observed. Marked deterioration in locomotor performance, degeneration of dopaminergic neurons along with decrease in dopamine (DA) pool was observed in PQ exposed w1118 flies while resistance towards all the above observed parameters was observed in mth1 flies. In contrast, flies that overexpress mth in their dopaminergic neurons when exposed to PQ exhibited more susceptibility to oxidative stress, behavioral deficit as well as increased neuronal death. The study demonstrated that mth mutation benefits the organism from PQ induced PD like symptoms which strengthens the possible role of GPCR in neurodegenerative disorder.

458B

Search for the modifiers of amyloid-β-42 mediated cell death in Drosophila eye. Andrew Steffensmeier[1], Oorvashi Roy Puli[2], Meghana Tare[2], Madhuri Kango-Singh[2], Amit Singh[2]. 1) Pre-Med, University of Dayton. 300 College Park Drive, Dayton, Ohio, 45469; 2) Department of Biology, University of Dayton. 300 College Park Drive, Dayton, Ohio, 45469.

Alzheimer's disease (AD) is an age-related, progressive neurodegenerative disorder. The reason for Alzheimer's neuropathology is the generation of large aggregates of Aβ42 that are toxic in nature, and induce oxidative stress, aberrant signaling and many other cellular alterations that trigger neuronal cell death. However, the exact mechanisms leading to cell death are not clearly understood. We employ a Drosophila eye model of AD to study how Aβ42 causes neurodegeneration. Misexpression of higher levels of Aβ42 in the differentiating photoreceptors of the fly retina rapidly induced aberrant cellular phenotypes and cell death. We looked for the modifiers of neurodegeneration phenotype of Aβ42 n the eye disc as well as the adult eye using a gain-of-function approach. Here we present our findings on the genetic interactions of one of the modifier which can rescue the amyloid plaque mediated cell death in the Drosophila eye.

459C

Identifying the molecular mechanism of nicotine-mediated rescue in a fly model of Parkinson's disease. Jared Techau[1], Gerald B. Call[2], Lori M. Buhlman[1]. 1) Biomedical Sciences, Midwestern University, Glendale, AZ; 2) Dept. of Pharmacology, Midwestern University, Glendale, AZ.

Parkinson's disease (PD) is characterized by the death of dopaminergic neurons in the substantia nigra pars compacta, which leads to motor and non-motor dysfunctions. It exists in both sporadic and familial forms, where prior studies have shown that nicotine exhibits neuroprotective effects in decreasing incidence of sporadic PD and delaying onset of motor symptoms (Quik et al., 2009). We have previously demonstrated that nicotine can rescue a familial model of PD in *Drosophila melanogaster* that have a heterozygous *parkin* loss-of-function mutation through behavioral assays such as climbing, flight, and olfaction as well as in lifespan. We sought to determine whether this protective effect of nicotine was mediated through activation of the Drosophila nicotinic acetylcholine receptor (DnAChR). A nonselective nAChR antagonist, mecamylamine, has been administered to determine if the neuroprotective effects of nicotine are antagonized or diminished in the same behavioral assays in the *park*^{25}heterozygotes. Our initial results indicate that nicotine may be eliciting its protective effects by acting through a DnAChR-independent manner in both climbing and flight assays. This data might indicate that nicotine is producing its protective effect by directly affecting other cellular targets directly, (e.g., mitochondria) in these flies.

460A

Study of the Intracellular Handling of Dopamine using Drosophila. Antonio Tito[1,2,3], Dongsheng Chen[1,3], Zhen Xu[1,3], Yanning Rui[1,3], Zhihua Chen[1,3], Sheng Zhang[1,2,3]. 1) Center of Metabolic and Degenerative Diseases, IMM, Houston, TX; 2) Department of Neurobiology and Anatomy, GSBS, Houston, TX; 3) UT-HEALTH, Houston, TX.

Dopamine (DA) is an important neuromodulator regulating many important behavioral roles in humans such as reward-driven behavior and motor control. Accordingly, both its intracellular packaging and release are tightly regulated in the dopaminergic neurons in the brain. Within the cell, dopamine is stored within acidic vesicles through membrane-bound Vesicular Monoamine Transporters (VMAT). These vesicles release their contents to the synaptic cleft to modulate neuronal response, while the cell surface DA transporters (DAT) re-uptake extracellular DA to terminate its action. Mis-regulation of proper DA storage mechanisms has been suspected to play a role in the selective degeneration of dopaminergic neurons in familial and sporadic cases of Parkinson's disease (PD), which is characterized by the loss of dopaminergic neurons in the substantia nigra, a brain region that controls motor output from the striatum. The cellular machineries that control DA

biogenesis and function are highly conserved in Drosophila. To study the mechanisms regulating in vivo handling of DA and their potential role in PD, we have developed transgenic fly lines that allow targeted overexpression or knockdown of the fly homologues of VMAT and DAT in different fly tissues, including dopaminergic neurons. Our results show that modulating their expression level and pattern leads to a variety of animal phenotypes. Using these reagents, we are testing whether manipulation of intracellular DA handling affects the survival of dopaminergic neurons.

461B

Molecular study of age-related hearing disorders using *Drosophila*. Leo Tsuda, Yasuhiro Omata, Yasutoyo Yamasaki, Young-Mi Lim. Animal Models of Aging, National Center for Geriatrics and Grontology, Aich, Japan.

Cell survival of sensory neurons is essential for the long-term maintenance of sensory functions. Its defect leads to the onset of age-related sensory defects suffered by a great number of aged human populations, such as hearing loss and retinitis pigmentosa. Noise induced hearing loss (NIHL) is thought to be a model system for studying the pathological nature of age-related hearing disorders. Research toward the molecular mechanism of NIHL, however, has been hampered due to the lack of efficient assay systems in model organisms. In this work, we tried to establish a model system to analyze the formation of NIHL using fruit fly and mouse. In the previous study, we have revealed that Ebi, a fly homologue of TBL1, which is involved in age-related hearing disorder in humans, forms a complex with AP-1 and represses expression of the pro-apoptotic genes in photoreceptor cells. In this time we monitored the survival of auditory sensory neurons by physiological methods, and found that Ebi and TBL1 is required for protecting sensory cells from toxic effect induced by sound stimulation. Thus studying ebi and TBL1 in the sensory cells survival might lead to reveal the molecular mechanism of NIHL and age-related hearing disorders.

462C

Vesicular trafficking in the pathogenesis of Parkinson's disease. Katerina Venderova[1], Sarah Wong[1], Jieyun Cao[1], Radek Linhart[1], Melody Tran[1], Casey Ardrey[1], Christine Hsu[1], Anne Huynh[1], Jong Min Park[1], Brian Phi[1], Gina Stassinos[1], Edwin Yadidi[1], Matthew Seaman[2]. 1) University of the Pacific, Stockton, CA; 2) University of Cambridge, Cambridge, UK.

Parkinson's disease (PD) is the most common movement neurodegenerative disorder. Its treatments are purely symptomatic and thus unable to halt or slow down the progression of the neuronal death. This is largely due to an incomplete understanding of molecular pathways involved in the disease process. To address this gap, we have previously generated a Drosophila model of PD that overexpresses the most common causative gene of PD, leucine-rich repeat kinase 2 (LRRK2), and employed this model in a genome-wide modifier screen. Several of the uncovered LRRK2 genetic interactors played a role in vesicular trafficking, and we have selected VPS35 for further studies. VPS35 is a core component of the retromer complex that is essential for sorting and recycling specific cargo proteins from endosomes to the trans-Golgi network. We observed that overexpression of VPS35 significantly ameliorated the mutant hLRRK2 eye phenotype. We next exposed the flies to rotenone - a neurotoxin commonly used in PD research. As we have shown previously, overexpression of mutant hLRRK2 makes flies more sensitive to rotenone, both in terms of lifespan and loss of dopaminergic neurons. Strikingly however, overexpression of VPS35 markedly extended the lifespan of mutant LRRK2-overexpressing flies. Furthermore, VPS35 overexpression significantly protected from the locomotor deficits observed in mutant LRRK2 flies, as assessed by the negative geotaxis assay. This protection was seen throughout the lifespan, and confirmed with two independent VPS35 lines. We are currently processing brains of these flies for staining and quantification of dopaminergic neurons. From our experiments we conclude that LRRK2 regulates the retromer pathway and that this pathway plays a role in PD pathogenesis.

463A

Buildup Arsenal for Functional Study of Huntingtin in Drosophila. Zhen Xu[1], Yanning Rui[1], Zhihua Chen[1], Dongsheng Chen[1], Antonio Tito[1], Yamin Sun[1], Sheng Zhang[1,2,3]. 1) Center for Metabolic & Degenerative Diseases , the Brown Foundation Institute of Molecular Medicine; 2) the Graduate School of Biomedical Sciences (GSBS); 3) Department of Neurobiology and Anatomy, The University of Texas Health Science Center (UTHEALTH) at Houston.

Although Huntingtin gene has been extensive studied since its identification in 1993, with many proposed cellular roles, its normal function remains not well-defined. Characterizing an Htt homolog in Drosophila (dhtt), a simple yet genetically tractable system will complement the established mammalian models for Huntingtin studies. We had established a null-mutant (dhtt-ko) and performed preliminary characterization of its phenotypes. Further, we found that expression of human Htt could rescue the dhtt mutant phenotypes, suggesting the evolutionally conserved Htt functions and also supporting the use of fruit fly to study human Htt. To further take advantage of the abundant tools available in Drosophila for functional investigation of Huntingtin, we decided to develop a set of toolkit that would allow convenient detection and isolation of endogenous dHtt protein. Pacman method, the recently developed genome-tagging technique in Drosophila, allows efficient tagging and transformation of a large gene in its genomic DNA context, with the expression of the tagged gene still under the control of its native regulatory elements, thus ensuing the normal pattern and expression level of the tagged protein. Using this approach, we have successfully generated a set of genome dhtt transgenic lines with different fluorescence and epitope tags that allow convenient tracking and isolation of endogenous dHtt protein. This set of tools will greatly facilitate in vivo analysis and manipulation of this large protein in Drosophila. Establishing dhtt-ko mutant and a collection of genome-tagged dhtt lines enable us to carry out a detailed functional study of dHtt protein, which in turn should provide critical insights into the normal function of Htt and help decipher the mechanisms underlying HD.

464B

Characterizing the interaction of neuron and glia by electroretinogram. Po-An Yeh, Henry Sun. Molecular Biology, Taipei, Taiwan.

Neurodegenerative diseases have been intensively studied. An increasing body of evidence shows that glia played an important role in the progression or propagation of neurodegeneration pathology. Nonetheless, the molecular mechanism of the crosstalk between neuron and glia is still unclear or under debate. Fly retina, a very regular and well organized structure, has been utilized to study neural diseases. In addition, electroretinogram (ERG) can diagnose very subtle deficit before the photoreceptor neurons completely lose their function or die. Taking advantage of this system, we attempted to investigate the interaction between neuron and glia. We found that expression of several polyglutamine-expanded proteins exclusively in glial cell resulted in reverse ERG signal without apparent morphological and anatomical deficit. Conversely, expressing these in retina did not cause acute neuronal deficit, suggesting that glia cells, instead of neurons, are more vulnerable to these polyglutamine-expanded proteins. These data gives a new vista to explore the effect on neuronal function by manipulating the surrounding glia. This tool will facilitate us into underlining the pathological mechanism of human neurodegenerative disorders.

465C

RAF2 promotes the autophagic degradation of the Amyloid-β peptide. Yan Zhang[1], Diego Rincon-Limas[1], Pedro Fernandez-Funez[1,2]. 1) Neurology, University of Florida, Gainesville, FL; 2) Neuroscience, University of Florida, Gainesville, FL.

Alzheimer's disease (AD) is an incurable neurodegenerative disorder clinically characterized by progressive cognitive impairment. Both the Amyloid-β (Aβ) peptide and Tau are key pathological hallmarks of AD, but Aβ42 seems to have a leading role in AD pathogenesis. To gain insight into the cellular mechanisms regulating Aβ42 neurotoxicity, we performed a genetic screen using our fly model expressing human Aβ42. The screen identified RING-associated factor 2 (RAF2) as a potent suppressors of Aβ42 neurotoxicity in the eye. We also found that RAF2 suppresses Aβ42 neurotoxicity in the mushroom bodies, suggesting that RAF2 protective activity is conserved in brain neurons. RAF2 also rescued the eye phenotype of APP; BACE, a more physiological model of AD, indicating that RAF2 targets mature, secreted Aβ42. Interestingly, RAF2 co-expression reduces the levels of Aβ42, suggesting the RAF2 promotes Aβ42 degradation. RAF2 is a new protein with a zinc rfinger in its C-terminus, but its biological function is unknown at this time. To determine the molecular mechanisms mediating RAF2 neuroprotection, we cloned the full-length cDNA of RAF2 and three deletions constructs. Co-expression of these constructs demonstrated that SRS is the only critical domain for Aβ42 neuroprotection. We also learned that both RAF2 and RAF2-ΔSRS inhibit Notch signaling, suggesting that RAF2 interferes with vesicle trafficking. To support this, we found that RAF2 co-localizes with endo-lysosomal vesicles in S2 cells. Finally, overexpression of RAF2 in flies induced the accumulation of autophagosomes. These results led us to hypothesize that RAF2 exerts its protective activity by promoting the degradation of Aβ42 contained in endocytic vesicles. In conclusion, we have identified a new protective mechanism against Aβ42 that implicates the interaction of endocytic trafficking and autophagy. Since these pathways are highly conserved in humans, we propose that promoting RAF2 activity may results in neuroprotection in AD patients.

466A

***Drosophila* as a model to study the genetic mechanisms of parental high-fat diet and its effects on the trans-generational initiation of obesity and heart dysfunction.** Ryan Tyge Birse, Hannah Catan, Kathryn Reardon, Sean Oldham, Rolf Bodmer. Program of Development and Aging, Sanford-Burnham Medical Research Institute, La Jolla, CA.

Given the early onset of the obesity epidemic, it is plausible that the metabolic state of the pregnant mother may contribute to the susceptibility of the offspring to obesity. Studies have shown that the diet of the pregnant mother correlates with disease type and its postnatal appearance. These studies led to a theory of maternal influences on disease causation, which states that the uterine nutritional environment is a critical determinant for disease development in the offspring. Although maternal effects in these cases have been shown, the genetic and mechanistic basis has yet to be elucidated. Therefore, it would be beneficial to study the central aspects of obesity in parallel with the control of heart function, in a simplified system. We have recently established the *Drosophila* as a tool for discovering not only the conserved genetic mechanisms that maintain heart function but also the genetic mechanisms of metabolism, as it relates to heart function. We find that there is a persistent transgenerational effect on lipid metabolism and heart function in offspring from parents on a high fat diet (HFD). We also found that a HFD induces a metabolic shift to glycolysis and lipogenesis. We also show that tissues specific overexpression (OE) of Bmm lipase can protect the progeny from the adverse effects of a maternal HFD. To further investigate Bmm activity I have expressed Bmm in the embryonic, larval and adult fatbody (FB) which induced a postnatal protection for the progeny from parents on a HFD. Finally I investigated the affects of sirtuin (Sir2) OE in the FB and heart since it is an epigenetic regulator known to be involved in metabolism. From these studies I found strikingly similar phenotypes to those of the Bmm OE and we have also found that Sir2 OE caused an increase in Bmm transcript levels. Therefore this study elucidates a potential epigenetic mechanism working through Sir2 and Bmm that regulates the effects from a maternal diet on its progeny.

467B

QTLs associated with female pupal weight on a high fat diet. Kelly Dew-Budd[1], Ronglin Che[2], Alison Motsinger-Reif[2], Laura

Reed[1]. 1) Department of Biological Sciences, University of Alabama, Tuscaloosa, AL; 2) Department of Statistics, North Carolina State University, Raleigh, NC.

In 2011 the CDC found that greater than one third of Americans were obese. To determine the genotype-by-diet interaction that is contributing to these effects, we used *Drosophila melanogaster* to model human obesity caused by a high fat diet. Using previously genotyped recombinant inbred DSPR lines, we tested the effect of a high fat diet on female pupal weight compared to larvae raised on the normal laboratory diet. We were able to find multiple quantitative trait loci that corresponded to the change in weight caused by increased fat intake. These QTLs define genomic regions that will be of high interest in future studies to determine the genetic mechanism of phenotypic changes brought on by diet.

468C

Myosin storage myopathy mutations cause age dependent muscle degeneration and cardiac dysfunction in a Drosophila model. Meera Cozhimuttam Viswanathan[1,2], William Kronert[1], Girish Melkani[1], Anthony Cammarato[2], Sanford Bernstein[1]. 1) Department of Molecular Biology and SDSU heart institute. San Diego State Univ, San Diego, CA; 2) Johns Hopkins School of Medicine, Baltimore, MD.

Myosin storage myopathy (MSM) is a rare congenital disorder caused by mutations in the β-cardiac MHC rod and characterized by subsarcolemmal accumulation of β-cardiac myosin that has a hyaline appearance. These mutations map near to or within the assembly competence domain that is crucial to filament assembly. We hypothesize that mutations change hydropathy or charge of residues in the heptad repeat thus altering interactions necessary for assembly of coiled-coil rod dimers or thick filaments causing aggregation. We have made a Drosophila model for MSM for pursuing mechanistic investigations, which makes it possible to examine interactions between wild-type and mutant full-length myosins, as the majority of mutant alleles are dominant. We introduced the R1845W, L1793P or the E1883K mutation into Drosophila MHC transgene and expressed each in the jump/ indirect flight muscles (IFM). Our studies show a severe reduction in the flight and jump ability of both homozygous and heterozygous transgenic flies with an age-dependent loss of muscle function. Electron and confocal microscopy of the IFM of transgenic lines show myofibrillar disarray with large areas of granular/ filamentous inclusions similar to hyaline bodies found in affected humans. In addition, heterozygotes of at least two mutants show restrictive cardiomyopathy phenotypes with arrhythmia that mirrors cardiomyopathy reported in human subjects. Life spans of the MSM mutants are also reduced compared to transgenic control. We plan to study in vitro filament forming ability of the mutant myosin to determine if defective filament formation or instability of the myosin filaments is the basis of MSM. Our study will be an important step in exploring the mechanistic basis of MSM, and identify potential therapeutic approaches by over-expressing myosin chaperones or autophagic response.

469A

Altering the balance of *prickle* isoforms changes NMJ bouton morphology and predisposes flies to seizures by lowering the seizure threshold. Salleh Ehaideb[1], Katie Cranston[1], Atulya Iyengar[1], Atsushi Ueda[1], Alexander G. Bassuk[2], David Gubb[3], Chun-Fang Wu[1], J. Robert Manak[1,2]. 1) Dept of Biology, Univ of Iowa, Iowa City, IA; 2) Dept of Pediatrics, Univ of Iowa, Iowa City, IA; 3) CIC bioGUNE, Biscay Technology Park, Derio, Spain.

prickle participates in the non-canonical WNT signaling/planar cell polarity (PCP) pathway. We previously reported that fly *prickle* mutants are seizure-prone, and that mutations in Prickle orthologues are associated with seizures in flies, mice and humans. *prickle* encodes two adult isoforms, pk^{pk} and pk^{sple}. Flies heterozygous for pk^{sple} mutations display pronounced seizures even though no planar cell polarity defects are visible, suggesting that the PCP and seizure phenotypes can be genetically separated. Remarkably, the pk^{pk} mutants are actually less seizure-prone than controls, which suggests that pk^{pk} andpk^{sple} act antagonistically. Consistent with this hypothesis, overexpression of the pk^{pk} isoform in brain, motor neurons and muscles (which recapitulates the imbalance of pk^{pk} and pk^{sple} isoforms seen in thepk^{sple} heterozygote) strongly induces fly seizures in an otherwise wild-type fly. Both pk^{sple} and pk^{pk} mutants show an increase in terminal bouton numbers at the larval neuromuscular junction (NMJ), withpk^{sple} mutants showing an increase in large boutons compared to controls, and pk^{pk} mutants showing an increase in small boutons compared to controls. Using the ElectroConvulsive Seizure (ECS) stimulation paradigm, we show that the pk^{sple} flies have a lowered seizure threshold compared to controls, similar to observations made for other seizure-prone mutants in flies and mice. Finally, we show that protein encoded by the pk^{pk} transcript co-localizes with proteins involved in synaptic vesicle fusion.

470B

Metformin reduces seizure-like activity in the Bang-sensitive paralytic mutants *easily-shocked* and *technical knockout*. Daniel R. Kuebler, Bryan Stone. Dept Biology, Franciscan University, Steubenville, OH.

The Bang-sensitive (BS) paralytic mutants are susceptible to seizure-like activity (SLA) following a variety of insults. The SLA that occurs in the BS mutants is characterized by violent uncoordinated contractions of the legs, wings and abdomen that cause the flies to spin and move violently. These mutants have proven to be a valuable model for investigating the etiology of seizure disorders as they have been used to identify genetic and pharmacological suppressors of seizure susceptibility. In addition, previous work with the BS mutants has identified an association between alterations in metabolism and the amount and intensity of SLA. We have found that the drug metformin, which is used to treat type II diabetes, reduces SLA intensity and duration in two of the BS mutants *easily-shocked* (*eas*) and *technical knockout* (*tko*). Metformin is known to decrease oxidative phosphorylation and increase glycolysis in mammalian cells. We have examined its effect on metabolism in these BS mutants

as well as its effect on glycolytic gene expression to see if these correlate with its ability to suppress SLA.

471C

Development and Validation of an Aged Adult onset Alzheimer's Disease model in *Drosophila melanogaster*. Siddhita D Mhatre, Sarah Michelson, Janine Gomes, Daniel Marenda. Department of Biology, Drexel University, Philadelphia, PA.

Late-onset Alzheimer's disease (LOAD) is a progressive neurodegenerative disorder that involves the accumulation of β-amyloid (Aβ) plaques and neurofibrillary tangles in the brains of elderly patients. Those that are afflicted often experience symptoms including memory loss, confusion, and behavioral changes. In an attempt to analyze this disorder, we developed a novel "Aged AD" model using*Drosophila melanogaster*. Through the Gal4-UAS system, we are able to express low levels of human AD genes - amyloid precursor protein (APP) and β-site APP cleaving enzyme (BACE) - specifically in the fly's nervous system. Advantages of our model include the onset of behavioral and neuropathological symptoms later in the fly's lifespan due to gradual accrual of Aβ within the central nervous system (CNS) adding age as the key factor in this model. Our model provides us with a comparable timeline for the disease pathology of LOAD in humans, and it will be an excellent instrument for the rapid testing of small molecules for therapeutic intervention *in vivo*.

472A

Paraquat-induced Oxidative Stress in a Drosophila von Hippel Lindau Mutant. Anna Moyer, Marleshia Hall, Janis O'Donnell. Biological Sciences, University of Alabama, Tuscaloosa, AL.

von Hippel Lindau (VHL) is a human disease, caused by mutations of the VHL tumor suppressor gene, that results in the development of highly vascularized tumors in the central nervous system, retina, and kidney cells. In mammals, VHL is responsible for the degradation of hypoxia inducible factor- 1 (HIF1) during normoxic conditions, a conserved function in Drosophila. Loss of the Drosophila VHL (dVHL) gene in embryos results in alterations in tracheal migratory behavior, a functional counterpart of vascularization in vertebrates. However, little research exists that seeks to determine the consequences of the loss of VHL in adult Drosophila. Our current research focuses on whether this loss results in susceptibility to the herbicide paraquat, which we have found to promote tracheal branch extension in adult brains that is coordinated with a neuroinflammatory response. Data have shown that loss of a single copy of dVHL causes sensitivity to 1-, 3-, and 5 mM paraquat compared to control flies. Further studies have been conducted to determine whether loss of this gene results in the induction of the inflammatory response and what other factors may be contributing to this increased sensitivity.

473B

Modulators for Prominin and EYS function in photoreceptor morphogenesis. Jing Nie, Simpla Mahato, Andrew Zelhof. Biology, Indiana University Bloomington, Bloomington, IN.

To accommodate the phototransduction machinery, photoreceptor cells of invertebrate and vertebrate animals have developed different strategies to expand their apical membrane: the tightly packed microvilli in invertebrate rhabdomeric photoreceptors and the tightly stacked membrane discs in vertebrate ciliated photoreceptor cells. Despite the morphological difference, our work has demonstrated that these two fundamental photoreceptor cell types utilize shared structural molecules, the transmembrane protein Prominin and the extracellular protein EYS, to drive the morphogenesis of their respective phototransduction compartments. Prominin and EYS are critical components in photoreceptor cells in that mutations in either of these genes cause defects in morphogenesis leading to retinal degeneration. Here we will present data from our proteomic and genetic approaches to uncover modulators required for Prominin and EYS function in Drosophila photoreceptor morphogenesis. Our findings provide insights into not only Drosophila eye development but also human retinal diseases.

474C

Drosophila heart as a model to study the genetic basis underlying Ischemia/Reperfusion (I/R)-induced cardiac injury: HIF1α and small HSPs. Sarah Piloto, Rolf Bodmer. Development and Aging, SBMRI, La Jolla, CA.

Ischemic heart attack is one of the leading causes of cardiac dysfunction-related mortality in the United States. An ischemic heart attack occurs when insufficient oxygen is available for normal function leading to cardiomyocyte death. Reperfusion or the return of oxygen to the tissue after an ischemic event adds another insult, further exacerbating cardiac dysfunction. To gain a better understanding of the genetic mechanisms mediating ischemia-reperfusion-induced cardiomyopathy, we use the Drosophila heart to elucidate novel genetic mechanisms involved in the cardiac response to I/R. Genetic mechanisms involved in cardiac development and function, including sarcomeric structure and ion channel physiology, are remarkably conserved between flies and vertebrates, but interestingly, flies are relatively resistant to low oxygen levels. Using extreme hypoxic conditions (1% oxygen for 18 hours), we find that heart function is relatively well preserved when reoxygenated after this hypoxia treatment. Using this protocol, we have identified Hif1α and Hsp23 as mediators of the fly's cardiac response to I/R. Hif1α/Sima is a key regulator of the hypoxia response coordinating autocrine and paracrine signals to compensate for decreased oxygen levels, and Hsp23 is a small molecular weight chaperone that may participate in maintaining cardiomyocyte proteostasis in the protection against I/R-induced injury. sima null mutants do not survive our I/R regimen; however analysis of heart-specific knock-down (KD) or sima heterozygotes reveals that sima is required for maintenance of cardiac contractility and rhythmicity after I/R. Similarly, we find decreased contractility and increased arrhythmias in hearts with reduced Hsp23 levels. Taken together, Drosophila turns out to be well-suited model system to study the genetic mechanisms that underlie

I/R-induced cardiac injury, and Hif1α and Hsp23 are key regulators in the cardiac response to I/R and can potentially serve as a sensitized model system to identify genetic and small molecule modifiers of a cardiac I/R response.

475A

Effects of Freeman Sheldon Syndrome Y583S and R672C Myosin Mutations on Indirect Flight Muscles of Drosophila. Deepti Rao, Anju Melkani, Sanford Bernstein. Department of Biology, San Diego State University, San Diego, CA.

Myosin, the molecular motor, interacts with actin filaments in the presence of ATP to produce muscle contraction. Mutations in the human embryonic myosin heavy chain cause Freeman Sheldon Syndrome (FSS), which is characterized by multiple congenital muscle contractures affecting facial and limb skeletal muscles. Structural analysis of myosin heavy chain reveals that most of the FSS mutations lie near the groove between the ATP binding site and actin binding site. These mutations are predicted to create structural changes in the ATP binding site, disrupting the binding of nucleotide to myosin. We hypothesize that FSS myosin along with ADP remains constantly bound to actin, leading to permanent contractures. Our overall aim is to identify the biochemical, structural and functional defects caused by FSS myosin mutations, using Drosophila melanogaster as the model organism. In vitro mutagenesis was performed to produce two myosin transgenes with the Y583S and R672C mutations. Lines containing transgenes were crossed into the indirect flight and jump muscle endogenous myosin null background to obviate the masking effect of wild-type myosin. Lines with near to wild-type expression of myosin were chosen to perform further studies. The homozygous and heterozygous transgenic flies showed a drastic reduction in their flight and jump ability when compared to controls indicating that sarcomere structure is compromised. Immunofluorescence confocal microscopy of the young homozygotes showed disorganization of myofibrils. Electron microscopy of the indirect flight muscles of young homozygotes showed thickening of Z-discs and diverging myofibrils, which indicates sarcomere disruption. ATPase and in vitro motility assays will help in understanding the effect of the mutations on the rate of ATP hydrolysis during the chemomechanical cycle and the ability of mutant myosin to translocate actin in the presence of ATP respectively. Overall, this model will yield insights into the mechanistic basis of FSS and may allow us to identify therapeutics to ameliorate FSS symptoms.

476B

A genetically tractable model of noxious cold detection in Drosophila larvae. Heather Turner[1], Christian Landry[2], Michael Galko[1]. 1) Biochemistry and Molecular Bio, MD Anderson, Houston, TX; 2) ProDev Engineering, Houston, TX.

An organism's comfort and even its survival depends on the ability to detect and avoid noxious thermal stimuli thus preventing tissue damage. In some disease states, patients cannot perceive or experience painful and maladaptive perception of innocuous cold and heat. Currently, our understanding of the basic biology of noxious cold perception is gravely minimal. Our goal is to determine the genetic basis for noxious cold perception using the genetically tractable Drosophila model. We have developed a novel "cold probe" that allows focal application of a defined noxious cold stimulus (3-15 ºC), and found that Drosophila larvae produce a mutually exclusive set of primary reactive behaviors, distinct from the previously described aversive "corkscrew" behavior seen in response to a high temperature probe. These behaviors include a tail raise (TR) of approximately 45-90°, a combined head and tail raise (HT) of 45-90°, and a full-body scrunching (SC) behavior. Below 12º C, the resulting behaviors occur in approximately 60% of larvae, and are consistent and reproducible. We probed 13 transient receptor potential (TRP) whole animal mutants to determine the possible contributions of each of the TRP channels on producing the cold-specific behaviors. Notably, we found that two mutants previously reported to affect perception of noxious heat, pyrexia and dTRPA1, both show an increase in one of our observed behaviors and a decrease in another. Alternatively, brivido mutants, recently reported to affect ambient cold preference, display exactly the opposite phenotype. Furthermore we found that the peripheral multidendritic (MD) sensory neurons that innervate the Drosophila epidermis play a significant role in producing these behaviors. Surprisingly, however, the class 4 nociceptive MD sensory neurons, which are required for noxious heat and mechanical sensation, are not required for producing cold evoked behaviors. Taken together, our unique tool and assay should allow us to uncover the cellular and molecular/genetic basis of noxious cold perception in Drosophila.

477C

Establishing an in vivo functional analysis system for renal gene discovery in Drosophila pericardial nephrocytes. Fujian Zhang[1], Ying Zhao[1], Zhe Han[1,2]. 1) Department of Internal Medicine, Division of Molecular Medicine and Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI.

The glomerular podocyte plays a central role in the mammalian renal system. Most known renal disease genes are involved in podocytes function, but understanding of the podocyte biology has been hindered by the low accessibility of mammalian nephrons in vivo. The Drosophila nephrocyte shares remarkable similarities to the glomerular podocyte, making it a potential ideal model to study podocyte biology. However, the lack of functional readout for nephrocytes makes it hard to exploit the power of Drosophila genetics. Here, we present a novel functional analysis of nephrocytes and established an in vivo genetic screen system for renal gene discovery. We found that nephrocytes efficiently uptake secreted fluorescent protein. We generated a transgenic line carrying secreted fluorescent protein that is accumulated in nephrocytes, and combined it to a nephrocyte specific driver for targeted gene knockdown to identify genes required for nephrocyte function. To validate this system, we examined the effects of knocking down sns and duf, the Drosophila homologues of nephrin and Neph1, respectively, in pericardial nephrocytes. We found that sns or duf knockdown completely abolished ANF-RFP protein

accumulation in pericardial nephrocytes. Ultra-structure analysis demonstrated that sns is required for nephrocyte diaphragm and lacunar structure formation that are essential for protein uptake. Our preliminary genetic screen also identified Mec2, which encodes the homologue of mammalian Podocin, another slit diaphragm component linked to renal disease. These findings suggested that the functional analysis system we developed has made the Drosophila pericardial nephrocyte an ideal in vivo model to help identify genes involved in podocyte biology and to facilitate the renal disease gene discovery.

478A

Dg-Dys-Syn1 signaling in *Drosophila* regulates stress related miRNA profile. Evgeniia V Edeleva, April K Marrone, Halyna R Shcherbata. MPRG of Gene Expression and Signaling, Max Planck Institute, Goettingen, Germany.

Muscular dystrophies (MDs) are fatal inherited neuromuscular disorders associated with deficiencies in the dystrophin-glycoprotein complex (DGC). Components of the DGC are evolutionary conserved from flies to humans making *Drosophila melanogaster* a good model for better understanding of DGC function and identifying novel mechanisms of its action. Using *D. melanogaster* as a model we previously found that stresses accelerate the onset of MDs and can even induce severe muscle degeneration symptoms in *wt* animals. miRNAs are good candidates to act as stress response factors as they allow for a quick cellular response with no transcriptional reorganization and synthesis of new cellular proteins. We analyzed miRNA profiles in dystrophic and *wt* animals under normal conditions and stress imposed by high temperature. After careful analysis of microarray data we grouped analyzed miRNAs into those linked to dystrophy and/or stress. Stress related miRNAs are of particular importance as they show that the DGC has a more general role in cellular homeostasis regulation compromised under stress. The DGC serves as a scaffold for multiple proteins, including Syn and nNOS. It was already shown in vertebrates that a pathway involving Dys-Syn-nNOS signaling regulates histone modifications modulating transcription of multiple genes (including miRNAs) in response to different conditions. We further showed that similar pathway involving Dg-Dys-Syn1 signaling exists also in flies suggesting that Dg-Dys-Syn1 via specific HDACs regulates expression of miRNAs implemented in stress response.

479B

Tests of Evolutionary Mechanisms for the Maintenance and Origin of Chromosomal Rearrangements in *Drosophila pseudoobscura*. Gwilym D. Haynes[1], Zachary L. Fuller[1], Ian S. Leopold[1], Atousa Janshahil[1], Shannon Duggan[2], Dianhuiz Zhu[2,3], Stephen Richards[2], Stephen W. Schaeffer[1]. 1) Biology, The Pennsylvania State University, University Park, PA; 2) Human Genome Sequencing Center, Baylor College of Medicine, One Baylor Plaza, Houston TX 77030; 3) Chevron, 1500 Louisiana St, Houston, Texas, 77002.

Drosophila pseudoobscura has over 30 different gene arrangements on the third chromosome that were generated by a series of overlapping inversions. Four classes of hypotheses can be used to explain how inversions are established in populations including direct effects of the mutation, indirect effects of recombination suppression, selective sweeps of an adaptive mutation, and genetic drift. Next generation sequences of 50 isochromosomal strains for seven inversion types within *D. pseudoobscura* were used to test these hypotheses about the origin and maintenance of the third chromosome gene arrangements. The third chromosome is segregating for over 1.3 million SNPs. SNP phylogenies from segments across the chromosome are consistent with the cytological phylogeny except for the central region, which departs from the accepted cytological phylogeny because two arrangements clustered with unexpected chromosomes, despite monophyly of the arrangements. Nucleotide and amino acid polymorphism for the 2831 annotated genes on the third chromosome found that 2662 genes had segregating amino acid variation. Premature stop codons or reading frame extending mutations were found to be segregating in 240 genes. Significant linkage disequilibrium of amino acid variation with chromosomal arrangement was found in 89 to 475 genes with amino acid LD being distributed across the chromosome. These results suggest that indirect effects of recombination suppression is the likely mechanism for the establishment and maintenance of the chromosomal polymorphism in *D. pseudoobscura*. We are testing genes in LD for evidence of positive selection with analyses of synonymous and nonsynonymous variation and of extended homozygosity.

480C

Y chromosome variants tip the epigenetic balance. Bernardo Lemos, Alan Branco, John Gibbons, Cristina Valente. Molecular & Integrative Physiological Sciences, Harvard School of Public Health, Boston, MA.

The Drosophila Y chromosome is an unusual molecule. In D. melanogaster the chromosome is ~40 megabases, but harbors only ~14 protein coding genes. We have recently developed intra-specific and inter-specific Y chromosome introgression to uncover Y-linked regulatory variation: the quantitative effects that tracts of Y-linked chromatin exert on gene regulation. Here we combine several published datasets with ongoing experiments addressing allele specific expression analysis to provide new insights into the mechanisms by which Y chromosomes modulate genome-wide expression levels and epigenetic states. Our studies point to mechanisms that include both genetic and epigenetic components.

481A

Molecular Evolutionary Genetics of Mexican Chromosomal Rearrangements in *Drosophila pseudoobscura*. Ian S. Leopold, Stephen W. Schaeffer. Department of Biology, The Pennsylvania State University, University Park, PA.

Drosophila pseudoobscura has a wealth of chromosomal arrangements segregating on the third chromosome in the United States and Mexico. A recent sample of chromosomes collected from San Pablo Etla in Oaxaca, Mexico found the common Tree

Line arrangement and a second common arrangement thought to be Cuenavaca based on cytological analysis. Cuernavaca is derived from the Santa Cruz arrangement by a single inversion step. Cytological analysis of these Cuernavaca and Santa Cruz chromosomes revealed a chromosomal looping pattern that was consistent with two rather than one inversion steps from Santa Cruz. DNA was extracted from two novel Cuernavaca strains and six Tree Line strains of Drosophila pseudoobscura. These DNAs were used to PCR amplify eighteen previously identified genetic markers for the third chromosome. These amplified samples were then purified and sequenced. Phylogenetic analysis was performed on the sequences using MEGA software. The phylogenies show that these Cuernavaca sequences are closely related to the Santa Cruz and Tree Line phylad. These data also served as quality control information for next generation sequence data collected by the Baylor College of Medicine.

482B

Differences in gene expression in *Drosophila* eye imaginal disc underlie morphological diversification. Isabel Almudi[1], Montserrat Torres[2], Saad Arif[1], Maria D. Santos Nunes[1], Maarten Hilbrant[1], Alistair McGregor[1], Nico Posnien[2]. 1) Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom; 2) Georg-August-University Göttingen Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology Department of Developmental Biology Ernst-Caspari-Hause (GZMB) Göttingen, Germany.

Animals exhibit a huge variation in size and form. In the last decade, the genetic basis for the evolution of particular traits have been identified but, nevertheless, our understanding of the evolution of complex morphological features, and how their underlying genetic changes arose and spread in populations is still limited. The compound eye of insects is a paradigmatic example of the tremendous variation exhibited by some of these complex traits: the number of its constituent ommatidia can range from 1 in some worker ants to 30,000 in dragonflies, while ommatidia size can vary from 5 to 50 µm in diameter. We have also found considerable variation in ommatidia size and number within and among species of the *Drosophila melanogaster* subgroup (*D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. sechellia*). For example, *D. mauritiana* has larger eyes than its sibling species, which is mainly due to differences in ommatidia size. By contrast, differences in eye size among populations of *D. simulans* are due mainly to variation in ommatidia number. In order to study the genetic and developmental origin of these differences in eye size and shape, we investigated the developmental stages at which differences in eye size first arise among different strains of *D. mauritiana* and *D. simulans* in concert with gene expression profiling in the eye-antennal imaginal discs using RNA-Seq. By comparing our RNA-Seq datasets from these different strains at different developmental points, we have identified differentially expressed genes that lie in QTL for differences in eye size, and therefore, that could be responsible for the variation in ommatidia size and number.

483C

Genetic and selective responses to artificial selection on wing shape. Jose D Aponte, Ellen Kosman, Andres Plata Stapper, Zach Boudreau, Mollie Taylor, Lisa Hollensead, Karalyn Aronow, Don Levitan, David Houle. Biological Science, Florida State university, Tallahassee, FL.

To assess if available standing genetic variation could generate novel varieties of wing shape, we selected in 2 directions—expansion and contraction (referred to as “up” and “down”, respectively) of the 2nd and 5th longitudinal veins in a lab-maintained *Drosophila melanogaster* population. In just 16 generations, we were able to select out of the range of variation present in the subgenus *Sophophora* entirely in the “up” direction and partially in the “down” direction. Interestingly, the intersections of the 3rd and 4th vein with the margin of the wing were also altered outside of the range of the subgenus despite not having been direct targets of selection. We then asked if the constraint in shape variation found in *Sophophora* is due to a selective pressure related to flight or mate preference. We performed a mark-release-recapture experiment to assay flight differences among the selected populations. We also performed a mate choice assay to detect differential mating preferences among the selected populations. We discovered that, while there is no difference in flight performance between selected and control flies, females tend to mate with unselected, wild-type males. Finally, we measured the magnitude of genetic response attributable to the phenotypic response seen in the selected populations. As the placement of the 2nd and 5th longitudinal veins is controlled by Decapentaplegic signalling, we measured expression of several downstream components of the core pathway and found no significant differences among selection treatments. Taken together, we conclude that sexual selection maintains wing shape variation and that 2nd and 5th longitudinal vein placement is likely influenced by factors outside of the canonical pathway.

484A

Intragenic epistasis underlying climatic adaptation in natural *Drosophila* populations. Emily L Behrman[1], Alan O Bergland[2], Dmitri Petrov[2], Paul S Schmidt[1]. 1) Biology, University of Pennsylvania, Philadelphia, PA; 2) Biology, Stanford University, Stanford, CA.

Epistatic variance for fitness-related traits can fundamentally affect the efficacy of natural selection and adaptation in the wild; however, the extent of non-additivity among alleles in natural populations is generally unknown. Previous mapping in *Drosophila melanogaster* has identified the couch potato gene (*cpo*) is involved with climatic adaptation by controlling the propensity of a reproductive diapause associated with temperate climate overwintering. A screen of *Drosophila* wild populations sampled across spatial and temporal scales identified three novel SNPs in *cpo* that vary predictably with climate in independent clines; however, certain multi-SNP combinations vary non-randomly with geographic location. This suggests that

epistatic interactions among independent SNPs in *cpo* influence organismal performance and fitness-related traits that underlie adaptation to climate. Here, this hypothesis is tested using DGRP inbred lines to create populations that are constant for each of the three-SNP allelic combinations. The performance and fitness of these multi-locus alleles is assessed using a comprehensive phenotypic screen, which shows significant variation for all traits investigated and pervasive epistasis among SNPs for these traits. There are emergent properties associated with combinations of SNPs; the northern allelic combination, as defined by the three SNPs whose allele frequency increases with latitude, is characterized by non-additive increases in traits associated with higher fitness in northern environments (e.g., cold tolerance). In contrast, the southern three SNP allelic combination exhibits non-additive increases in traits associated with southern environments (e.g., fecundity). The changes in life history phenotypes correspond with differential transcriptional profiles among the allelic combinations. This demonstrates that emergent properties of SNP combinations underlie adaptive life history evolution in natural populations of *Drosophila*.

485B

Evidence of Blastoderm Dpp Gradient Conservation in Drosophila. Juan S. Chahda, Priscilla Ambrosi, Claudia M. Mizutani. Biology, Case Western Reserve University, Cleveland, OH.

The dorso-ventral axis of the Drosophila blastoderm is partly patterned by Decapentaplegic (Dpp)/BMP-4. High levels of Dpp in the dorsal ectoderm activate genes that specify the amnioserosa and dorsal epidermis, and lower levels of Dpp present in lateral regions of the embryo contribute to neuroectoderm specification via gene repression. The scaling properties of this morphogen have not been fully investigated across species. Here, we quantified the blastoderm expression of dpp and its target genes - rhomboid and race - in multiple Drosophila species and found that, between D. melanogaster and D. sechellia, the expression domain of rhomboid and race is relatively constant, compared to significant differences observed in the dpp expression domain. In D. simulans, we detected similar expression of rhomboid, but did not detect the expression of race within the embryo trunk, even though race is expressed in D. sechellia, a recently diverged sister species. We also show that the expression domain of dpp tends to scale with embryo width across evolutionary time. In order to test the dynamics of the Dpp gradient, we used mutants to expand zygotic dpp expression, and thus Dpp activity, and reduced peak Dpp activity by disrupting its extracellular regulation using multiple mutant vkg alleles. Vkg is a type IV collagen that extracellularly binds Dpp and its signaling complex. We have evidence that the Dpp gradient may not scale when augmented or reduced within the blastoderm, indicating the absence of a blastoderm Dpp “expander” that allows the gradient to scale in the growing wing disc. We are currently investigating if vkg embryos also exhibit increased Dpp activity in lateral regions of the embryo, which would result in altered gene expression profiles in the neuroectoderm. These results will help us understand the mechanisms of Dpp gradient conservation during evolution.

486C

Investigating the Mechanism of Sex Determination in Branchinecta lindahli. Michael J. Colgan[1], Janice Krumm[1], Alexis Nagengast[2]. 1) Department of Biology, Widener University, Chester, PA; 2) Department of Biochemistry and Chemistry, Widener University, Chester, PA.

Establishing the sex of an organism requires the coordination of complex gene pathways via transcriptional and posttranscriptional regulatory mechanisms. *Branchinecta lindahli* is a freshwater crustacean of the order Anostraca, and members of this order are important biomonitors for their ecosystems as well as a common food stock in the fish industry. A better understanding of their biology has economic and conservation benefits. The genetic sex determination mechanism of *B. lindahli* is currently unknown. PCR is being performed on *B. lindahli* cDNA to detect the sex determination gene: doublesex. *doublesex*has been identified in the closely-related crustacean *Daphnia magna* (Kato et al. 2011 *PLoS Genetics* 7(3)). Because *doublesex* is conserved in the sex determination pathway of *D. magna* and other related arthropod species, the presence of a *doublesex*-like gene is also expected in *B. lindahli*. To identify other genes that influence sex determination, we will hybridize *B. lindahli* cDNA to *Drosophila*microarray chips to identify the conservation of genes used in the sex determination pathways between these species. Future work will include sequencing putative *doublesex*-like genes and determining their activity in *B. lindahli* sex determination.

487A

Evolution of a novel wing pigmentation pattern in Drosophila : when engrailed crosses the line. Héloïse D. Dufour, Cédric Finet, Shigeyuki Koshikawa, Jane E. Selegue, Sean B. Carroll. HHMI/UW Madison, Madison, WI.

Color patterns in animal play crucial ecological, physiological and behavioral roles. However, the molecular mechanisms underlying the evolution of complex color patterns are still largely unknown. Here, we investigate the generation and evolution of a complex white and black spot pattern in the wing of the fruit fly Samoaia leonensis. We show that the white spots correlate with engrailed pupal expression. This is an unexpected result as engrailed, which encodes a homeodomain transcription factor, plays a crucial role in the establishment of the posterior identity of Arthropod segments, where it is expressed. This role and expression pattern has been highly constrained for the last 500 million years. Earlier in development though, in the imaginal disc, the engrailed expression is restrained to its posterior canonical pattern in S. leonensis. This suggests that the engrailed role in establishing the posterior identity is maintained, while the gene is later recruited and redeployed to repress dark pigmentation. Transgenesis was established in this species to test this hypothesis. Furthermore, by collecting closely related species and reconstructing their phylogenetic relationships, we show that the spotty engrailed

pattern likely evolved on a black wing background, thereby providing a visible phenotype for selection to act on. Those results make an example of how, once their role in body plan establishment is accomplished, crucial develomental genes can acquire discrete new functions.

488B

Cis-regulatory divergence at the *Insulin Receptor* locus contributes to evolution of a reproductive morphology between two *Drosophila* species. Delbert A. Green[1], Cassandra G. Exatavour[2]. 1) Molecular and Cellular Biology, Harvard University, Cambridge, MA; 2) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

An open question in evolutionary genetics and evolutionary developmental biology is the precise genetic architecture of morphological change between species. We extend the list of such closely examined traits to *Drosophila* ovariole number, a reproductive morphology with direct impact on fitness. Previously we determined that differences in terminal filament (TF) number, and hence ovariole number, between*D. melanogaster* (Oregon R strain) and *D. sechellia* (Robertson strain) are due to differences in somatic gonad cell precursor establishment and also subsequent cell proliferation rate. Here, we analyze the genetic and molecular basis for ovariole number difference between these two species. Interspecific genetic analysis indicates that the *Drosophila Insulin receptor* (*InR*) promoter contains sequence that controls ovariole number difference between these species. A putative null coding mutation in *D. melanogaster InR* can complement the *D. sechellia InR* locus in hybrids. This suggests a model in which *InR*expression level, versus differential activity from species-specific receptors, is responsible for the difference. Consistent with this hypothesis, *InR* is more highly expressed in whole female larvae of *D. melanogaster* than of *D. sechellia*. Additionally, we find that ovariole number in both species is hypersensitive to poor larval nutrition compared to body size. However, *D. melanogaster* shows a significantly greater reduction in both traits than does *D. sechellia*. Taken together, these results indicate that in *Drosophila*, one mechanism of controlling reproductive capacity is through modulating nutritional sensitivity. We propose that this is achieved through species-specific regulation of *InR* expression via cis-regulatory sequence evolution at the *InR* promoter.

489C

Trans-generational medication in *Drosophila sechellia*. Balint Z. Kacsoh, Zachary R. Lynch, Nathan T. Mortimer, Todd A. Schlenke. Biology, Emory University, Atlanta, GA.

Drosophila species are regularly infected by parasitoid wasps, which lay eggs in the hemocoels of fly larvae. Flies attempt to kill wasp eggs using an immune response termed melanotic encapsulation, whereby hemocytes migrate towards and adhere to the wasp egg, eventually resulting in a blackened capsule. Surprisingly, a sister species of *D. melanogaster*, *D. sechellia*, lacks the ability to melanotically encapsulate any of the 15 wasp species tested. *D. sechellia* has evolved numerous specializations to live on the fruit of *Morinda citrifolia*, which contain high levels of octanoic acid that are toxic to other flies. I hypothesized that *D. sechellia* might use octanoic acid as a medication to prevent or cure wasp infections in the absence of the canonical melanotic encapsulation response. I found that *D. sechellia* adults preferentially lay eggs on oviposition sites with higher levels of octanoic acid in the presence of wasps, and that eggs laid on octanoic acid food have significantly higher eclosion success in the presence of wasps. Adult flies sense the wasps by sight, increase their own octanoic acid resistance after seeing wasps, and remember having seen the wasps for the rest of their lives. *D. sechellia* alter their oviposition behavior in response to multiple larval parasitoid wasp species but not against a pupal parasitoid, and can also distinguish male and female wasps. Altogether, my data demonstrate a novel, trans-generational prophylactic medication behavior that *D. sechellia* use as one of their main defenses against parasitoid wasp infection.

490A

A faster-X effect for gene expression in Drosophila embryos. Alex T. Kalinka[1], Melek A. Kayserili[1], Dave T. Gerrard[2], Pavel Tomancak[1]. 1) Max Planck Institute, Dresden, Germany; 2) University of Manchester, Manchester, UK.

The X chromosome is present as a single copy in the heterogametic sex, and this hemizygosity is expected to drive unusual patterns of evolution on the X relative to the autosomes. Theory suggests that the single copy of the X in males could facilitate faster evolution of the X, although this faster evolution could be either adaptive or non-adaptive. We measured gene expression across the chromosomes in several*Drosophila* species, and also in several inbred strains of *D. melanogaster* for both embryos and adults. We found that gene expression is evolving significantly faster between species in the embryos (an average excess of ~20%), yet harbours significantly less variation within inbred strains (on average ~10% lower). Furthermore, expression divergence of genes on Muller's D element is significantly greater along the branch leading to the obscura sub-group, in which this element segregates as a neo-X chromosome. In adults, the excess of X chromosome divergence is much lower than in the embryos, yet they also harbour significantly lower levels of gene expression variation on the X in inbred strains. The X chromosome also appears to fix mutations at a lower rate in mutation accumulation lines, suggesting that random genetic drift is not acting more strongly on the X. Overall, our results are consistent with there being an excess of adaptive evolution on the X chromosome in *Drosophila* embryos, and highlight the importance of biological context for understanding how chromosomes evolve in different species.

491B

Effects of extreme temperatures on embryonic development in *Drosophila* species from different climates. Steven G. Kuntz[1], Michael B. Eisen[1,2]. 1) Department of Molecular and Cell Biology, University of California, Berkeley, CA; 2) Howard

Hughes Medical Institute, University of California, Berkeley, CA.

Drosophila is a globally distributed genus with species living in most tropical, temperate, and subtropical climates. Although species have evolved myriad phenotypic differences, affecting pigmentation, behavior, and metabolism, their morphology is highly conserved, making the genus attractive for studying the genetic control of development. However, while conducting genome-wide analyses in embryos of diverse *Drosophila* species, we encountered complications arising directly from their distinct ecological niches. Most significant was the different temperatures at which each species prefers to develop and live, forcing us to compare embryos at either disparate or suboptimal temperatures. With little literature on how temperature differentially affects development in *Drosophila* species, we recorded time-lapse images spanning the entirety of embryogenesis of 12 geographically diverse species at precisely controlled temperatures (15°C to 32.5°C) and used a combination of manual and automatic curation of the resulting movies to measure when 34 developmental landmarks were reached in embryos for each species at every temperature. Tropical species from different clades exhibit similar, though not identical, temperature-dependent developmental timecourses, but two groups of temperate flies reveal unique responses to temperature extremes. *D. virilis* and *D. mojavensis* exaggerate their growth slowing when cold, while *D. pseudoobscura* and its close relatives arrest development from heat shock at temperatures up to 5°C colder than tropical species. To investigate the effect of these developmental differences on gene expression, we have sequenced mRNA from single embryos of 5 species sampled at precise developmental landmarks at different temperatures and have identified genes relevant to environmental adaptation and genomic experiment design and analysis. Our characterization of environmental species collections will be a broadly useful *Drosophila* community resource.

492C

Divergence of the *yellow trans*-regulatory network plays a significant role in pigmentation diversity between species. Richard W. Lusk, Cassandra D. Kirkland, Gizem Kalay, Patricia J. Wittkopp. University of Michigan, Ann Arbor, MI.

Genes are expressed according to the interaction between their *cis*-regulatory sequences and the *trans*-regulatory network that interprets them. While there are now several examples linking *cis*-regulatory changes to morphological variation, we know much less about the contributions made by variation in the *trans*-regulatory network. In this work, we use the *yellow* pigmentation gene to investigate this variation on a large scale. *yellow* is required for the production of black pigment, and the expression pattern of *yellow* in pupae prefigures the pigmentation pattern of adults. Here, we examine the activity of twelve *yellow* regulatory regions, taken from six species, in transgenic *D. melanogaster* and *D. virilis* hosts, allowing us to separate the contributions to pigmentation diversity made by variation acting in *cis*and in *trans* to this gene. We find that *D. virilis*, which is largely unpatterned, nevertheless maintains a complex *trans* regulatory network upstream of *yellow* which is capable of specifying complex patterns of expression. Moreover, although the two species' networks share this complexity, they otherwise appeared to have diverged, with the two hosts specifying sometimes strikingly different expression patterns from identical *cis*-regulatory sequences. We use these differences to outline where changes in *cis* and *trans* have generated pigmentation diversity and where different sets of regulators appear to underlie similar pigmentation phenotypes, highlighting how divergence of the *trans*-regulatory network can shape the evolution of phenotypic diversity between species.

493A

A dictionary of genetic effects as a unified representation of the genotype-phenotype map. Eladio J Márquez[1], Rosa Moscarella[1], David Aponte[1], Washington Mio[2], David Houle[1]. 1) Dept of Biological Science, Florida State University, Tallahassee, FL; 2) Dept of Mathematics, Florida State University, Tallahassee, FL.

We are building a “dictionary” of genetic effects by systematically manipulating gene expression at target genes, and observing their phenotypic effects. Our model phenotype is the size and shape of the Drosophila wing. We characterize phenotypic responses as the entire multivariate set of responses across the wing, rather than specifying a small number of traits a priori. We manipulate gene expression quantitatively using the mifepristone GeneSwitch system coupled with RNAi-induced transcriptional changes, allowing us to model phenotypic responses as a function of gene expression level. These data probe the genotype-phenotype map, directly providing information about features of the map such as non-linearity of effects and robustness to developmental perturbation, as a function of the role played by a gene in the developmental circuitry. Our approach ensures that the effects inferred for all genes remain comparable, by describing the “terms” of the dictionary as a difference vector of localized changes relative to a reference wing shape. These vectors provide a common “language” that facilitates their implementation in any study quantifying the same features, thus providing a powerful tool to link patterns of variation, irrespective of its nature, with putative causal factors whether they are genetic or developmental. We demonstrate practical uses of the Dictionary in a genomic-wide association study, a comparative analysis of interspecies divergence, and an analysis of ecological variation. These examples demonstrate how a catalog of known cause-effect functions can shed light on the direct causation of large-scale phenomena as long as a common, phenomic language is adopted to ensure wide comparability.

494B

Convergent evolution of hybrid inviability in Drosophila. Daniel R. Matute[1], Jackie Gavin-Smyth[2]. 1) Human Genetics, Univ Chicago, Chicago, IL; 2) Ecology and Evolution, Univ Chicago, Chicago, IL.

Postzygotic isolation causes reduced gene flow between species after the zygote gets formed, for instance by causing hybrid

inviability or sterility. Dissecting the genetic basis of hybrid inviability not only reveals the role of molecular evolution in keeping species distinct, but also sheds light on coevolution required for genes to interact normally in pure species. Using high-resolution mapping we found that dorso-ventral specification is prone to breakage in two different hybrids (D. melanogaster/D. santomea and D. melanogaster/D. sechellia) and that two specific genes (dl and fog) lead to hybrid inviability in these two interspecific hybrids. These results demonstrate that the independent evolution of a developmental trait in two lineages involved changes to the same molecular mechanisms and that postzygotic isolation can have the same genetic basis in different interspecific hybrids.

495C

Genetic analysis of differences in eye and face morphology between *Drosophila simulans* and *Drosophila mauritiana*. Alistair P. McGregor[1], Saad Arif[1], Maarten Hilbrant[1], Corinna Hopfen[2,3], Isabel Almudi[1], Maria D. S. Nunes[1], Nico Posnien[1,4], Linta Kuncheria[1], Kentaro Tanaka[5], Philipp Mitteroecker[6], Christian Schötterer[2]. 1) Oxford Brookes University, Oxford, United Kingdom; 2) Institute for Population Genetics, Vetmeduni Vienna, Vienna, Austria; 3) Max Planck Institute for Biology of Ageing, Cologne, Germany; 4) Department of Developmental Biology, Georg August University, Göttingen; 5) Department of Population Genetics, National Institute of Genetics, Mishima, Shizuoka, Japan; 6) Department of Theoretical Biology, University of Vienna, Vienna, Austria.

D. melanogaster subgroup species exhibit considerable variation in head and eye morphology. While all of these species exhibit a negative correlation between eye and face size, *D. mauritiana* generally has bigger eyes composed of larger ommatidia and conversely a narrower face than its sibling species. To better understand the evolution of these differences, we carried out QTL mapping of eye size and face width differences between *D. mauritiana* and *D. simulans*. We found that the major loci responsible for the differences in eye and face size between these species map to the chromosome X and 3L respectively, and to distinct regions of chromosome 2. We confirmed this finding by independently introgressing regions of chromosome X and 3L from *D. mauritiana* into *D. simulans*, which resulted in flies with larger eyes but no significant difference in face width for the X chromosome region and vice versa for the region on chromosome 3L. Fine mapping of these regions identified a number of candidate genes for these differences in eye size and face width. We also found that the difference in face width is detectable earlier in the development than the difference in the size of the retinal field. Our results suggest that different loci that act at different developmental stages underlie changes in eye and face width. Therefore, while there is a negative correlation between these traits in *Drosophila*, we show genetically that they also have the potential to evolve independently.

496A

Diversity and dynamics of chorion patterning across *Drosophila* species. Matthew G. Niepielko, Robert A. Marmion, Kenneth Kim, David Luor, Chelsea E. Ray, Nir Yakoby. Center for Computational and Integrative Biology, Rutgers University, Camden, NJ.

Drosophila oogenesis is an established model system for studying patterning dynamics and morphogenesis of epithelial cells. During oogenesis, a mono layer of follicle cells overlying the developing oocyte is guided by multiple cell signaling pathways to fold into the 3D *Drosophila* eggshell. Eggshell morphologies are highly diverse among *Drosophila* species, and we hypothesized that changes in gene patterning should reflect this diversity. Here, we focus on one of the major family of genes that pattern the *Drosophila* eggshell, the chorion proteins. Using *in situ* hybridization, we screened for the patterns of all nine chorion protein genes in three species (*D. melanogaster*, *D. nebulosa*, and *D. willistoni*). We found that most genes are expressed dynamically during mid and late stages of oogenesis. Applying an annotation matrix code to fully capture the complexity of all gene patterns, we compared the annotation matrices among species. Pattern annotations were sufficient to cluster the species according to their phylogenetic associations. Employing genetic manipulations and drugs, we analyzed the fundamental components of the patterns. Strikingly, these results were correlated with the clustering domains. Specifically, each component of the pattern is regulated jointly or independently by two major signaling pathways; the bone morphogenetic protein (BMP) and the epidermal growth factor receptor (EGFR). Our results provide strong evidence that complex gene patterns are combinatorially assembled from simple patterns.

497B

Evolution of clasper morphology between *Drosophila simulans* and *D. mauritiana*. Maria D.S. Nunes[1], Kentaro Tanaka[1], Corinna Hopfen[2,3], Christian Schlötterer[2], Alistair P. McGregor[1]. 1) BMS, Oxford Brookes University, Oxford, United Kingdom; 2) Institute for Population Genetics, Vetmeduni, Vienna, Austria; 3) Max Planck Institute for Biology of Ageing, Cologne, Germany.

Male sexual characters are often among the first traits to diverge between closely related species. Identifying the genetic basis and evolutionary forces underlying this rapid evolution has great potential to allow us to understand the processes of animal diversification and the evolution of new species. Several traits of the genital arch have evolved in closely related species of *Drosophila*. In this study we focused on the evolution of the clasper, a structure important for correct positioning and attachment of the male to the female during mating. The size, shape and bristle number of this structure have evolved dramatically between *D. mauritiana* and *D. simulans* and it is likely to have affected their mating behavior. In order to map the genetic basis of these differences we generated a QTL map for clasper area and bristle number. We found two major QTL for clasper area, one on the right arm of the 2nd chromosome and another on the right arm of the 3rd chromosome, while clasper

bristle number mapped to the X chromosome. Using marker-assisted recombination mapping, we have introgressed each of those QTL regions from *D. mauritiana* into *D. simulans* and used available gene expression data on male genital discs to identify candidate genes that cause these evolutionary changes . As well as studying how these genes cause variation in clasper morphology, since the clasper develops from the same tissue as other divergent terminalia structures, we are currently testing whether our candidate genes have pleitropic effects on anal plate and posterior lobe morphology. These results are important not only for understanding the genetic basis of genitalia evolution but also because they provide a platform for further research to test for differences in copulation behavior and isolation mechanisms between species.

498C

Septin evolution following gene duplication. Ryan S. O'Neill, Denise V. Clark. Biology, University of New Brunswick, Fredericton, New Brunswick, Canada.

Septins are cytoskeletal components that assemble into oligomeric complexes and polymers, associate with cell membranes, actin filaments and microtubules, and can act as a scaffold for recruiting proteins and preventing diffusion of membrane bound proteins. In *Drosophila*, septins are involved in many biological processes, including cellularization during embryogenesis. *Drosophila melanogaster* has 5 septins:*Sep1*, *Sep2*, *pnut*, *Sep4*, and *Sep5*. Based on phylogenetic analysis, *Sep1*, *pnut*, and *Sep4* are group 2B septins, whereas *Sep2* and *Sep5* are group 1B septins. *Sep5* arose via retrotransposition of *Sep2*, between 62.2 and 62.9 Mya, and is not found outside of the subgenus *Sophophora*. Our work aims to assess functional redundancy and diversification of *Sep2* and *Sep5*. Since retrotransposition does not duplicate transcriptional regulatory elements, we investigated the evolution of *Sep2* and *Sep5* expression patterns across the sequenced *Drosophila* species using *in situ* hybridization. *Sep2* is expressed ubiquitously during embryogenesis; this pattern is conserved across species, regardless of the presence of *Sep5*, suggesting that *Sep2* has maintained its ancestral function. The expression pattern of *Sep5* has diversified across species, possibly indicating prolonged functional diversification. However, *Sep5* is always co-expressed with ubiquitous *Sep2*, and both *Sep2* and *Sep5* interact with *Sep1* and *pnut*, so it is not clear if these paralogs have different functions. Selection analyses of coding regions do not reveal clear patterns of positive selection acting specifically on either *Sep2* or *Sep5*; most codons appear to be under pervasive purifying selection. However, multiple sequence alignment of *Sep2* and *Sep5* reveals that ~14% of amino acid positions were substituted early in the evolution of *Sep5*, and these amino acid differences between *Sep2* and *Sep5* are highly conserved; these differences include amino acids in the G1 and G3 GTPase domains, the Sep1 motif, and the N-terminal coiled coil domain.

499A

A role for male genitalia in mate recognition: Aedeagus shape evolution results in pseudocopulation in the *Drosophila mojavensis* species cluster. Maxi Polihronakis Richmond, Therese Markow. Cell and Developmental Biology, University of California, San Diego, La Jolla, CA.

The primary role of the aedeagus during copulation is to transmit sperm to the female. However, due to the vast morphological diversity of these structures, especially among arthropods, the aedeagus also has been hypothesized to play a role in mate recognition through mechanical and/or sensory mechanisms. In a previous analysis quantifying patterns of aedeagus variation in the *Drosophila mojavensis* species cluster, we found evidence that this structure is involved in mate recognition due to a pattern consistent with oscillating bouts of stabilizing selection between divergence events in combination with directional selection occurring at the time of divergence. In order to test this prediction from a mechanistic perspective, we conducted reciprocal mating experiments between all taxa in the *D. mojavensis* species cluster and measured the degree of pseudocopulation, or the ability of males to achieve the appropriate copulatory position after females agreed to mate. We also recorded time to each copulatory attempt, copulation duration, and resulting progeny. The results of the pseudocopulation experiment revealed varying degrees of post-copulatory isolation among *D. mojavensis* cluster taxa. Copulatory attempts between the sister species *D. arizonae* and *D. mojavensis* resulted in the highest frequency of pseudocopulation and often ended with the male getting stuck such that neither party could terminate copulation. The degree of pseudocopulation between the *D. mojavensis* subspecies was variable and dependent on the population of origin of both the male and the female. These results support a mate recognition role for the aedeagus. Whether this role is sensory and/or mechanical is discussed in light of the known shape differences among the taxa studied, and whether certain shape combinations are more likely to result in failed copulation attempts.

500B

Measuring the effects and rates of microsatellite instability in the morphogen concentration-sensitive enhancers of *Drosophila*. Clinton Rice, Albert J. Erives. Department of Biology, University of Iowa, Iowa City, IA.

Developmental enhancers are important sites of functional evolution in animal genomes. The neurogenic ectoderm enhancers (NEEs) located at *vnd*, *brk*, *vn*, and *rho* drive expression of key regulators of embryonic development in response to maternal Dorsal, which acts as a morphogen to pattern the dorsal/ventral axis. Different NEEs are able to activate their target genes over different ranges of the Dorsal nuclear gradient. We have found that these enhancers are canonical for the *Drosophila* genus, and that the NEE at *vnd* is also present in mosquitos. Among the functional transcription factor binding sites that comprise NEEs are motif-invariant Dorsal and Twist binding sites, separated by an 8-18 bp spacer. Changes in the length of this spacer alter the concentration-response threshold, leading to wider or narrower expression stripe widths for reporter genes or correct expression stripe widths in embryos of different sizes. Spacer evolution apparently occurs via

expansion and contraction of CA-microsatellite tracts within and around Twist binding sites. The four canonical NEEs are enriched in similar microsatellites that are presumed to be relic Twist binding sites and spacers. Here, we are using these microsatellite sites to measure both the deleterious effects and relative rates of mutations within the NEEs. Using multiplexed genotyping, we are assaying for length variants and sequencing to determine whether microsatellite expansion or contraction has occurred. To determine the level of selection acting at these loci, we are comparing the rates of microsatellite length variation between adults (i.e. post-selection) and failed embryos, as well as between microsatellite repeats inside and outside of the functional spacer region. We are also using DNA repair mutant *spellchecker1* (*spel1*) to exacerbate microsatellite instability and improve our ability to measure relative rates of change.

501C

The evolution and development of limb regeneration: a perspective from studies on the red flour beetle, *Tribolium castaneum*. Yuichiro Suzuki. Department of Biological Sciences, Wellesley College, Wellesley, MA.

Many, if not all, metazoans have the ability to regenerate parts of their bodies. Despite the independent evolution of appendages in the protostome and deuterostome lineages, many species in both of these groups are capable of regenerating. To compare the mechanisms underlying limb regeneration across metazoans, the genetic regulation underlying larval leg regeneration was investigated in the red flour beetle,*Tribolium castaneum*. Knockdowns of several key factors known to be involved in the early stages of vertebrate limb regeneration also affected wound healing and blastema growth in *Tribolium*. In contrast, the re-patterning of limbs was found to involve a reversion to the embryonic mode of appendage patterning. Because vertebrate and insect limbs are patterned differently during embryogenesis, the mechanisms underlying re-patterning differ between vertebrates and insects. These findings suggest that studies on the earliest stages of regeneration may shed light on the cellular mechanisms common to regeneration across all metazoans.

502A

Chill coma recovery analysis a major climatic adaptation tool among drosophila species. Pankaj K. Tyagi[1], Shruti Tyagi[1], Sudhir Singh[2]. 1) Dept Biotechnology, Meerut Institute of Engineering and Technology, Meerut, Uttar Pradesh, India; 2) Department of Biotechnology, NIMS University, Jaipur Rajesthan INDIA.

We examine in six drosophila species (D. birempes, D. takahshi, D. kikkawai, D. melenogatesr, D. ananassae and D. jambulina) for the short and longer-term effects of three different conditions recovery temperatures in degree centigrade, cold duration in hrs and age in days on measures of cold resistance, particularly chill coma recovery. Data of recovery temperature and on recovery time showing a negative relation (non linear pattern with a plateau above 25°C). Duration of cold stress and on recovery time, age and on recovery time both are showing a positive relation (looks like a linear pattern). The most common in all three conditions the D. takahshi is the coldest tolerant and D. ananassae the less cold tolerant species. In comparison of sex differentiation in chill coma recovery time was consistently larger for males than for females in all three conditions. Although theses relationships are well-known and previously published, but we have the opportunity to discuss them according to the different species used, according to their geographical distributions, origins and this pattern is assumed to reflect differences in their thermal adaptation, especially in their cold tolerance species as well as the less cold tolerant species in a global collection of six drosophila species.

503B

Regulation of wingless by Abd-B and Doublesex and the evolution of male abdominal segment reduction in Drosophila. Wei Wang, John Yoder. Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL.

Male-specific reduction in adult abdominal segment number is a trait shared by all Cyclorrhaphan diptera. As such this is as an attractive model for investigating developmental and evolutionary mechanisms underlying morphological innovation. Drosophilid females possess 7 abdominal segments, while the terminal segment in males (A7) is significantly reduced during pupation. A major mechanism promoting male A7 reduction is sex- and segment specific repression of the morphogen encoding wingless (wg) gene by the Hox protein Abdominal-B (Abd-B) and the sex-determination transcription factor Doublesex (Dsx). To investigate whether Abd-B and Dsx directly regulate wg expression we have performed a systematic molecular screen to identify cis-regulatory elements (CREs) governing wg expression in the Drosophila melanogaster pupal abdomen.Two distinct abdominal CREs were identified. One CRE promotes abdominal-specific expression (wg1) while the second CRE(wg2) drives reporter expression in additional imaginal tissue including the genital disc. Both CREs contain multiple putative Abd-B binding sites as well as Dsx consensus sites.While these potential regulatory sites are largely conserved in wg2, Dsx binding sites in wg1 have been lost within several lineages of the Drosophila genus group. Interestingly, these losses correlate with modified male A7 morphology suggesting that evolutionary alteration to wg regulation promoted partial restoration of male A7 in some species.We will present comparative functional analyses of these CREs investigating 1) their direct regulation by Abd-B and Dsx and 2) their relative contributions to wg expression in these diverse lineages. This study will provide a critical genetic context in which to explore the role of wg regulation in the evolution of segment reduction as well as provide insight into constraints acting on, as well as the degree of evolutionary flexibility within, a deeply fixed trait.

504C

Regulation of Diverse Modes of Segmentation in *Coleoptera* (Beetles). Jie Xiang[1], Alison Heffer[1], Leslie Pick[1,2]. 1) Program in Molecular & Cell Biology, University of Maryland, College Park, MD; 2) Department of Entomology, University of Maryland,

College Park, MD.

A hierarchy of genes regulating segmentation in *Drosophila melanogaster* has been well-characterized. *Drosophila* is a highly specialized long-germ insect with all segments specified simultaneously at the blastoderm stage prior to gastrulation. However, in short- and intermediate-germ insects, only anterior segments are formed before gastrulation; additional segments are added sequentially as the embryo grows. Expression data and functional studies suggest that orthologs of some *Drosophila* gap and pair-rule genes function differently in other arthropods. To understand how patterning mechanisms evolved, we are examining *ftz* and *ftz-f1* sequence, expression and function in short, intermediate- and long-germ beetles, *Tribolium casteneum*, *Dermestes maculatus* and *Callosobruchus maculatus*, respectively. While both *Tc-ftz* and *Tc-ftz-f1* are expressed in pair-rule stripes, *Tc-ftz-f1* is also expressed ubiquitously in pre-blastoderm embryos. Functional studies using RNAi revealed a role for *Tc-ftz-f1* in regulating alternate Engrailed stripes, establishing *ftz-f1* as a pair-rule gene in *Tribolium*. In addition, *Tc-ftz-f1* is required at later stages of embryogenesis for cuticle development. Preliminary *in situ* hybridization in the other beetle species suggests striped patterns of *ftz* and *ftz-f1* expression at early stages of development. RT-PCR result suggests *Dmac-ftz-f1* is maternally deposited, similar to *Tc-ftz-f1*. Expression patterns of *ftz* and *ftz-f1* in these two species will be further examined and interaction of their protein products will be tested. Next, RNAi will be performed to study functions of *ftz* and *ftz-f1* in these beetles. These studies will shed light on the evolution of the genetic basis underlying segmentation across arthropod taxa.

505A

Inbreeding reveals mode of past selection: stabilizing selection for sperm length but directional selection for sperm competition success and male attractiveness in Drosophila melanogaster. Outi Ala-Honkola[1,2], David Hosken[3], Mollie Manier[2], Stefan Lüpold[2], Elizabeth Droge-Young[2], Kirstin Berben[2], William Collins[2], John Belote[2], Scott Pitnick[2]. 1) Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland; 2) Department of Biology, Syracuse University, Syracuse, NY, USA 13244; 3) Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, UK.

Directional dominance is a prerequisite of inbreeding depression. Directionality arises because selection fixes alleles increasing fitness and eliminates dominant deleterious alleles, whereas deleterious recessive alleles are hidden from selection and hence maintained at low frequencies. Thus, directional dominance should be high for traits under directional selection, but low for traits under stabilizing selection or for traits weakly linked to fitness, and hence such traits are predicted to exhibit little-to-no inbreeding depression. Here, we quantify the extent of inbreeding depression in a range of male reproductive characters and use it to infer the mode of past selection on them. The use of transgenic populations of Drosophila melanogaster with red or green fluorescent-tagged sperm heads permitted in vivo discrimination of competing male sperm and quantification of characteristics of ejaculate composition, performance and fate. We found that male attractiveness (i.e., mating latency) and competitive fertilization success (i.e., P2) both show some inbreeding depression, suggesting they have been directional selection, whereas sperm length showed no inbreeding depression suggesting it has been under stabilizing selection. Despite having measured several sperm quality (sperm viability in female reproductive tract, offspring viability, in vivo sperm swimming speed) and quantity (ejaculate size, the number of sperm in storage) traits, we were unable to discern the mechanism underlying the lowered competitive fertilization success of inbred males.

506B

Opposing fitness effects contribute to maintenance of polymorphism at a QTN in *Aldehyde dehydrogenase*. Mahul Chakraborty, James Fry. Department of Biology, University of Rochester, Rochester, NY.

Resistance to ethanol is a crucial physiological adaptation in Drosophila melanogaster. Quantitative variation in this trait follows a worldwide clinal pattern, wherein flies from temperate populations are more resistant to ethanol than their tropical counterparts. Mitochondrial aldehyde dehydrogenase (DmALDH) contributes to this adaptation by detoxifying acetaldehyde, the breakdown product of ethanol. An *Aldh*replacement SNP changes a highly conserved leucine residue, located close to the predicted active site, to phenylalanine. The *Phe* allele is rare in the tropics but present in most temperate populations, suggesting it may be beneficial for ethanol metabolism. Nonetheless, the frequency of the *Phe* allele is usually no greater than 10-20%, raising the question of why it does not sweep to fixation. *In silico* analysis suggests that the substitution reduces the volume of the active site, resulting in improved fit for acetaldehyde, but poorer fit for larger aldehydes, which are continuously generated as byproducts of normal respiration. This prediction was confirmed by kinetic studies using purified recombinant enzyme: the *Phe* form, compared to the *Leu* form, has a higher turnover rate for acetaldehyde, but lower turnover rate for larger aldehydes. Consistent with the kinetic data, transgenic flies homozygous for the *Phe* allele are more resistant to ethanol than those homozygous for the *Leu* allele. In the absence of ethanol, however,*Phe* flies have markedly lower overall fitness than Leu flies. This difference is likely due in whole or part to lower ability of the *Phe* form to detoxify aldehydes generated by normal mitochondrial oxidative stress, as suggested by lower resistance of *Phe* flies to elevated oxidative stress than *Leu* flies. Thus, the advantage of *Phe* arising from faster ethanol detoxification is undermined by its deleterious effect on an important ancestral function, protection from mitochondrial oxidative stress. Our results give a rare example of an ecologically-relevant fitness trade-off caused by a single SNP.

507C

Sperm utilization and fertility of mitochondrial introgression genotypes in Drosophila. James A. Mossman, David M.

Rand. Ecology and Evolutionary Biology, Brown University, Providence, RI.

We test the hypothesis that mtDNA-Y chromosome interactions affect male fertility. Different mtDNAs have been associated with male infertility in humans and have been linked to variation in OXPHOS activity in sperm (Ruiz-Pesini et al. 2000). Even synonymous mutations in mtDNA have been associated with poor semen quality (Holyoake et al. 2001). Synonymous polymorphism in human and animal mtDNA is extensive and may account for a substantial proportion of variation in male fertility. Mutations in genes affecting spermatogenesis disrupt mitochondrial morphology (Hales and Fuller 1997). Since mitochondria provide the energy to power sperm motility, sperm function provides a stringent testing ground for mitochondrial performance. However, the statistical association between the mtDNA and the Y chromosome in all male offspring of a given mated pair of animals is 100%. Yet there is no means by which a Y and mtDNA can be transmitted to the same offspring (in mammals and insects, barring paternal leakage which is very rare). These rules of transmission dictate that modifier mutations arising in a population that suppress deleterious mtDNA effects cannot be transmitted by males, which may explain the higher incidence of mitochondrial disease in males (Frank and Hurst 1996). The lack of co-transmission between Y and mtDNA means that beneficial interactions are not promoted, and deleterious interactions can accumulate. The Y chromosome also carries important fertility factors in humans (Lahn and Page 1997) and Drosophila (Carvalho et al. 2000). While a single-gene study reported no DNA polymorphism on the Y (Zuroycova and Eanes 1999), a recent survey of other Y-linked genes (Carvalho et al. 2001) have uncovered polymorphism (A. Clark and B. Carvalho, Dros. Res. Conf. 2002). Given the important role that mtDNA and Y chromosomes play in fertility, it is surprising that no experimental manipulation of these markers has been conducted to dissect their relative contribution to animal fertility.

508A

Viability in strains of *Drosophila melanogaster* submitted to artificial selection for wing shape divergence. Libéria Torquato, Blanche Bitner-Mathé. UFRJ, Rio de Janeiro, Brazil.

Many studies suggest that artificial selection and inbreeding can lead to a decrease in viability. To obtain strains of *D. melanogaster* with divergent wing shape, we have performed a program of artificial selection with two biological replicates (named 1 and 5) at 22ºC. For each biological replicate, three strains were established: one with selection for elongated wings (L strain), one with selection for rounded wings (R strain), and a control strain with no selection applied (C strain). In this study, we investigated a possible effect of selection on the viability of each strain, in the 123th generation. Vials with 30 eggs from each strain were reared at 25ºC until adult emergence. The viability was calculated as the percentage of adults that actually emerged. And the effects of selection (L, R or C) and replicates (1 and 5) were tested using ANOVA. If selection has had a significant influence on viability in this selection program, we would expect to observe decreased viability in the selection strains (L and R) from both replicates, so that the interaction between selection and replicate would be non-significant. But this was not the case. We observed a highly significant interaction between these effects, as follows. Replicate 1 presented a significant lower viability in the selected strains (L and R strains) relative to the non-selected control strain (C strain). In contrast, no significant difference in viability was observed across L, R or C strains from replicate 5, this contrasting result indicates that selection might not be the factor affecting viability, which leaves genetic drift as an alternative hypothesis to explain the lower viability observed in replicate 1.

509B

Purging of deleterious mutations through sexual selection: negative evidence. Jing Zhu, James Fry. Biology Dept, University of Rochester, Rochester, NY.

Simple population-genetic models, when parameterized with available estimates of mutation rates, predict that recurrent deleterious mutations should severely depress the reproductive capacity of populations of higher eukaryotes, including *Drosophila*. One way that the mutational genetic load could be mitigated is through sexual selection: if deleterious mutations are eliminated primarily because of their effects on male mating or fertilization success, then little reduction of population mean fitness need occur. The sexual selection hypothesis requires that there be substantial overlap between mutations that reduce female fitness and those that reduce male mating and fertilization success, and that selection against deleterious mutations is on average considerably stronger in males than in females. We tested these assumptions by allowing spontaneous mutations to accumulate in an outbred population in the near-absence of selection, using the “Middle Class Neighborhood” (MCN) design. After more than 70 generations of mutation accumulation, we created lines from this population whose genomes were derived primarily from either males that were consistently successful at obtaining matings in competitive mating trials ("stud" lines), or males that were consistently unsuccessful ("dud" lines). Males from stud lines (descendents of the original males) had substantially higher mating success than males from the dud lines, but females from the stud and dud lines did not differ in reproductive output, giving no evidence that mutations that depressed mating success also depressed female fitness. Moreover, relative to stud and dud lines from a control population in which selection had been allowed to operate, the stud and dud lines from the MCN population showed similar declines in male mating success and female reproductive output, giving no evidence for generally stronger selection in males.

510C

The Drosophila Early Ovarian Transcriptome Provides Insight to the Molecular Causes of Recombination Rate Variation. Andrew Adrian[1,2], Josep Comeron[1,3]. 1) Biology, University of Iowa, Iowa City, IA; 2) Interdisciplinary Graduate Program in Informatics, University of Iowa, IA; 3) Interdisiplinary Program in Genetics, University of Iowa, IA.

Evidence in yeast indicates that elevated expression is correlated with increased levels of double-strand breaks (DSB). Our recent studies of recombination maps across the D.melanogaster genome also indicate an excess of DSBs within annotated transcripts relative to intergenic sequences. As cells that undergo DSB formation (and recombination via DSB-repair) represent only a small fraction of the whole individual or even the gonadal tissue, present transcriptomes poorly represent the relevant chromatin state and expression patterns during recombination. We investigated the expression profile during early Drosophila meiosis in females, utilizing mRNA-Seq. Our analysis provides a glimpse at the most relevant patterns of expression during DSB formation and repair, and may provide insight into a complex relationship between gene expression and local recombination rates. We also note that expression patterns of nuclei from early meiotic regions of the gonad are enriched for genes involved in morphogenesis and cellular differentiation to a greater extent than the complete ovary. Additionally, we have identified more than 30 novel genes. Lastly, we detect a set of genes with a maternally derived expression pattern and find a bias towards X chromosome-expressed genes. These results indicate that the Drosophila early meiotic environment possesses a distinct pattern of expression and may reveal clues pertinent to recombination landscape patterning.

511A

Variability of 5' and 3' untranslated regions of *Dras1* gene in the *Drosophila virilis* species group. Anna I. Chekunova[1], Prokhor A. Proshakov[1], Maxim I. Barsukov[1], Ekaterina Sivoplyas[1], George N. Bachtojarov[2], Svetlana Yu. Sorokina[1], Vladimir G. Mitrofanov[1]. 1) Dept Genetics, Koltsov Inst Dev Biol, RAS, Moscow, Russian Federation; 2) Mechnikov Research Institute of Vaccines and Sera, RAMS, Moscow, Russian Federation.

Investigation of conservative genes polymorphism in groups of closely related species is particularly interesting from evolutionary point of view because the revealed variability should be neutral. *Ras1* gene is highly conservative. Product of its expression - Ras1 protein - takes part in mitotic activity regulation of cell. Mutations in this gene frequently lead to cancerogenesis. *Ras1* activity regulation is also evolutionary conservative. Previously, we studied the variability of several exons and introns of *Dras1* gene in the *drosophila virilis* group, which serves as a convenient model for studying of molecular evolutionary processes at the early stages of divergence. In this part of study the nucleotide sequences of enhancer region of *Dras1* gene and its 3'- region were analyzed in the *virilis* species group. As sequences comparative analysis shows, the characters of evolution of these two sequence fragments greatly differ. Enhancer region of *Dras1* gene revealed significant polymorphism presented both by nucleotide substitutions and indels of 1-22 bps of length. Alignment of this sequence fragment with the same fragment of *D.melanogaster, D.mojavensis* and *D.grimshawi* only was possible for short motives (approx. 10 nucleotides), which may be functionally important, such as DRE elements. The great amount of variable sites, including phylogeneticaly informative sites, was found among the *virilis* group species. In contrast, 3'- region was much more conservative. The greater part of sequence polymorphism (including insertion of 9 bps that is nearby polyadenilation site) allows to distinguish the species at phylad and subphylad levels. Some substitutions were species specific. The study was supported by RFBR grant N 12-04-00926-a and the program of the Presidium of RAS "Wildlife: Current status and problems of development".

512B

Extended open reading frames in *Drosophila* associated with small introns are a useful genomic tool for the identification of rapidly evolving coding sequence and splice junctions. Robert C. Eisman, Thomas C. Kaufman. Dept Biol, Jordan Hall A505, Indiana Univ, Bloomington, IN.

Genome reduction in the genus *Drosophila* relative to many other insects, is primarily due to the deletion of significant regions of intergenic and intronic sequence. In this study of the evolution of orthologous*centrosomin* genes within several insect Orders, we show the additional loss of many small introns (<100 bp) in Drosophila and two mosquitoes has resulted in exon fusions. Exon fusions appear to be due to the imprecise loss of introns and are associated with rapidly evolving protein sequence. Interestingly, many of the remaining small introns in *Drosophila cnn* are either in the same reading frame as adjacent coding exons or are covered by long overlapping reading frames of two adjacent coding exons. Our data from *Drosophila* and other insects suggest these extended reading frames may arise as an intermediate step when introns are reduced in size and may help buffer against the potentially deleterious effects expected when the fusion of coding exons includes small intronic fragments. These effects are also buffered by simple protein folds encoded by extended open reading frames and a relaxed splicing mechanism. Additionally, *cnn*-like extended reading frames are present in approximately 3% of the genes in *D. melanogaster* and are useful tools for the identification of rapidly evolving protein coding regions and changes in the intron-exon structure of genes in the genus *Drosophila*, as well as other insect Orders.

513C

Evolution of a heterochromatic domain, the Muller F element, in Drosophila / Sophophora. SCR Elgin[1], M Burg[2], J DiAngelo[3], A Haberman[4], C Jones[5], L Kadlec[6], SCS Key[7], J Leatherman[8], GP McNeil[9], H Mistry[10], A Nagengast[10], DW Paetkau[11], S Parrish[12], L Reed[13], S Schroeder[14], S Smith[15], M Wawersik[16], L Zhou[17], CD Shaffer[1], W Leung[1]. 1) Washington U MO; 2) Grand Valley St MI; 3) Hofstra U NY; 4) Oberlin OH; 5) Moravian PA; 6) Wilkes U PA; 7) NC Central U NC; 8) Northern Colorado CO; 9) York/CUNY NY; 10) Widener U PA; 11) St Mary's IN; 12) McDaniel MD; 13) Alabama-Tuscaloosa AL; 14) Webster U MO; 15) Arcadia U PA; 16) William & Mary VA; 17) U Pittsburgh PA.

The Muller F element in Drosophila is unusual because it exhibits both heterochromatic and euchromatic properties.

Students in the Genomics Education Partnership are analyzing this region (and Muller D euchromatic reference regions) in several Drosophila species to chart the evolution of this unique domain and its genes. Students have generated >4 million bases of high quality sequence and manually curated >1000 gene models from 4 species: D. erecta, D. virilis, D. mojavensis and D. grimshawi. Muller F elements have a higher repeat density than euchromatic domains; we find that their genes are larger, with more exons and larger introns, and show lower codon bias. D. mojavensis has the highest repeat density among these F elements, which partially accounts for the larger size of the banded region (1.7 Mb vs. 1.2 Mb in D. melanogaster). The distribution and types of repeats found in these regions are being analyzed. Histone modification enrichment patterns are largely conserved among F elements. Despite a large number of gene rearrangements, most of the genes found on the D. melanogaster F remain on the F elements in the other species, but there have been at least 13 transposition events. Analysis of the subset of such genes found in a euchromatic domain in at least one species shows that these genes typically adopt the properties of their local genome environment, with some exceptions. The carefully sequenced and annotated domains generated by GEP students provide a high quality resource for these and other types of analyses. Support: HHMI grant 52005780 & NIH grant R01 GM068388 to SCRE.

514A

Evolution of piRNA clusters in Anopheles gambiae M and S forms. Phillip George[1], Igor Sharakhov[1], Chantal Vaury[2], Silke Jensen[2]. 1) Department of Entomology, Virginia Tech, Blacksburg, VA, USA; 2) Laboratoire Génétique, Reproduction et Développement (GReD), Clermont-Ferrand, France.

The piRNA pathway is known to be an important mechanism in the suppression and control of transposable element (TE) mobilization in many genomes including the fruit fly, Aedes mosquitoes and mice. Hybrid dysgenesis in Drosophila is caused by a lack of maternally loaded piRNAs, which was absent in the non TE-carrying female. However, a role of the piRNA pathway in reproductive isolation between species is not yet established. Members of the Anopheles gambiae complex (including incipient species M and S) represent a promising system for addressing this gap. We have sequenced piRNAs from the Mali strain (M form) and Zanu strain (S form) of A. gambiae. A library of total small RNAs ranging from 20-35 nt was taken from the total RNA pool. 26-32 nt sequences were kept, and redundant RNA sequences were removed. A program NucBase was used to count and map the RNAs that interacted with the PEST, S and M genomes of A. gambiae. The highest piRNA clusters are found in the pericentromeric and intercalary heterochromatin. We have also identified the top 15 piRNA clusters. They have been further analyzed to determine the TE content difference between the M and S forms. The clusters are similar, but there are noticeable differences in the TE classes and the non-TE content in the clusters. The number of piRNAs that uniquely map to these clusters also is visibly different. We hypothesize that the divergence in the piRNA pathway in the malaria mosquito plays an important role in speciation due to the protection or lack of protection toward conferred TEs in offspring. The TE derepression in F1 hybrids, although not documented in A. gambiae as of yet, could be one possible mode of speciation.

515B

Young retrogene detection in Drosophila. Tatiana A. Gurbich[1], JJ Emerson[2], Doris Bachtrog[1]. 1) Integrative Biology, University of California, Berkeley, Berkeley, CA; 2) Ecology and Evolutionary Biology, University of California, Irvine, Irvine, CA.

Retroposed genes are duplications that originate when mature mRNA of the parental gene is reverse transcribed and inserted in a new location in the genome. Because of the nature of this mechanism, retroposition is not only a source of novel genes, but is also a tool by which genes can change their genomic location. Studying young retrogenes can provide insight into selective forces that shape chromosome content. Detecting young retrogenes in assembled genomes can be problematic due to low divergence of exonic sequence between the parental gene and the retrocopy, which can lead to the retrocopy not being assembled at all. Retrogenes are also likely to be surrounded by repetitive DNA sequence which results in these regions often being unassembled. These properties of young retrogenes can lead to them being undetected in analysis. In this study, we implemented a suite of methods that can be used to detect retrogenes from raw sequencing data without relying on an assembled genome. We show that with our methodology we can detect more retrogenes than was possible previously. We also present data on young retropositions in *Drosophila miranda* that originated since *D.miranda*'s split from *Drosophila pseudoobscura* ~1.5 million years ago.

516C

Lack of association between piRNA abundance and the deleterious capacity of transposable element families in *Drosophila melanogaster*. Erin S. Kelleher, Daniel A. Barbash. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Transposable elements (TEs) are genomic parasites whose selfish propagation can disrupt functional sequences, and in extreme cases is associated with sterility and cancer. Homologous TE insertions further threaten genome integrity by acting as substrates for ectopic recombination. The piRNA pathway defends animal genomes against the harmful consequences of TE infection by imposing small-RNA mediated silencing, predominantly in the germline. Because silencing is targeted by TE-derived piRNAs, piRNA production is posited to be central to the evolution of genome defense.

We harnessed genomic data sets from *Drosophila melanogaster*, including measures of piRNA, mRNA, and genome-wide abundance of TE families, along with estimates of TE-family age structure and risk of ectopic recombination, to address fundamental questions about the functional and evolutionary relationships between TE families and their regulatory piRNAs.

We demonstrate that TE transcription, the degree of participation in the "ping-pong" cycle, and the number of insertions in piRNA clusters together explain the majority of variation in piRNA abundance between TE families. These results provide the first robust statistical support for the prevailing model of piRNA production. Intriguingly however, we discover that the most transpositionally active TE families, with the greatest capacity to induce harmful mutations or disrupt gametogenesis, are not necessarily the most abundant in the piRNA pool. Additionally, we find no evidence that piRNA abundance responds to selection against ectopic recombination. Our observations reveal that the deleterious capacity of a TE family is not associated with piRNA abundance, and point to more complex models of host adaptation to TE infection.

517A

Evolutionary Constraints on DNA Shape. Tevfik H Kitapci, Tianyin Zhou, Remo Rohs, Sergey V. Nuzhdin. University of Southern California, Los Angeles, CA.

Is DNA Shape, specifically minor groove width, under natural selection? In this study we analyze SNPs coming from full genome data of 66 Drosophila melanogasters. We use a ChIP-seq dataset to annotate transcription factor binding sites. Using high-throughput minor groove width prediction method based on Monte Carlo simulations we analyze SNP frequency distributions. Our preliminary results suggest that there might be selection on DNA minor groove width in transcription factor binding sites.

518B

Rapid evolution of the *Responder* satellite in the *melanogaster* species subgroup. Amanda M. Larracuente, Daven C. Presgraves. Biology, University of Rochester, Rochester, NY.

Responder (*Rsp*) is a satellite DNA repeat found in the pericentric heterochromatin of chromosome *2* in *Drosophila melanogaster*. *Rsp* is well-known for being the target of *Segregation Distorter* (*SD*)— a meiotic drive system found on chromosome *2* of *D. melanogaster*. We studied the evolution of this satellite in *D. melanogaster* and its close relatives. We find that *Rsp* is not a satellite cluster restricted to pericentric heterochromatin: *Rsp* repeats occur throughout the genome, including the euchromatin. Contrary to previous reports, we find the *Responder* satellite in *D. simulans* and *D. sechellia*. The repeats in these species are considerably diverged at the sequence level compared to *D. melanogaster* and have a strikingly different genomic distribution. Thus, *Rsp* has diverged in both sequence and genomic location on a short time scale of <240,000 years. We contrast the evolution of this satellite between one species where it is, and species where it presumably is not a target of segregation distortion.

519C

The functional and evolutionary significance of nested genes. Grace Y C Lee[1], Hsiao-Han Chang[2]. 1) Ecology and Evolution, University of Chicago, Chicago, IL; 2) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

The distribution of genes in the genome is not random: there are gene deserts with few functional genes as well as genomic regions where genes are densely packed and partially or entirely overlap. An especially interesting class of overlapping genes is in which one gene is completely nested within an intron of another gene (nested and including gene, respectively). Even though the coding sequences of these nested/including gene pairs do not overlap, their intimate structures and the possibility of shared regulatory sequences raise questions about the evolutionary forces governing the origination of nested genes and their subsequent functional and evolutionary impacts. We found ~7% of genes in the *Drosophila melanogaster* genome are in nested gene structure. Nested genes tend to be more recently derived, under less evolutionary constraint, and more narrowly expressed than other genes, while including genes show the opposite patterns. Surprisingly, expression levels of nested/including gene pairs are less likely to be positively correlated than the expression levels of randomly selected pairs of adjacent yet non-overlapping genes. Interestingly, there are significantly more nested genes in *trans* orientation to their including genes than are in *cis* orientation. We hypothesized that this is due to selection against potential erroneous mRNA splicing when nested/including gene pairs are in *cis* orientation. Consistent with this hypothesis, we found that *cis*-nested genes are more likely to have only one exon and to have stronger tissue-specific expression than *trans*-nested genes, while there is no obvious difference between *cis*- and *trans*- including genes. Also, transposable elements that are *trans*-nested in introns are enriched in the reference genome and occur at higher population frequencies than *cis*-nested transposable elements.

520A

Candidate genes contribute to behavioral isolation revealed by comparative genomic approach. Juan Li[1], Lan Jiang[1], Chung-I Wu[1,2], Chau-Ti Ting[3], Xuemei Lu[1]. 1) Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100029, People's Republic of China; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637; 3) Department of Life Science, Institute of Ecology and Evolutionary Biology, & Institute of Zoology, National Taiwan University, Taipei, Taiwan, ROC.

Two behavioral races, M (for cosmopolitan) and Z (for Zimbabwe) of Drosophila melanogaster provide a great model to study the genetic basis of racial differentiation. When given a choice, females from the Zimbabwe race mate only with males from its congener whereas females from the cosmopolitan race mate readily with males from both races little discriminatorily. A series of genetic analyses showed that the Z/M behavioral isolation is mainly contributed by two major autosomes, and several fragments of the third chromosome are crucial in either male behavior or female preferences. However, very little was

known about the genetic locus and the evolution of racial differentiation genes. To address this question, we have generated a reference genome of Z race by deep sequencing. By comparing to the published reference genome (M race), 0.8% of the sites have diverged between the two genomes. In addition, 104 copy number variations were identified. Of which, we narrow down to around 12 candidate regions that may contribute to the M/Z racial differentiation by analyzing a small sample from the DPGP2 genomes.These results provide a general framework on mapping behavioral genes underlying racial differentiation in D. melanogaster.

521B

Sex-specific embryonic gene expression at different stages of sex chromosome evolution. Susan E. Lott[1,4], Jacqueline E. Villalta[2], Doris Bachtrog[3], Michael B. Eisen[1,2,3]. 1) Dept. of Molecular and Cell Biology; 2) Howard Hughes Medical Institute; 3) Dept. of Integrative Biology, University of California, Berkeley, CA; 4) Dept. of Evolution and Ecology, University of California, Davis, CA.

The most significant form of natural genetic variation in many species is the difference in sex chromosome dose between males and females. In Drosophila, females have two X chromosomes, while males have one X and one Y. However, the composition of these sex chromosomes has shifted dramatically in many lineages. Several fusions of sex chromosomes with autosomes have occurred along the lineage leading to *D. miranda* - the first ~15 MYA, before the divergence of *D. miranda* from *D. pseudoobscura*, and the second after the divergence of these species, ~1.5 MYA. The resulting neo-X chromosomes are gradually acquiring the properties of sex chromosomes, becoming targets for molecular mechanisms that compensate for differences in X chromosome dose between sexes. We have recently shown that *D. melanogaster* possess at least two dosage compensation mechanisms: the well-characterized MSL-mediated dosage compensation active in most somatic tissues, and a second system active during early embryogenesis. To investigate the evolutionary constraints on sex chromosome expression and evolution, we used single embryo mRNA-seq to characterize gene expression in female and male embryos of *D. pseudoobscura* and *D. miranda*, across the first eight hours of embryogenesis. We observe imperfect dosage compensation at the onset of zygotic transcription, which improves through developmental time with establishment of MSL-mediated dosage compensation. Surprisingly, the young neo-X chromosome of *D. miranda* is better compensated than the ancestral X and older neo-X chromosomes, and is better compensated in the embryo than in the adult. This suggests differences in how early zygotic dosage compensation and MSL-mediated dosage compensation evolve, with the former being a more general, less gene-specific mechanism which can evolve over a short period of evolutionary time.

522C

Rapid evolution and differential expression of transcripts associated with sex chromosome meiotic drive in stalk-eyed flies. Josephine A. Reinhardt[1], Richard H. Baker[2], Gerald S. Wilkinson[1]. 1) Biology, University of Maryland College Park, College Park, MD; 2) Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, NY.

Sex chromosome meiotic drive causes a distortion of population sex ratios in many dipterans. In the stalk-eyed fly, *Teleopsis dalmanni*, males carrying the meiotic drive X chromosome parent almost exclusively daughters. The drive haplotype (X^D) is present at a high frequency in natural populations covering a wide geographic, genetic, and temporal distance. The X^D haplotype associates with a large portion of the X chromosome (which is novel in *T. dalmanni* relative to other dipterans) and is thought to be maintained by a chromosomal rearrangement suppressing recombination with standard X chromosomes. In addition, the drive haplotype is known to cause a variety of pleiotropic effects in males. To understand how drive may be operating genetically, we obtained Illumina transcriptomes from *T. dalmanni* testes that carried the meiotic drive haplotype (X^D) and standard X chromosomes (X^{ST}). We identified hundreds of transcripts that were differentially expressed between X^D and X^{ST} testes. When compared with other transcripts, genes that were differentially expressed in X^D testes were more likely to be X-linked and to show testes-biased expression. The majority of these genes - particularly those that were the most strongly differentiated - had no orthologs in diptera and had low protein-coding potential. These results imply that drive-associated differences in gene expression occur on the novel X chromosome and may be driven by rapid evolution of genes, including putative noncoding RNA genes. Finally, we found that hundreds of X-linked transcripts carry fixed differences between X^D and X^{ST}samples while only a handful of such differences were found in autosomal genes. This supports the hypothesis that the X^D haplotype long ago ceased recombination with X^{ST}, revealing a genomic mechanism for the maintenance of the drive phenotype.

523A

Copy number variation and the limits of natural seleciton in *Drosophila yakuba* and *Drosophila simulans*. Rebekah L. Rogers[1], Julie M. Cridland[2], Ling Shao[1], Kevin R Thornton[1]. 1) Ecology and Evolutionary Biology, University of California, Irvine, CA; 2) Department Of Evolution and Ecology, University of California, Davis, CA.

Gene duplications are key contributors to adaptive evolution and mophological diversity. We have used paired-end Illumina sequencing to identify segregating duplications in populations of *Drosophila yakuba* and *Drosophila simulans*. We find evidence for natural selection favoring duplications in *D. simulans* especially those that fall on the X chromosome. Furthermore, we see overrepresentation of duplicate genes involved in rapid evolutionary processes such as immune defense, and evolution of chemosensory and photosensory perception, chitin cuticle formation, and chorion development. We identify large numbers of abnormal gene constructs such as chimeric genes and recruitment of formerly noncoding sequence. In addition, many tandem duplications may disrupt or silence genes, suggesting that tandem duplications can serve as forces of destruction in addition to

their role in generating new genes. While we observe large numbers of segregating gene duplications in both species, the likelihood that a duplication of any single gene is present in the population is still low. Hence, evolution is likely to be severely limited by mutation with respect to duplicate genes, especially in cases where environments change rapidly or often.

524B

The short life cycle of orphan genes in the *Drosophila obscura* group explains the paradox of conserved gene number across species. Christian W. Schloetterer, Nicola Palmieri, Carolin Kosiol, Viola Nolte. Inst f Populationsgenetik, Vetmeduni Vienna, Wien, Austria.

It is well understood that orphan genes emerge at a high rate, frequently from previously non-coding DNA. Despite that a high fraction of the recently emerged genes quickly acquires essential functions, the total number of genes remains remarkably stable across species. To shed more light on this apparent paradox we studied orphan genes in the *Drosophila obscura* group. Using RNA-Seq we identified 1143 putatively protein coding orphan genes in *D. pseudoobscura*, which is consistent with the previously published high emergence rate of orphan genes. Through phylogenetic analysis of *D. affinis*, *D. lowei*, *D. miranda* and *D. persimilis* we dated the birth and death process of these orphan genes. Contrary to neutral expectations, we found that most orphan genes pseudogenized (lost function) shortly after their emergence and only few orphans are phylogenetically conserved. This short life cycle of orphan genes reconciles their high birth rate with the stability of gene number across species.

525C

Transfer of mitochondrial DNA fragments into the nuclear genome in flies and cell line of *D.virilis*. Svetlana Y. Sorokina[1], Denis A. Romanov[2], Boris V. Andrianov[2], Ilya A. Zakharov[2]. 1) Dept Genetics, Koltsov Inst Dev Biology, Moscow, Russian Federation; 2) Dept insect Genetics, Vavilov Inst Gen Genet, Moscow, Russian Federation.

We have developed a method of experimental detection of *D.virilis* Numt-sequences, based on site-specific insertion of *Tv1*-retrotransposon into mitochondrial microsatellite (AT)n, located at the spacer region between *atp6* and *cox3* mitochondrial genes. This approach allows us to detect the recent events of Numt-sequences insertions into nuclear genome. We show that *D.virilis* cell line, as well as *D.virilis*fly strains, have the fragments of *atp6* mitochondrial gene of equal size (261 bps) in their nuclear genomes. In both cases *atp6* fragments are associated with LTR of *Tv1*-retrotransposon. But comparative analysis of these sequences revealed different origins of *atp6* Numt-sequences in flies and cell line. *Atp6* Numt-sequences of all five studied *D.virilis* strains from different geographic localities have 18 point mutations in comparison to functional mitochondrial *atp6* gene sequence of *D.virilis*. These mutations lead to seven aminoacid substitutions, but do not lead to stop-codon formation. Coincidence of *atp6*Numt-sequences of all *D.virilis* strains studied allows us to suppose an ancestral status of *D.virilis atp6* Numt-sequence from flies. In contrast, all *atp6* Numt-sequences from *D.virilis* cell line found were completely identical to homological mt-sequence of *D.virilis* with one exception - C-8 clone that has only one synonymous substitution. However, *Tv1* parts of all Numts revealed were different in length and nucleotide sequence. These facts suggest the recent origin of *atp6* Numt-sequences in *D.virilis* cell line through a series of independent events. The study was supported by RFBR grant N 11-04-01630-a and the program of the Presidium of RAS “Wildlife: Current status and problems of development”.

526A

Selection driven signatures of domestication in Drosophila. Craig E Stanley, Rob J Kulathinal. Dept. of Biology, Temple University, Philadelphia, PA.

Genomic analyses highlight potential regions under selection during domestication of Drosophila melanogaster Craig E. Stanley Jr.* & Rob J. Kulathinal Session: 07 Evolution and quantitative genetics Drosophila melanogaster, one the first genetic model systems (and arguably the most important), has been studied in the laboratory setting for over a century. While many studies have focused on the time required for selection to act on laboratory populations, the effect of inadvertent selection on laboratory lines of Drosophila remains unknown. With more than 12 generations per year and the presence of similar laboratory environments with relatively constant selective pressures, there has been ample opportunity for selection to act. Additionally, anecdotal phenotypic evidence has been reported between the behavioral and life history traits of laboratory and wild D. melanogaster. Here, we compare the genomes of laboratory vs. wild-derived flies in order to understand the heritable genomic alterations that have occurred across decades of domestication. Using data from Flysnp, DGRP, and DPGP, we identify a high fraction of SNPs unique to the laboratory strains of D. melanogaster. Within genes, unique SNPs are overrepresented in non-coding regions, more specifically, in 5’ and 3’ UTRs. Genes containing unique SNPs are also enriched for neural and sensory gene ontological classes. Additionally, a combination of whole genome assemblies and RNAseq data were used to conduct individual-level (SNP analysis) along with population-level (tests of selection) analyses. Moreover, six genome sequences from D. simulans (DPGP) were used to polarize allelic changes. Results from this study provide a genetic basis into observed phenotypic differences between laboratory and wild-caught D. melanogaster.

527B

Identifying misregulated genes contributing to male lethality in *D. melanogaster/D. simulans* hybrids with RNA-seq. Kevin HC Wei, Andrew G Clark, Daniel A Barbash. Molecular Biology and Genetics, Cornell, Ithaca, NY.

Mutations in the *D. melanogaster* (*D. mel*) gene *Hybrid male rescue* (*Hmr*) suppress male larval lethality in F1 hybrids produced by *D. mel* females mated to *D. simulans* (*D. sim*) males. However, the function of *Hmr* within *D. mel* and the

mechanism by which it induces male lethality in hybrids remains poorly understood. To investigate the role of *Hmr* in both *D. mel* and hybrid genomes during larval development, we profiled the transcriptomes of 72hr-old larvae with RNA-seq to identify genes misregulated in *Hmr* mutants (*Hmr⁻ D. mel*) compared to wildtype *D.mel* (*wt D. mel*), and genes differentially regulated in rescued hybrids (*Hmr⁻* hybrid) compared to lethal hybrids (*wt* hybrid). In *Hmr⁻ D.mel*, we only detected 20 genes significantly misregulated; almost all of them (18) are upregulated, strongly indicating that *Hmr* is a repressor. Comparing between the *Hmr⁻* and *wt* hybrids, we identified a large set of 473 differentially expressed genes. In contrast to the over-representation of upregulated genes in*Hmr⁻ D. mel*, fewer genes are up-regulated (154) than down-regulated (319) in *Hmr⁻* hybrids. Additionally, of the differentially expressed autosomal genes between the hybrids, significantly more *D. mel*alleles are misregulated (345) than *D. sim* alleles (265), suggesting the *D.mel* genome is under greater perturbation in the *wt* hybrids. To characterize developmental defects contributing to hybrid lethality, we compared the expression profiles of our 72 hr-old larva to that of L2 and early L3 larva generated by the modENCODE consortium. Consistent with previous reports that hybrids are developmentally delayed, hybrids in general are more L2-like than *D. mel*, and *wt* hybrids have the most genes expressed at L2-like levels. Of the 42 genes expressed at L2-like levels only in *wt* hybrids, we find a striking enrichment for genes expressed in the larval central nervous system (31) and the thoracioabdominal ganglion (37), suggesting a substantial developmental lag for the nervous system of *wt* hybrids.

528C

Sex-Specific Adaptation Drives Early Sex Chromosome Evolution in Drosophila. Qi Zhou, Doris Bachtrog. Integrative Biology, University of California, Berkeley, Berkeley, CA.

Most species' sex chromosomes are derived from ancient autosomes and show few signatures of their origins. We studied the sex chromosomes of Drosophila miranda, where a neo-Y chromosome originated only approximately 1 million years ago. Whole-genome and transcriptome analysis reveals massive degeneration of the neo-Y, that male-beneficial genes on the neo-Y are more likely to undergo accelerated protein evolution, and that neo-Y genes evolve biased expression toward male-specific tissues—the shrinking gene content of the neo-Y becomes masculinized. In contrast, although older X chromosomes show a paucity of genes expressed in male tissues, neo-X genes highly expressed in male-specific tissues undergo increased rates of protein evolution if haploid in males. Thus, the response to sex-specific selection can shift at different stages of X differentiation, resulting in masculinization or demasculinization of the X-chromosomal gene content.

529A

Patterns of gene co-expression evolution throughout development in the Drosophila pseudoobscura group. Kawther Abdilleh, Carlos Machado. Department of Biology, Univ Maryland, College Park, MD.

Gene co-expression analyses are proven methods used to determine correlated expression patterns from high-throughput gene expression data. Here, using a developmental time-course microarray dataset for D. pseudoobscura and D. persimilis, we compare the expression profiles of genes expressed throughout development in both males and females of these species. We find that genes showing patterns of statistically correlated expression profiles cluster together and share common molecular functions and participate in similar biological processes. Further, our results suggest that clusters consisting of housekeeping genes more often than not also physically cluster together on chromosomes. Intraspecific comparisons between the sexes suggests a correlation between expression level, sex-bias and cluster membership such that genes highly expressed in one sex are overrepresented in certain clusters. Taken together, our results here provide further insights into interspecific and sex-biased gene co-expression evolution.

530B

Parthenogenesis as an alternative reproductive strategy in *Drosophila*. Chia-chen Chang[1], Shu Fang[2], Chau-Ti Ting[3], Hwei-yu Chang[1,2]. 1) Department of Entomology, National Taiwan University, Taipei, Taiwan 10617, ROC; 2) Biodiversity Research Center, Academia Sinica, Taipei, Taiwan 11529, ROC; 3) Department of Life Science, Genome and Systems Biology Degree Program, Institute of Ecology and Evolutionary Biology, Institute of Zoology, and Research Center for Developmental Biology and Regeneration Medicine, National Taiwan University, Taipei, Taiwan.

Parthenogenesis has independently evolved multiple times in *Drosophila* lineages, and most of them are facultative. Given that most *Drosophila* species reproduce bisexually, the facultative parthenogenesis is not an adaptive reproductive strategy in long-term evolution. Nonetheless, some characteristics for the facultative parthenogenesis may be temporarily advantageous. To test this idea, we investigated several fitness components of a parthenogenetic strain in *D. albomicans* compared to their bisexual counterparts. The fertility of the parthenogenetic strain was low, only 10% *via* parthenogenesis and 60% *via* sexual reproduction compared with a bisexual strain. Females from the parthenogenetic strain produced both parthenogenetic and sexual offspring after mating. The number of parthenogenetic offspring produced by mated females was comparable with that by virgin females, suggesting that parthenogenetic ability was not obviously disturbed by mating. We also found that 70% of F_1 offspring produced *via* bisexual reproduction could perform parthenogenesis albeit their fertility was extremely low. Genetic variation of the parthenogenetic strain might be extreme low because gamete duplication was the predominant mechanism (91.8%). Despite the low fertility, several features including producing both parthenogenetic and sexual offspring after mating, high parthenogenetic capability of sexually produced F_1 females, and multiple mechanisms for diploidization, might explain why the facultative parthenogenesis could be maintained in the population. Our findings suggest that facultative parthenogenesis is an alternative reproductive strategy.

531C

Sexually attractive traits as activity indicators of nutrient-sensing pathways. Tatyana Y Fedina[1], Tsung-Han Kuo[2], Ingrid Hansen[2], Klaus Dreisewerd[3], Herman A Dierick[2], Joanne Y Yew[4], Scott D Pletcher[1,2]. 1) University of Michigan, USA; 2) Baylor College of Medicine, USA; 3) University of Münster, Germany; 4) National University of Singapore, Singapore.

Purpose: Sexually attractive traits are thought to reflect an individual's health and reproductive potential, but the underlying molecular mechanisms through which they do so are generally unknown. Insulin/insulin-like growth factor signaling (IIS) is a systemic nutrient sensor known to modulate aging and reproduction across species. Here we investigate IIS effects on sexual attractiveness of *D. melanogaster* female cuticular hydrocarbons (CHCs). **Methods:** We used *chico*, *dfoxo* mutants, *chico;dfoxo* double mutants, and gsTub5>Akt^{RNAi}/Pten/InR/dFoxo/TOR^{TED} flies whose adults were exposed to RU486 to activate transgene expression. GC-MS and a novel mass-spec technique were used to obtain comprehensive CHC profiles. Male choice was quantified as a relative time spent courting one of the two decapitated females in two-choice attractiveness assay. **Results:** Genetic manipulations of IIS pathway consistently and predictably altered CHC profiles and the expression of CHC synthesis enzymes (*eloF, desat1, desat2, desatF*); this effect was independent of dFOXO and involved the nutrient-sensing Target-of-Rapamycin pathway. Manipulations that reduced IIS also reduced attractiveness, while females with increased IIS were more attractive to males. Decreased IIS shifted CHC composition to favor long-chain compounds. **Conclusions:** *D.m.* female IIS pathway activity is reflected in CHC composition that is used by males to choose females with increased IIS (=high immediate fecundity). This is the first study to dissect the link between the activity of a conserved nutrient sensing pathway in the adult and sexual attractiveness. We further hypothesize that different attractiveness traits across species may represent the activity of a few conserved molecular regulators of growth and reproduction. In support of this hypothesis, subsequent studies in rhinoceros beetles have shown that IIS activity during larval development can be reflected in adult ornaments.

532A

Epistasis plays a dominant role in the genetic architecture of *Drosophila* quantitative traits. Wen Huang[1], Robert Anholt[2], Trudy Mackay[1]. 1) Department of Genetics, North Carolina State University, Raleigh, NC; 2) Department of Biology, North Carolina State University, Raleigh, NC.

Genetic interaction or epistasis is important for canalization and speciation. However, the role of epistasis in controlling quantitative trait variation remains controversial. We performed genome-wide screens for single nucleotide polymorphisms (SNPs) associated with three life history traits (starvation resistance, startle response, and chill coma recovery time) in the recently developed *Drosophila melanogaster*Genetic Reference Panel (DGRP) and in a synthetic outbred population, derived from this panel through advanced intercrossing (Flyland). The genetic architecture for all three traits was highly polygenic in both the DGRP and the Flyland populations. Although there was no overlap between SNP associations in the two populations, genes associated with the quantitative traits in either population were highly connected in common epistatic networks. Furthermore, population/background specific associations could be explained by changes in allele frequencies of many SNPs that constituted the genetic contexts through epistasis in the two populations. We extended the analysis to all pair-wise interactions between SNPs in the genome and found extensive epistasis. Particularly for chill coma recovery, there was a marked enrichment of significant single SNP associations among SNPs participating in pair-wise interactions. In addition, we also showed by simulation that epistasis could induce substantial additive variance. Taken together, these results suggest a dominant role of epistasis in the genetic architecture of *Drosophila* quantitative traits, with additivity an emergent property from underlying epistatic genetic architecture.

533B

The effects of thermal stress on embryonic development: from cellular defects to whole-organism survival. Brent L. Lockwood, Kristi L. Montooth. Department of Biology, Indiana University, Bloomington, IN.

It is a widely held tenet in the field of developmental biology that embryos are 'canalized' to develop normally despite environmental perturbation. However, in ectothermic organisms like *Drosophila*, changes in environmental temperature likely alter cellular processes, thus making development more challenging in thermally variable environments. Are ectothermic embryos actually vulnerable to thermal stress, and what cellular structures and developmental processes are most vulnerable? Here we investigate the effects of thermal stress on early stage embryos of *Drosophila melanogaster* by measuring whole-organism survival, the cellular structures that mediate this stress, and the mechanisms that may buffer this stress during development. We find that exposure of eggs to heat stress causes disruption of early mitotic events and a significant decrease in survival to adulthood. We also find that some genotypes are more tolerant to heat stress than others, suggesting that thermal tolerance in embryos has a genetic basis. We discuss our progress using confocal fluorescence microscopy to assess the effects of heat stress on cytoskeletal proteins that coordinate early development.

534C

Evolution of behavioral defenses against parasitoid wasps in the melanogaster subgroup. Zachary Lynch, Balint Kacsoh, Todd Schlenke. Biology Department, Emory University, 1510 Clifton Rd, Atlanta, GA 30322.

Parasites reduce the fitness of their hosts and thus impose strong selective pressures on hosts for the evolution of defense mechanisms. However, molecular, cellular, and behavioral defense mechanisms can be costly to employ. Therefore, if multiple

possible defenses against a particular parasite exist, we might expect hosts to specialize in one form of defense. Preliminary evidence for this type of tradeoff was found in the sister species *Drosophila melanogaster* and *D. simulans*, both of which are attacked by the parasitoid wasp *Leptopilina boulardi* in nature. *D. simulans* melanotically encapsulates and kills *L. boulardi eggs*, whereas *D. melanogaster* does not show this ability. However, *D. melanogaster* reduces its oviposition rate in the presence of wasps to limit the overall likelihood of offspring infection. Thus,*D. melanogaster* appears to rely on behavioral defense to compensate for its poor cellular defense. I am assaying behavioral and cellular defense abilities of the nine species in the melanogaster subgroup to look for repeated patterns of tradeoffs among immune mechanisms. In particular I will assay three behavioral immune mechanisms: (i) reduced oviposition rate in the presence of wasps, (ii) oviposition in food sources that are more toxic to wasps than flies, such as ethanol for *D. melanogaster* and octanoic acid for *D. sechellia*, in the presence of wasps, and (iii) increased consumption of toxins by infected larvae. I expect that species with strong cellular immune defenses such as *D. simulans* and *D. yakuba* will rely less on behavioral immune defenses.

535A

Elucidation of the sex-determination pathways in an organism with monogenic sex determination. Meaghan L Pimsler[1], Sing-Hoi Sze[2], Corbin D Jones[3], Jeffery K Tomberlin[1], Aaron M Tarone[1]. 1) Entomology, TAMU, College Station, TX; 2) Computer Science and Engineering, TAMU, College Station, TX; 3) Biology Department, UNC, Chapel Hill, NC.

Research has shown sex-specific differences in gene expression in Diptera, an examples of which is Sex-lethal (Sxl) in Drosophila. The standard system in flies is one in which males are heterogametic and males and females are produced in approximately even proportions. However, Chrysomya rufifacies (Diptera: Calliphoridae) exhibits monogenic sex determination with single sex offspring clutches and homomorphic sex chromosomes. As there are no known morphological differences in the immature stages, genetic markers for quick sex-specific screening of larvae will be useful to look at differences between males, male-producing females, and female-producing females. Our objective was to identify markers of the three types of adults using transcriptomics. A single male and female were isolated together. Upon oviposition, the two adults were collected for subsequent RNA extraction after the progeny had eclosed and the progenitor female had been identified. RNA were sequenced with Illumina HiSeq and the transcriptomes were assembled with ASplice. Predicted transcripts were compared with information in the FlyBase database to identify homologous genes and predict functions. Transcripts were assembled that shared homology with four genes identified from Drosophila sex-determination pathways: Sxl, da, dsx, and fru. Male and female isoforms were also assembled for Sxl and dsx. On average, only about ten percent of the genes were differentially expressed between males and females and about sixty percent were male biased. In comparison, only about one percent of the transcriptome was statistically different between female types and about eighty percent of them were male-producer biased. This is surprising as the current model of sex determination in this species is that the female producers are heterozygote dominant for a product that is incorporated during gametogenesis and determines the sex of the offspring.

536B

Cis-regulatory determinants of Y-linked gene expression variation. Timothy Sackton, Jun Zhou, Daniel Hartl. Organismic & Evol Bio, Harvard Univ, Cambridge, MA.

Over the past five years, our lab has demonstrated that variation on the Y chromosome in Drosophila has significant effects on the expression of hundreds of autosomal and X-linked genes. This phenomenon is not solely attributable to expression of Y-linked genes, as it is observable in XXY females (where transcription from the Y is absent) suggesting that structural variation on the Y chromosome plays a role in modulating expression elsewhere in the genome. Recently, we have shown that genes that are susceptible to Y-linked regulatory variation (YRV) are significantly more likely to be located in intercalary heterochromatin and present in the nuclear periphery than expected. However, it remains unclear if the expression effects of Y-linked variation are solely driven by the genomic location of the target genes, or if cis-regulatory sequences can impact the susceptibility of a gene to YRV. In order to address this question, we measured allele-specific expression using RNA-seq in *D. simulans* intraspecific crosses where in all cases the genomic background is the same, but each F1 strain carries a different *D. simulans* Y chromosome. In this design, cis-regulatory effects can be detected if we observe genes whose expression differs between alleles in some Y chromosomal contexts but not others. This kind of allele-by-Y effect can only easily arise if a particular Y chromosome shifts the expression of one allele at a locus but not the other, presumably due to cis-regulatory sequence on only one of the two alleles. Here, we present the results of this analysis, which demonstrate the impact of cis-regulatory sequences on YRV.

537C

A genetic and molecular analysis of mating choice in *D. simulans*. Rui Sousa-Neves, Youngmin Chu, Emma Yang, Joseph Schinaman, Sebastian Chahda. Biol, Case Western Reserve Univ, Cleveland, OH.

Female mating choice is an ancient and wide spread process of decision-making in the animal kingdom that allows females to recognize and mate with conspecific males and reject males of other species. Besides its evolutionary significance, this behavior serves as a platform to identify genes and circuits required for decision-making. *Drosophila simulans*, and *Drosophila sechellia* are three closely related species of *Drosophila* with very similar genomes and a marked difference for the males that they prefer. Here we analyzed F2 hybrids between *D. simulans* and *D. sechellia* for their ability to mate or reject D. simulans males. Our results show that a set of dominant genes are required for this choice. The X- chromosome bears two of these genes, Mate choice XA (Macho-XA) and Mate choice XB (Macho-XB). In addition, two other dominant genes are localized on the

second and third chromosomes. We genetically and molecularly mapped the two X- chromosome genes to relatively small intervals. In addition, we found that sex appeal segregates as one or two autosomal genes suggesting that Macho-2 and Macho-3 encode sex appeal.

538A

The effect of sex-ratio meiotic drive on sequence evolution and gene expression in *Drosophila affinis*. Robert Unckless, Andrew Clark. Department of Entomology, Cornell University, Ithaca, NY.

Organisms must adapt not only to their external environment, but also to challenges from within their own genome. Sex-ratio meiotic drive occurs when one sex-chromosome contains elements that are able to disable or kill the opposite sex-chromosome resulting in highly skewed sex-ratios in offspring. Several consequences of sex-ratio meiotic drive have been examined both theoretically and empirically, including extinction, the evolution of reproductive isolation and polyandry. One of the first sex-ratio systems discovered was in X-drive *Drosophila affinis*. Through a combination of genome and transcriptome sequencing we examine the effect of the X-drive system in *Drosophila affinis* on both gene expression and sequence evolution in tightly linked regions of the X-chromosome. Several candidates for the causative locus for drive have emerged and drive appears to have had a significant impact on gene expression both in linked regions and genomewide.

539B

Dissecting the sources of genetic variation in regulation of gene expression within D. simulans isolates. Hosseinali Asgharian[1], Rita M Graze[2], Bradley J Main[1], Marta L Wayne[2], Alison M Morse[2], Lauren M McIntyre[2], Sergey V Nuzhdin[1]. 1) Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 2) Molecular Genetics and Microbiology, University of Florida, Gainesville, FL.

Variation in cis and trans controllers of gene expression (e.g. change in DNA sequence of a promoter, or change in protein sequence or abundance of a transcription factor) can result in gene expression variation. Understanding the nature of this variation is essential to the study of phenotypic and genomic evolution. We used overall and allele-specific expression profiling in Drosophila simulans lines to estimate the prevalence of gene expression variation due to cis, trans and cis-by-trans effects. Allelic imbalance in the heterozygous F1 offspring of two isogenic lines (referred to as full heterozygotes) is due to cis effects. Differential expression of the same allele in the heterozygous F1 vs. the homozygous parental line is due to a trans effect. Introgression lines with a common reference background and a varying natural allele for a large portion of the 3rd chromosome were constructed. F1 introgression heterozygotes were made by backcrossing the introgression to the reference line. The difference in allelic imbalance for genes within the introgression region in the full heterozygotes vs. in the introgression heterozygotes reflects cis-by-trans interactions. At FDR=0.2, 60% (5454/9090) of autosomal genes in females, 49% (744/1507) of X-linked genes in females and 48% (4361/9089) of autosomal genes in males showed evidence for cis variation. In contrast, genes showing trans variation was only ~1.5% of genes in most settings, the exception being autosomal genes in females where it was 6.2% (565/9104). Cis-by-trans interactions were seen in 8.0% (77/967) and 2.7% (26/968) of genes in females and males, respectively. The connection of cis, trans and cis-by-trans variation with sex-biased expression, functional category (GO annotation) and belonging to the tentative groups of transcription factors or target genes was explored.

540C

The evolutionary consequences of seasonality: assessing demography and balancing selection in real time. Alan O. Bergland[1], Emily Behrman[2], Katherine O'Brien[2], Paul Schmidt[2], Dmitri A. Petrov[1]. 1) Dept. of Biol., Stanford Univ, Stanford, CA; 2) Dept. of Biol., Univ. of Penn., Philadelphia, PA.

Assessing the change in genetic composition of a population through time provides a means to understand the evolutionary forces acting on that population. For *D. melanogaster* living in temperate environments, seasonal climatic fluctuations cause severe demographic events and act as strong selective agents. To identify the evolutionary impact of seasonality in flies, we performed whole genome resequencing of large samples of flies collected in a temperate, Pennsylvania orchard during the spring and fall over three consecutive years and contrast changes in allele frequency through time with changes in allele frequency along a North American latitudinal cline. We demonstrate that populations collected through time are as differentiated as populations separated by 5-10° latitude. Using forward simulations, we show that such levels of differentiation are more consistent with models that incorporate temporal variation in selection coefficients and population size than purely demographic models. Accordingly we performed a genome-wide scan for SNPs that vary in frequency in a cyclic fashion and conservatively identify ~1000 such sites that strongly vary in frequency by ~20% between the seasons and infer that per-generation selection coefficient acting on these SNPs is on the order of 10%. We show that a large fraction of the 'seasonal' SNPs can evolve independently under simple models of natural selection and that patterns of polymorphism surrounding seasonal SNPs are consistent with adaptive evolution. We also show that frequencies of seasonal SNPs following the first frost of the winter respond in a predictable way. Finally, we contrast seasonal SNPs with clinal SNPs and find that while seasonal SNPs are weakly clinal, there is surprisingly little overlap. Taken together, our results highlight the abundance of balancing selection driven by seasonal variation in selection coefficients and corroborate models put forward by T. Dobzhansky over 50 years ago.

541A

Evidence of positive selection on sex biased genes in *Drosophila melanogaster*. Joseph R. Boland, Matthew E. B. Hansen, Craig E. Stanley, Jr., Rob J. Kulathinal. Department of Biology, Temple University, Philadelphia, PA.

Darwinian selection can effectively drive the fixation of alleles, leading to increased genetic diversity between populations and species. Using a windows-based, bioinformatics analysis of whole genome sequences from 300+ individuals across natural populations of Drosophila (from the DPGP and DGRP projects), we evaluate the presence of positive Darwinian selection on sex-biased genes. RNA-seq data from males and females (from the modENCODE project) were used to identify loci that exhibit differential gene expression between the sexes. The presence of selection in local populations of D. melanogaster from North America, Africa, and Europe was assessed by identifying regions that have a high density of derived or fixed alleles in addition to a employing a variety of tests of neutrality. Positively selected genes were also characterized according to enriched ontological classes (GO) and tissue-specificity.

542B

How looks like Drosophila in different Romanian ecosystems. Gallia A. Butnaru[1], Cristina Chelu[2], Cristina Popescu[3]. 1) Prof. Dept of Genetics, Banat Univ of Agricultural Sci ences and Veterinary Medicine from Timisoara, Romania; 2) Ingenuity Systems Inc., Redwood City, California, Romanian Branch; 3) West University "Vasile Goldis" from Arad, Romania.

To answer to ecological questions we have in mind the A. Krogh phrase "for many problems there is an animal on which it can be most conveniently studied". The Drosophila ecotypes were used as comparative model for the Trichogramma sp. studies. The Drosophila and Trichogramma ecotypes were collected from the same natural and anthropogenic polluted areas. To point out the "evolutionary development" and to establish the adaptive differences among the Drosophila and Trichogramma ecotypes was the main purpose of our research. Drosophila ecotypes characterization was based on two criteria: 1) morphological dimorphism and 2) evolutionary distance among them. 15 ecotypes were compared with w1118, ebony and Oregon genotypes. According to the first criterion there were two distinct groups; with a strong differentiation between females and males in case of Timisoara, Socodor, Nadab, Maru and Tg. Jiu ecotypes being closely related to w1118 genotype. Among Bucovat, Govora, Barzava, Rosia Montana, and Monorostia the sexual dimorphism was less expressed. The second criterion, genetic polymorphism was checked by RAPD method. The 10 markers used pointed out two distinct groups. The bands type emphasizes absence and presence of unique bands in Timisoara and Socodor respectively. The most interesting "ecotype" was PN - black body, sepia eye, long life cycle and an unusually pupation. Partially reproductive barrier is among PN and other strains and ecotypes. The size of PN L3 imaginal wing discs is significantly lower then the wild type. The most atypical phenotype of PN was observed in the fertilized eggs which exhibit 6 - 8 appendices. It is amazing its evolutionary development.

543C

Constraints on the evolution of plasticity in *Drosophila melanogaster*. Brandon S. Cooper[1], Loubna A. Hammad[2], Kristi L. Montooth[1]. 1) Department of Biology, Indiana University, Bloomington, IN; 2) METACyt Biochemical Analysis Center, Department of Chemistry, Indiana University, Bloomington, IN.

When environments vary greatly, alleles that enable the matching of the phenotype to the current environment should be favored by selection. Antagonistic pleiotropy and mutation accumulation, however, can create negative genetic correlations in fitness across environments leading to decreased performance of generalist relative to specialist genotypes. Our previous work has shown that an increased degree of cellular plasticity evolves in an experimentally variable environment, consistent with the selective advantage of an environmentally sensitive allele(s) with associated costs in constant environments. This evolution of increased cellular plasticity enables specialization within generations in environments that vary among generations. Here, we extend this work to natural populations by evaluating the evolution of cellular generalization and specialization in populations of *Drosophila melanogaster* from Vermont, Indiana, and North Carolina. To evaluate the evolution of cellular generalization, we quantified plasticity in two measures of cell membrane lipid composition: (1) change in the ratio of phosphatidylethanolamine to phosphatidylcholine and (2) change in lipid saturation in cool (16ºC) relative to warm (26ºC) developmental conditions. Within each developmental environment we also evaluated the evolution of cellular specialization in environments that differ in mean temperature. We will discuss these data in the context of (1) measures of genetic correlations between developmental and reversible plasticity of the cell membrane, and (2) our data from experimentally evolved populations that support the selective advantage of environmentally sensitive alleles that modify plastic responses of the cell in variable environments. While these alleles increase reproductive fitness, they also decrease tolerance to certain environments, resulting in antagonistic pleiotropy that may constrain the evolution of cellular plasticity in nature.

544A

Genomic basis of latitudinal differentiation among North American populations of Drosophila melanogaster. Thomas Flatt[1,2], Daniel K. Fabian[2], Martin Kapun[2], Viola Nolte[2], Robert Kofler[2], Paul S. Schmidt[3], Christian Schlötterer[2]. 1) Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland; 2) Institut für Populationsgenetik, Vetmeduni Vienna, Vienna, Austria; 3) Department of Biology, University of Pennsylvania, Philadelphia.

Understanding the genetic basis of clinal adaptation is a major but still largely unresolved problem in evolutionary genetics. Drosophila melanogaster, an ancestrally tropical insect that has spread to temperate regions and become cosmopolitan, offers a powerful opportunity for identifying the population genomic basis underlying clinal differentiation. Here we have applied genome-wide next generation sequencing of DNA pools ("pool-seq") to three populations collected along the North American

east coast (Southern Florida, Pennsylvania, Maine) in order to investigate patterns of latitudinal differentiation. Comparing the genomes of these populations is of particular interest since they exhibit strong clinal differentiation in a number of important life history traits, including body size, fecundity, lifespan and adult reproductive diapause. We document extensive latitudinal differentiation at the genic level, with many of the most strongly differentiated genes being involved in central signaling pathways such as the insulin/TOR, ecdysone, torso, EGFR, TGFβ/BMP, JAK/STAT, immunity and circadian rhythm pathways. Several of these pathways, and many of the candidates we have identified, have been previously implicated in the regulation of life history. Remarkably, the majority of our candidate genes and SNPs is located on chromosome 3R, especially within the inversion In(3R)Payne. Our results thus provide genome-wide evidence consistent with pervasive spatially varying selection along the well-known North American cline, with many candidates implicated in life history regulation and exhibiting parallel differentiation along the previously investigated Australian cline.

545B

Do Males Matter? Exploring Male-Mediated Effects on Female Meiotic Recombination. Chad M. Hunter, Nadia Singh. Department of Genetics, North Carolina State University, Raleigh, NC 27695.

Homologous recombination is a critical genetic process as well as a driving evolutionary force. Rates of crossing-over are highly variable within and between species due to both genetic and environmental factors. Early studies in Drosophila implicated female genetic background as a major determinant of recombination rate and recent work has highlighted male genetic background as a possible mediator as well. This latter result was puzzling since Drosophila males do not undergo meiotic recombination. We used classical genetics to address the question of how maternal and paternal genetic background affect crossover rate. We devised a two-step crossing scheme exploiting visible markers to measure rates of crossing over in a 33 cM region of the *D. melanogaster* X chromosome. In total, we measured crossover rates in females from ten inbred lines crossed to males from each of the same ten inbred lines. Our experimental design facilitates measuring the contributions of female genetic background, male genetic background, and male by female interaction effects on rates of crossing-over in females. Our results indicate that paternal genotypes do not significantly affect female meiotic crossover rates in Drosophila and that instead, maternal genotypes explain the majority of the observed variation in crossover rate. Our results have implications for deciphering the molecular and genetic basis of recombination rate variation in Drosophila.

546C

Identifying natural genetic variation for Drosophila melanogaster resistance to parasitoid wasp infection. Kate J Hutchence, Todd A Schlenke. Biology Department, Emory University, Atlanta, GA.

Drosophila melanogaster is a model system for the study of innate immunity and parasitoid wasp species are some of the most common pathogens of fruit flies in nature. Although the melanotic encapsulation response flies amount against wasp eggs is well-characterized at the cellular level, the genetic basis for the response remains poorly understood. The Drosophila Genetic Reference Panel (DGRP) is an excellent resource for making associations between phenotypic variation and genotypic variation, and especially for identifying mutations that affect fly immune success against wasps in nature. I have performed an association study using 98 DGRP lines to investigate the genetic basis of variation in D. melanogaster resistance to infection by the parasitoid wasp Leptopilina clavipes. DGRP strains were exposed to wasp attack and four phenotypic measures were taken: the proportion of fly larvae that (i) avoided infection, (ii) that successfully survived wasp attack, (iii) that died as a result of an overactive immune response, or (iv) that were successfully parasitized by the wasp. Significant variation was observed for all traits and a number of candidate anti-wasp immune genes were identified. I am now genetically testing and functionally characterizing several of the candidate genes.

547A

Variation in gene expression during embryogenesis in Drosophila strains and species. Asli Kayserili, Alex Kalinka, Pavel Tomancak. Max Planck Institute for Cell Biology and Genetics Pfotenhauerstrasse 108, Dresden, Germany.

In nature, we observe phenotypic variation within species as well as between species. The ratio of between-species to within-species variation can tell us about the type of evolutionary forces acting on the underlying genes. To discover how different genes involved in embryogenesis are evolving, we measured the variation of gene expression in early embryos of D. melanogaster inbred lines from North Carolina (DGRP). We focus on gene expression because we can directly analyse the effects of genetic variations in the regulatory elements. In this study, we analyse the amount of variation seen in inbred lines, and compare it to five of the sequenced Drosophila species. In addition, we estimate the importance of local adaptation by comparing D. melanogaster strains from North America with D. melanogaster strains from Africa. Our results show that there is substantial variation in gene expression during Drosophila embryogenesis, which is important for divergence between both populations and species.

548B

Natural genetic variation in chromatin state assessed by Position Effect Variegation. Keegan J. Kelsey, Andrew G. Clark. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Epigenetic modifications result in changes in gene expression, and there is growing interest in quantifying how much inter-individual variation in gene expression is driven by differences in epigenetic states. Similarly, because natural selection operating on gene expression may do so by modification of epigenetic state, we need to quantify population genetic forces

acting on epigenetic differences. We screened for variation in Position Effect Variegation (PEV) by crossing 123 wild-derived lines from the Drosophila Genetic Reference Panel (DGRP) to a common reporter stock, 1712, carrying the *white-mottled-4* allele (*In(1)w^{m4}* or simply *w^{m4}*) and a second chromosome balancer. Male progeny displayed a wide range of PEV in eye pigmentation, which was quantified through custom image analysis. We first asked whether known suppressors and enhancers of PEV [*Su(var)* and *E(var)*] harbor nucleotide sequence differences that were correlated with PEV phenotypes, and identified several. We then performed genome-wide association tests using a linear mixed model and identified additional loci that are candidates for further involvement in establishment and/or maintenance of chromatin state. This framework will provide a quantitative assessment of the role of epigenetic contribution to heritable variation in gene expression, uncover additional genetic factors that influence chromatin state, and begin to quantify how selection may act on these factors.

549C

Fruit flies prophylactically medicate offspring after seeing parasites. Todd A. Schlenke, Balint Z. Kacsoh, Zachary R. Lynch, Nathan T. Mortimer. Biol/O Wayne Rollins Res Ctr, Emory Univ, Atlanta, GA.

Hosts have numerous defenses against parasites, of which behavioral immune responses are an important but under-appreciated component. Here, I describe a behavioral immune response Drosophila melanogaster utilizes against endoparasitoid wasps. I found that adult flies detect wasp presence by sight and undergo a neuropeptide F-mediated oviposition behavior switch to lay eggs in food containing toxic levels of alcohol, which protects hatched larvae from wasp infection. This change in oviposition behavior is retained even after wasps are removed in a process dependent on the canonical long-term memory gene Adf1. Flies respond to diverse female larval endoparasitoids but not against male larval or female pupal endoparasitoids, showing they have evolved specific wasp search images. Furthermore, the response evolved in parallel with ethanol tolerance multiple times across the genus Drosophila. My data uncover a novel behavioral immune response based on anticipatory medication of offspring, and outline a novel non-associative memory paradigm based on innate parasite search image recognition by the host.

550A

Experimental evolution in *Drosophila* uncovers the importance of phenotypic plasticity and canalization for the evolution of gene expression in a changed environment. Christian W. Schloetterer[1], Miguel Gallach[1,2], Viola Nolte[1], Pablo Orozco-TerWengel[1,3], Eszter Ari[1]. 1) Inst f Populationsgenetik, Vetmeduni Vienna, Wien, Austria; 2) present address: Center for Integrative Bioinformatics Vienna, Max F. Perutz Laboratories, University of Vienna and Medical University of Vienna. Vienna, Austria; 3) present address: Cardiff University, Wales, UK.

The evolutionary forces shaping gene expression divergence are still poorly understood. Gene expression divergence between natural populations in their native environment is determined by non-genetic (i.e., plastic) and genetic (i.e., potentially adaptive) factors. Their relative importance can be determined by measuring gene expression of natural populations evolved in different environments but assayed under the same conditions (i.e., common garden experiments). Here, we analyze patterns of gene expression in experimental populations that have evolved for one year under temperate and tropical temperature regimes in the laboratory. Our results show that plasticity contributes much more (up to 66 % of the total expressed genes) than genetic change (7 %) to temperature-specific gene expression patterns. This suggests that plasticity in gene expression is more important for the short term response to a novel environment (i.e., temperature regime). Both evolved populations showed more variability in gene expression among replicates when they were cultivated in a non-native environment rather than their native one. This transcriptome-wide decanalization of gene expression in non-native environments demonstrates how non-selective forces contribute to gene expression divergence, raising the question to what extent environmental heterogeneity needs to be considered for the interpretation of inter- and intra-specific patterns of gene expression evolution.

551B

Genome-wide fine-scale recombination rate variation in Drosophila melanogaster. Yun S. Song[1,2], Andrew Chan[1], Paul Jenkins[3]. 1) Department of EECS, University of California, Berkeley, CA, USA; 2) Department of Statistics, University of California, Berkeley, CA, USA; 3) Department of Statistics, University of Warwick, Coventry, UK.

Estimating fine-scale recombination maps of Drosophila from population genomic data is a challenging problem, in particular because of the high background recombination rate. To address this challenge, we have developed a new computational method which allows more accurate inference, and exhibits greater robustness to the effects of natural selection and noise, compared to a well-used previous method developed for studying fine-scale recombination rate variation in the human genome. As an application, we have performed a genome-wide analysis of genetic variation data for two Drosophila melanogaster populations, one from North America (Raleigh, USA) and the other from Africa (Gikongoro, Rwanda). Our study shows that fine-scale recombination rate variation is widespread throughout the D. melanogaster genome, across all chromosomes and in both populations. At the fine-scale, a conservative, systematic search for evidence of recombination hotspots suggests the existence of a handful of putative hotspots each with at least a tenfold increase in intensity over the background rate. We have compared the estimated recombination maps in the two populations and quantified the extent to which recombination rates are conserved. In general, similarity is observed at very broad scales, but substantial differences are seen at fine scales. The average recombination rate of the X chromosome appears to be higher than that of the autosomes in both populations, and this pattern is much more pronounced in the African population than the North American population.

We have also examined the correlation between various genomic features, including recombination rates, diversity, divergence, GC content, gene content, and sequence quality; the most notable difference between D. melanogaster and humans is in the correlation between recombination and diversity.

552C

The genetic architecture of diet-dependent immune defense in *Drosophila*. Robert Unckless, Susan Rottschaefer, Brian Lazzaro. Department of Entomology, Cornell University, Ithaca, NY.

The ubiquitous genetic variation may be explained, in part, by heterogeneity in environments experienced by individuals within and between populations. Two fundamental parts of an individual's life are nutrition and immunity and there are several examples of how the two may influence each other. Here we examine the influence of diet on immunity and the genetic basis behind this interaction. We raised flies from the *Drosophila* Genetic Resource Panel (DGRP) on two diets (high and low sugar), then infected each with *Providencia rettgeri* and measured bacterial load 24-hours post infection. We also measured several nutritional indices (including glucose, protein, triglycerides and weight) for each line. Flies reared on the high sugar diet had much higher bacterial loads and there was a significant negative correlation between immune response and glucose, but only on the high sugar diet. Genome wide association tests revealed several candidate genes for both immune and nutritional index variation. Immunity candidates were validated using classical mutants. Two genes (*crinkled* and *Indy* contained SNPs significant only on the high sugar diet. Interestingly, the crinkled SNPs showed an interaction with the glucose correlation with load with one allele showing a negative correlation between load and glucose and the other no correlation at all. Thus, we show that diet influences immunity in flies and that the genetic basis of this interaction is tractable.

553A

Variation at the Cyp6g1 locus between two populations of *Drosophila Melanogaster*. Srna Vlaho, Matthew Salomon, Sergey Nuzhdin, Daniel Campo. MOLECULAR AND COMPUTATIONAL BIOL, UNIVERSITY OF SOUTHERN CALIFORNI, LOS ANGELES, CA.

Understanding how different populations adapt to their local environment is fundamental to the study of how natural selection shapes the genetic makeup of different organisms by selecting for particular phenotypes. This study analyzes the variation in the Cyp6g1 gene between two populations of *Drosophila melanogaster* from Winters, CA and Raleigh, NC. This gene is of particular interest because it is known to confer resistance to the insecticide DDT. Previous work found that an increase in DDT resistance was associated with an increase in the copy number of the Cyp6g1 gene. In fact, a 330 kb region containing that gene was found to be highly divergent between the Raleigh and Winters populations of *D. melanogaster*. We genotyped a large number of individuals from both populations, and found that the BA and AA alleles, which contain two copies of the Cyp6g1 gene, were the most common alleles. Yet there were statistically significant differences between the allelic frequencies in Winters and Raleigh. The BA allele, which has been shown to confer a greater resistance to pesticides, was more common in the Winters population that was collected from an organic orchard, than in the Raleigh population that was sampled in a farmers market. It is possible that the most resistant allele was positively selected during many years, and now that the selecting agent DDT is not present anymore, the allele frequencies are coming back to equilibrium. In addition, we discovered a new genotype in one of the Raleigh lines that could give it an enhanced resistance to pesticides. We are currently performing functional experiments with our populations to determine a relationship between the genotypes we observed and the level of resistance to pesticides.

554B

An extreme test of mutational meltdown in small populations. Ronny C. Woodruff. Dept Biological Sciences, Bowling Green State Univ, Bowling Green, OH.

In small populations, deleterious mutations arise and, because of inbreeding and drift, can go to fixation (become homozygous) over time. This slow decline in fitness may ultimately lead to extinctions. This process is called mutational meltdown. We tested mutational meltdown in small populations of Drosophila melanogaster that were previously inbred by single brother/sister matings for over 150 generations. Mutational meltdown did not occur in small populations (Ne = 2 to 4). Only one line went extinct out of 52 tested lines. In fact, there were significant increases in average progeny sizes over time that had to be caused by new beneficial mutations.

555C

Molecular Evolution of the Synaptonemal Complex in the genus Drosophila. Lucas Hemmer, Justin Blumenstiel. Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS.

The synaptonemal complex (SC) is a highly conserved meiotic structure seen across eukaryotes that functions to hold the homologs together during meiosis and facilitate exchange. Three Drosophila-exclusive proteins have been identified as the components: C(3)G, C(2)M and Cona. Each protein is necessary for proper meiotic function; mutations lead to reduced crossing over and chromosomal non-disjunction. Despite the conserved nature of the SC and the key role that these three proteins have in meiosis, they display little apparent sequence conservation. We are performing a molecular evolution analysis to determine the nature of selection that might explain this lack of apparent conservation. To perform this analysis, we searched 23 genomes across the Drosophila genus using tblastn and the D. melanogaster protein sequences as our query.

Several species of Drosophila have no recognizable sequences corresponding to these crucial SC components. With the recognizable hits, the sequences were aligned using translation MAFFT and MUSCLE and analyzed by Branch-site REL and GA Branch for evolutionary rates. Preliminary studies have demonstrated that the components of the SC are evolving with very little constraint and with very little adaptation, leaving questions as to how such essential proteins are undergoing such high rates of evolution.

556A

Intragenomic conflict drives rapid evolution of piRNA pathway genes in *Drosophila*. Jeffrey P Vedanayagam, Daniel Garrigan. Department of Biology, University of Rochester, Rochester, NY.

Piwi-interacting RNAs (piRNAs) defend against transposable elements (TEs) in the *Drosophila* germline. The piRNA pathway is a complex interplay of several genes whose function is essential for both piRNA biogenesis and TE silencing. Here, we analyze coding sequence polymorphism data from 25 genes involved in piRNA pathway in the two closely related species *D. simulans* and *D. mauritiana*. Species-specific McDonald-Kreitman test finds that eight of the genes show evidence of a significantly increased rate of amino acid substitution. *D. simulans* has an increased rate in *armi*, *Hen1*, *spn-E*, *vas*,*aub*, *krimp* and *zuc* genes, while *D. mauritiana* has an increased rate of amino acid substitution in *csul* gene. *armi*, *spn-E* and *vasa* are putative RNA helicases, and *mael* and *zuc* are putative nucleases. All these genes are known to be crucial for piRNA biogenesis. *aub* is essential for ping-pong amplification of piRNAs, while *krimp* is essential to recruit *aub* to nuage - a peri-nuclear organelle where piRNA biogenesis occur. *csul* ensures the methylation of symmetric arginine residues of piRNA effector proteins during piRNA biogenesis. Subsequently, we find that the piRNA pathway is enriched for genes experiencing positive selection relative to three randomly chosen molecular pathways: miRNA pathway, Notch-signaling pathway and NK cytotoxicity pathway. Compared to these random pathways, we also find that the piRNA pathway is enriched for species-specific amino acid substitutions that are fixed in one species and absent in the other species, contributing to species differences. Furthermore, a sliding window K_A/K_S analysis of piRNA pathway genes to *D. melanogaster* shows that highly divergent regions correspond to key protein domains in these genes. Comparative genomic analysis of these protein domains in the *Drosophila* phylogeny reveals that these domains evolve three times faster when present in a piRNA pathway gene as opposed to a non-piRNA pathway gene. Overall, these results provide candidate loci for transgenic studies of the effect of piRNA machinery on hybrid fitness in interspecific crosses.

557B

Using Experimental Evolution to Study Temporal Responses of the Genome to Selection. Julien F. Ayroles[1,2], Lawrence G. Harshman[3], Jennifer Grenier[2], Andrew G. Clark[2]. 1) OEB, Harvard, Cambridge, MA; 2) MBG, Cornell, Ithaca, NY; 3) School of Biological Sciences, Univ. of Nebraska, Lincoln, NE.

For nearly a century, experimental evolution has been a favorite tool of biologists seeking to test evolutionary models. By coupling this approach with DNA sequencing, we can obtain a detailed view of the dynamics of evolutionary change. For example, how acccurately does the Wright-Fisher model capture the true variability in evolutionary trajectories of neutral alleles? How similar are the trajectories of selected alleles across replicates? To address these questions, we used a bulk segregant analysis to describe standing genetic variation associated with starvation resistance in a base population. Using next generation sequencing of DNA pools drawn from these starvation selection experiments, we quantified allele frequency dynamics over 15 generations for every segregating nucleotide in the genome in 4 selected and 4 control lines. We observed a strong phenotypic response associated with changes in allele frequency at hundreds of loci. Here, we present a novel analytical framework using methods borrowed from signal processing theory that enables the description not only of changes in allele frequency at individual loci, but also the covariance of allele frequency dynamics across linked sites. From the covariance matrix of allele frequency changes we assembled genetic networks for the selection response, highlighting the importance of non-additive effects in shaping evolutionary trajectories. Our analysis yields a fit to quantitative models for the temporal dynamics of genome-wide allele frequency changes across discrete generations, and the observed over-dispersion compared to Wright-Fisher provides an improved null model for assessment of the impact of selection on the genome.

558C

Adaptive trait dissection in non-model *Drosophila*: Using next-gen sequencing to fine-map a naturally-occurring polymorphism in the sexually-selected cuticular hydrocarbons of *D. serrata*. Stephen F. Chenoweth, Bosco Rusuwa, Francesca Frentiu. Biological Sciences, University of Queensland, Brisbane, Queensland, Australia.

A fundamental goal of evolutionary genetics is to establish links between the phenotypes that have facilitated adaptation, and their underlying genetic basis. The fly *Drosophila serrata* has proven to be an apt model system in which to investigate the evolution of traits affected by not only natural selection but also sexual selection. The cuticular hydrocarbons (CHCs) of *D. serrata*, function in mate and species recognition and also vary clinally along a latitudinal gradient, suggesting they are sensitive to climatic variation. However, the precise genetic underpinnings of CHC expression in *D. serrata* are yet to be determined. One conspicuous feature of the cline is a major-effect variant that segregates in tropical populations of *D. serrata* and declines in frequency towards temperate populations. To dissect this variant we have performed 1) a *de novo* genome assembly, 2) classic QTL mapping, supplemented with 3) next-generation bulked segregant analysis of advanced intercrosses and, 4) resequencing of multiple mutant and wild-type genomes. We isolate a small region a small region on 3R of the *D. serrata* genome enriched for sets of tandemly duplicated desaturase and elongase genes, each with likely functions in

the CHC biosynthetic pathway. This region is then further interrogated using expression analysis. Fitness assays from polymorphic populations show that the variant likely has sexually antagonistic fitness effects - decreasing the mating success of males but increasing female desiccation resistance. Our results suggest that the polymorphism may be maintained though a balance between opposing natural and sexual selection.

559A

Genetic basis of natural variation in cuticular hydrocarbons in the *Drosophila melanogaster* Genetic Reference Panel. Lauren Dembeck[1,3], Katalin Böröczky[2,3], Michael Maguire[1,3], Richard Lyman[1], Coby Schal[2,3], Trudy Mackay[1,2,3]. 1) Department of Genetics, North Carolina State University, Raleigh, NC; 2) Department of Entomology, North Carolina State University, Raleigh, NC; 3) W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC.

Drosophila melanogaster mate choice is strongly influenced by the presence of contact pheromones, which consist of cuticular hydrocarbons (CHCs), on the insect's cuticle. Hence, variation in CHCs can potentially alter mate choice leading to assortative mating and incipient speciation. We studied natural variation in CHCs using the *Drosophila melanogaster* Genetic Reference Panel (DGRP). The DGRP is a panel of inbred lines of *D. melanogaster* derived from a natural population at the Raleigh, NC, Farmer's Market. Complete genome sequences are available for the lines, which enable genome-wide association (GWA) analyses to uncover the genetic basis of natural variation in CHCs. We collected gas chromatography spectra of DGRP flies and quantified relative abundance of CHC components for both sexes. We identified 69 female and 36 male CHCs, including several dienes and methyl alkanes that were not previously described. A majority of the CHCs show heritable phenotypic variation including the known pheromonal compounds. Our preliminary GWAs did not identify obvious candidate genes; however, we find polymorphisms in or near many genes not previously known to affect natural variation in CHCs. Finally, we conducted correlation analyses of variation in CHCs with other traits such as mate choice and aggression. These results may offer insight into the link between variation in pheromone blend with behavior and incipient speciation.

560B

A genome-wide association approach to characterize natural genetic variation in the plastic response of mated lifespan and age-specific fecundity to diet in *Drosophila melanogaster*. Mary F. Durham[1], Michael M. Magwire[2], Jeff Leips[1]. 1) Dept Biological Sci, Univ Maryland, Baltimore County, Baltimore, MD; 2) Department of Genetics, N.C. State University, Raleigh, NC.

Nearly every organism experiences a wide array of variation in environmental conditions for which no single phenotype or genotype is universally advantageous. One method of maximizing individual fitness in these varying environments is to utilize phenotypic plasticity: the ability of a single genotype to produce one or more distinct, repeatable phenotypes in response to a change in a single environmental variable. Although the occurrence of phenotypic plasticity has been well demonstrated, the genetic mechanisms that drive plastic responses are poorly understood. There are two main hypotheses on the genetic mechanisms underlying phenotypic plasticity, “allelic sensitivity” and “gene regulation”. Allelic sensitivity posits that the effects of alleles on phenotypes are sensitive to the environment and so produce different phenotypes in different environments. In this case, alleles influencing plasticity will co-localize with the trait genes. The gene regulation hypothesis suggests that independent regulatory genetic components modulate the response of other genes that directly influence the focal phenotypes in varying environments, thus these plasticity genes are independent of the trait genes. Modification of the dietary regiment has been shown to result in phenotypic plasticity of lifespan and fecundity in a wide variety of organisms. We have completed a genome-wide association study on lifespan and fecundity of mated females under two different dietary regimes using the *Drosophila melanogaster* Genetic Reference Panel to identify naturally occurring candidate genes involved in the plastic response of lifespan and fecundity to changes in dietary protein content. We found evidence to support both the allelic sensitivity hypothesis and the gene regulation hypothesis of phenotypic plasticity.

561C

Building a better mousetrap: High-throughput, high-parameter analysis of *Drosophila* aggression gives novel insight into the genetic architecture of behavior. Bryn E. Gaertner, Landon Blakey, Kirsty Ward, Trudy F. C. Mackay. Genetics, North Carolina State University, Raleigh, NC.

Aggression, a physical confrontation resulting from disputes over territory or resources, is ubiquitous in the animal kingdom. Beneath this broad definition, individuals within and between species vary remarkably with respect to the circumstances and persistence of aggression, resulting in natural variation from passive and permissive to overt antagonism. Such is the case in a wild-derived genetic reference panel of *Drosophila melanogaster*, where in stressful (food deprived) conditions, inbred lines vary from almost no aggression to more than one aggressive encounter every second. Attempting to map the genetic basis to this trait has proven difficult, as epistasis is pervasive and a statistical signal may be lost because the term “aggression” covers many different behaviors, each of which may have its own unique genetic architecture. To address these issues, we have set out to build a novel experimental regime that allows for high-throughput recording of pairs of *Drosophila* males. These videos are subsequently analyzed for all described aggression subtypes, such as wing-flicking, boxing, kicking, and chasing. Using this approach, we have identified important differences in behavioral subtypes among inbred lines as well as their hybrid progeny. Because of the high-parameter method of phenotyping, we can then map the genetic basis of these differences to a handful of candidate genes.

562A

Understanding the effects of Insecticides using Genome-Wide Association Studies. Llewellyn Green[1,2], Josh Schmidt[1,2], Bec Smith[1,2], Paul Battlay[1,2], Charles Robin[1,2]. 1) Genetics, The University of Melbourne, Melbourne, Victoria, Australia; 2) The Bio21 Institute, Melbourne, Victoria, Australia.

The Drosophila melanogaster Genetic Reference Panel consists of 192 highly inbred lines, 162 of which have had their genomes sequenced [1]. These lines form a 'living library', where limitless numbers of flies of each genotype can be phenotype for any trait. The genetic variants contributing to the traits can then be identified using genome-wide association methods. We report on three insecticide screens of the DGRP and the experiments we have employed to validate the genes implicated by these screens. Three important perspectives into the genetic basis of insecticide biology arise from these studies. Firstly, we can identify polygenes underlying insecticide 'resistance' and assess them for 'footprints' of positive selection. This allows us to determine environmental exposure of non-target organism to insecticides, and address the debate of the role of polygenes and monogenes in insecticide resistance evolution. Secondly, we can identify genetic variants that affect resistance to new insecticides (specifically those that target the Ryanodine receptor) and thereby help predict the mechanisms of field resistance before it can arise and compromise pest control. Thirdly, we have gained insights into the biological processes that are perturbed by insecticides, which are supposed to have distinct modes of action. Perhaps not surprisingly these include several genes involved in neurogenesis and calcium homeostasis. Among the genes uncovered by these studies is a previously poorly characterized muscle associated, protein-kinase-like gene. We dub this gene 'knocked-up', as reduced function alleles increased resistance to a DDT-knockdown phenotype. [1]. Mackay et al. (2012) The Drosophila melanogaster Genetic Reference Panel. Nature 482 p173-178.

563B

Bayesian multi-phenotype genome-wide association for structured experimental designs. Anthony J. Greenberg[1,2], Gabriel E. Hoffman[1], Pavel Korniliev[1], Yuxin Shi[1], Susan McCouch[2], Jean-Luc Jannink[2], Jason Mezey[1]. 1) Dept BSCB, Cornell Univ, Ithaca, NY; 2) Dept PBG, Cornell Univ, Ithaca, NY.

The quest to understand the relationship between genetic and phenotypic variation underpins most fundamental research in evolutionary genetics. It is also critically important for progress in applied fields, such as plant and animal breeding to improve efficiency of food production. With the development of dense single nucleotide polymorphism (SNP) markers, it has become possible to assess genotype-phenotype relationships by conducting genome-wide association (GWA) studies. However, these studies are statistically and computationally challenging due to the presence of confounding factors, such as population structure, and the massive amount of SNP and phenotype data. In model organisms, such as Drosophila, phenotypic measurements for GWA are typically performed on inbred lines that are reared in a replicated experiment. Statistical methods developed so far are typically unable to both account for confounding effects and deal for complex experimental designs, especially when multiple phenotypes are considered at once. We developed a Bayesian hierarchical approach that jointly estimates quantitative-genetic parameters for multiple phenotypes, corrects the influence of population structure, and estimates pleiotropic and single-phenotype effects of individual SNPs. The method can incorporate experimental designs of arbitrary complexity, including crosses among lines. We test our method on extensive simulations and apply it to a data set of wing measurements in a set of D. melanogaster lines from the DGRP collection.

564C

Sperm length predicts female sperm loads in Drosophila species in the wild. Hiroto Kameyama[1], Esra Durmaz[2], Giovanni Hanna[1], Therese Markow[1]. 1) University of California, San Diego, Division of Biological Sciences, 9500 Gilman Dr., La Jolla, CA 92093; 2) Hacettepe University, Faculty of Science, Department of Biology, 06800 Cankaya, Ankara / TURKEY.

Sperm length in Drosophila varies from .35mm in D. subobscura to 56 mm in D. bifurca. In species characterized by long sperm, males produce far fewer sperm than do those in short sperm species. We predicted that females of the long sperm species should be sperm limited in nature. We tested this prediction by collecting, from nature, females of species with a range of different sperm lengths allowing them to use up their sperm by transferring them to fresh food vials daily and counting the progeny they produced. Species with relatively short sperm, such as D. subobscura, D. pseudoobscura, and D. melanogaster, contained sufficient sperm supply to produce offspring for up to two weeks, while species such as D. hydei, which produces a 23mm long sperm, ran out of sperm after one day and after having produced an average of 20 progeny. We discuss the implications of these observations for reproductive and evolutionary biology.

565A

Genetic dissection of genomewide expression variation in the Drosophila female brain. Elizabeth G. King[1], B. Sanderson[2], Casey L. McNeil[2], Anthony D. Long[1], Stuart J. Macdonald[2]. 1) Ecology & Evolutionary Biology, UC Irvine, Irvine, CA; 2) Department of Molecular Biosciences, University of Kansas, 1200 Sunnyside Avenue, Lawrence, Kansas 66045.

Drosophila melanogaster is widely employed as a model genetic system to understand fundamental aspects of the control of complex trait variation in populations. In addition, the system is increasingly recognized as an important translational model for the study of human neurodegenerative disease and the action of drugs of abuse. As with all complex, polygenic traits identifying the molecular pathways and causative genes responsible for variation in these phenotypes is challenging. Given the community interest in genetically dissecting neurobehavioral traits in flies, we took advantage of a novel resource for genetic analysis to characterize quantitative variation in transcript abundance in Drosophila heads. The Drosophila Synthetic

Population Resource (DPSR) is composed of over 1,600 genotyped Recombinant Inbred Lines (RILs) derived from a pair of highly-recombinant synthetic laboratory populations. These two populations were each initially founded by a different set of eight founder strains, ensuring high functional allelic diversity in the DSPR. We generated 600 heterozygous genotypes - the progeny of intercrosses between DSPR lines from the different populations - isolated RNA from mated adult female heads, and subjected each sample to microarray analysis. These data allow us to construct gene networks and capture the full biological complexity of the pathways involved in gene regulation in the Drosophila head and brain. Genomewide expression QTL (eQTL) analysis also provides a high-resolution picture of the location, effect, and population frequency of loci that influence expression variation in the head, and allows us to examine the relative abundance of cis- and trans-regulatory loci. In addition, researchers using the DSPR to genetically dissect neuronal or behavioral phenotypes will be able to exploit our eQTL data for a systems-level analysis of trait variation, and quickly home in on likely candidate genes.

566B

Developmental noise and evolution of a new stable bristle pattern in D. santomea. Virginie Orgogozo[1], Isabelle Nuez[1], Amir Yassin[1], Daniel Matute[2], David Stern[3], Jean David[1]. 1) Institut Jacques Monod, CNRS UMR7592, Paris, France; 2) University of Chicago, Chicago, USA; 3) Janelia Farm, Ashburn, USA.

How new robust phenotypes evolve despite developmental noise remains unclear. *Drosophila santomea* is unique among the nine *D. melanogaster* subgroup species in that it has lost a pair of bristles surrounding the phallus. All the tested *D. santomea* flies displayed 0 bristles and the other species 2 bristles at various raising temperatures, indicating that both the ancestral and the evolved phenotypes are stable. Nevertheless, a few hybrids between *D. santomea* and *D. yakuba* display an unstable phenotype, with only one genitalia bristle (left side for some individuals, right for others, fluctuating asymmetry). To map the genomic region(s) responsible for this loss of genitalia bristles, we performed backcrosses in both directions between *D. santomea* and *D. yakuba* and we genotyped all individuals with the Multiplexed Shotgun Genotyping (MSG) method. The largest effect is found on the left tip of chromosome X, which includes the *achaete-scute* gene complex, a complex encoding transcriptions factors that activate bristle formation. At least two other genomic regions are involved, and each corresponding *D. santomea* allele increases the probability of genitalia bristle loss. Interestingly, introgression lines carrying the entire *achaete-scute* gene complex from *D. santomea* in a *D. yakuba* background show that the *D. santomea* introgressed part is not sufficient in itself to cause hypandrial bristle loss. However, this introgressed region does reduce bristle number in presence of other *D. santomea* alleles (in hybrids between introgression lines and *D. santomea*). Our data are consistent with a threshold model where *D. santomea* alleles decrease the value of a continuous variable. Evolution of a new stable phenotype appears to require a large-effect QTL to pass over the instability range.

567C

Mitochondrial genotypes drive differential expression of nuclear genes under varied levels of hypoxia in Drosophila. David M. Rand, Yawei Ge, Nicholas Jourjine, Patrick Flight. Ecology & Evolution, Brown Univ, Providence, RI.

When organisms encounter reduced oxygen tension, or hypoxia, they reduce cellular demand for oxygen by down-regulating mitochondrial functions through altered expression of nuclear genes, mediated by the HIF pathway. Despite the central role mitochondria play in oxygen consumption, the effect of mitochondrial genotypes on the hypoxic response has not been examined. Here we use mtDNA introgression strains of Drosophila to examine the effects of alternative mtDNA-encoded genes on the nuclear transcriptional response to varied hypoxia. Flies carrying mtDNA from either D. melanogaster OreR, D. melanogaster Zimbabwe, D. simulans siI, or D. simulans siII on a D. melanogaster OreR nuclear chromosomal background were constructed using balancer substitutions and maternal cytoplasm from these four genotypes. We studied the expression profiles of these genotypes under two general conditions: 1) a gradient of hypoxic stress for 2 hours (normoxia, 6%, 3%, and 1.5% oxygen), and 2) a time course of 1.5% oxygen for 1, 2, 3, and 4 hours. Expression profiles of whole males were determined using Affymetrix 2.0 arrays. The mtDNA genotype design allows for association of alternative mtDNAs within a species, or fixed differences between Dmel and Dsim mtDNAs as drivers of nuclear gene expression, in trans. MtDNAs have subtle effects on gene expression under normoxia (~30 genes altered), but have pronounced effects at 3% (>200 genes altered) and 6% oxygen (~500 genes altered). These results are confirmed in the time course study, with gene expression effects peaking at 3 hours and subsiding by 4 hours. In each case the species-level differences between mtDNAs (Dmel vs. Dsim) drive different sets of genes than the individual mtDNA haplotypes within either species (OreR vs. Zimbabwe or siI vs. siII). These results provide strong evidence for mitochondrial retrograde signaling in the nuclear transcriptional response to hypoxia and offer the first evidence that genes in mtDNA play a critical role in modulating the transcriptional response to hypoxia under different levels of hypoxic stress.

568A

Verification of single nucleotide polymorphisms affecting sleep in *Drosophila*. Yazmin L. Serrano, Susan T. Harbison. Laboratory of Systems Genetics, National Heart Lung and Blood Institute, Bethesda, MD.

Human genome-wide association studies (GWAS) have been used to identify thousands of Single Nucleotide Polymorphisms (SNPs) associated with complex traits and disease. Demonstrating the causality of these SNPs is difficult in human populations; however, it may be possible to validate candidate polymorphisms and determine their likely mechanism of action in genetically tractable model organisms such as*Drosophila*. Here we employ an artificial selection procedure to validate candidate SNPs for sleep, a complex trait whose genetic basis is not well understood. In a previous study using the fully

sequenced*Drosophila* Genetic Reference Panel (DGRP), we observed significant genetic variation for night sleep duration. Association of 2,490,165 non-singleton SNPs in the DGRP with night sleep revealed 160 significant SNPs. We have chosen a subset of 96 SNPs from this study for further validation. We designed SNP genotyping assays based on Taqman PCR chemistry and verified their ability to distinguish allelic differences among the five longest- and five shortest-sleeping DGRP lines. We crossed these lines in a full diallel design and measured night sleep in the resulting progeny. Most of the progeny from crosses between long sleepers and short sleepers had intermediate levels of sleep, suggesting that the SNP haplotypes behave additively; however, some haplotypes were dominant. To determine the effect of single SNP alleles on sleep, we created an outbred population by mating the progeny of this cross randomly for 15 generations, allowing long-sleeper and short-sleeper alleles to recombine. From the outbred population, we created duplicate selection lines for long sleep and short sleep, along with an unselected control. We are currently measuring sleep and SNP genotypes in successive generations to verify the key SNPs involved in sleep.

569B

Genetic and plastic effects for body melanisation in cold adapted - D. takahashii. Shama Singh. ZOOLOGY, UNIVERSITY OF DELHI, DELHI, India.

Drosophila takahashii - exhibits broad variation for segmentwise melanisation resulting from genetic polymorphism and/or phenotypic plasticity. We analyzed segmentwise reaction norms for melanisation in D. takahashii. Wild individuals of D. takahashii were investigated for variation in frequency of body melanisation collected from highland localities of Indian subcontinent. Changes in melanisation of all segments showed higher effect of growth temperatures. Significant differences were observed between reaction norms (slope values) of melanisation in three anterior vs. posterior abdominal segments in this species. In the present study, first three abdominal segments showed continuous variation leading to plastic effects whereas, genetic polymorphism (heterozygote plastic effects) was observed for posterior abdominal segments (5th, 6th and 7th) due to variable dominance in 5th and 6th; and complete dominance in 7th segment. The present study has shown genetic variability as well plastic response for body melanisation in cold adapted- D. takahashii.

570C

Epistatic interactions are prevalent in Drosophila 3'UTR evolution. Ying Zhen, Peter Andolfatto. Ecology and Evolutionary Biology, Princeton University, Princeton, NJ.

It has been shown that a large fraction of noncoding DNA in Drosophila is evolutionarily constrained and may be target of substantial adaptive evolution. With the available population polymorphism data in Drosophila melanogaster and D. simulans, we identify candidate 3UTRs showing strongest evidence of recurrent adaptive evolution. In a panel of five these candidate 3'UTRs, we use GAL4-driven Luciferase-3UTR reporter assays in D. melanogaster transgenic lines to test the effects of 3'UTR divergence on the levels of gene expression divergence between species, controlling for chromosome location and genomic context. In addition, chimeric 3'UTRs are used to understand how substitutions interact and contribute to changes in gene expression. Our results indicate that in all of our five 3'UTR candidates sequence divergence is associated with differences in gene expression and that expression divergence is the result of complex interactions among substitutions, and epistatic interactions are prevalent. We also find that the interaction between substitutions and the final expression difference output is sex and tissue specific.

571A

Assaying functional divergence in the hybrid incompatibility gene *Hybrid male rescue* (*Hmr*) between *D. melanogaster* and *D. simulans*. Tawny N. Cuykendall, Daniel A. Barbash. Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY.

Hybrid lethality and sterility are examples of post-zygotic reproductive isolating barriers between species. Crosses between *Drosophila melanogaster* females and *D. simulans* males, which diverged ~3 MYA, produce viable but sterile daughters, and sons which die as 3rd instar larvae. Lethality is caused by an epistatic interaction in the hybrid background between *D. melanogaster Hybrid male rescue*(*Hmr*) and *D. simulans Lethal hybrid rescue* (*Lhr*). The hybrid lethal activity of *Hmr* is unique to the *D. melanogaster* ortholog. We are interested in explaining this functional divergence of *Hmr* between *D. melanogaster* and *D. simulans*. Our experiments have two goals: 1) to determine the intraspecific function of HMR and 2) to identify functions of *Hmr* that have diverged between orthologs. Using a 3X-HA epitope-tagged *Hmr* transgene injected in *D. melanogaster*, we find that HMR, like LHR, localizes to heterochromatin. We have used fluorescent in situ hybridization (FISH) to more finely map HMR to specific satellite sequences in the *D. melanogaster* genome, including dodeca, 2L3L, and AG-rich repeats. We have also generated a transgenic line expressing *D. simulans Hmr* in a *D. melanogaster*background and are currently testing whether *D. simulans* HMR localizes to heterochromatin in a foreign species background. Additionally, we are performing RNA-seq on transgenic lines expressing either*D. melanogaster Hmr* or *D. simulans Hmr* in an *Hmr* mutant background to investigate how HMR affects gene expression and whether it has diverged between these two species with respect to its function in gene regulation.

572B

Natural selection acts across species boundaries in *Drosophila simulans* and *D. sechellia*. Daniel Garrigan, Sarah Kingan, Cara Brand. Department of Biology, University of Rochester, Rochester, NY.

Until recently, it was believed that *Drosophila simulans* and *D. sechellia* diverged in allopatry. However, a whole-genome resequencing study reveals that at least 5% of these two genomes has experienced gene flow after their initial divergence. Here, we resequence a 20 kb region of chromosome *3R* that shows the most pronounced evidence for recent gene flow. We find a haplogroup that introgressed from *D. simulans* to *D. sechellia* approximately 10,000 years ago and occurs at high frequency in both species. In *D. sechellia*, a core region of the introgressed haplogroup is fixed in our sample, while in *D. simulans*, the core region is nearly fixed and the haplogroup varies in length among our samples. Patterns of both allele frequencies and linkage disequilibrium support the hypothesis that positive natural selection has recently elevated the introgressed haplogroup to high frequency in both species. Interestingly, the target of natural selection centers on a cluster of transcription factor binding sites adjacent to a homeobox transcription factor gene. However, the introgressed haplogroup also harbors two genes involved in olfaction that have hitchhiked to high frequency in *D. sechellia*. While we currently cannot detail the phenotype being targeted by natural selection, the resequence data are unique in that they show evidence for an adaptive mutation that is globally advantageous across divergent genomic backgrounds and ecological conditions.

573C

Nuclear introgression in *Drosophila subobscura* and *D. madeirensis* despite distinct mitochondrial genomes. Danielle Herrig, Ana Llopart. 143 Biology Building University of Iowa, Iowa City, IA.

Speciation typically occurs when a single species splits into two populations in which gene flow is severely reduced. Over time, the two populations accumulate genetic differences that eventually produce two independent species. While hybridization between two species, and thus the potential for gene flow, has been traditionally viewed as a reproductive mistake, recent studies suggest that it is not as rare as once believed. In this study, we examine if gene flow has occurred in the sister species *Drosophila madeirensis* and *D. subobscura*. *D. madeirensis* is endemic to the ecologically unique island of Madeira while *D. subobscura* is distributed throughout the palearctic region. Both species are now found on Madeira Island where they hybridize and produce partially fertile hybrids. We obtained the sequence of 26 loci randomly distributed throughout the genome in 33 lines (16 from each sister species and one from the outgroup *D. guanche*). Our data includes genes on the Y chromosome and the mitochondrial genome. To estimate gene flow after the split, we used maximum likelihood methods applied to multilocus data as well as individual genes. Our results indicate that there has been effective gene exchange between the two species. In particular we detect *D. subobscura* genes in a largely *D. madeirensis* genetic background. Interestingly, this signature of introgression does not include the mitochondrial genome and is limited to a small fraction of the nuclear genes.

574A

Formation of reproductive barriers in a hybrid zone of American and Caribbean *Drosophila melanogaster*. Joyce Kao, Sergey Nuzhdin. University of Southern California, Los Angeles, CA.

In order to understand the interaction between gene flow and adaptation in *Drosophila melanogaster*, multiple populations have been collected from different locations along the southeast US and the Carribean Islands. We specifically focus on studying the existence of reproductive barriers in populations collected from regions where two allopatric populations interbreed again after being separated for a long period of time by which each individual population has accumulated new alleles adapting to their separate environments. The southeast US and the Carribean Islands are a recent hybrid zone between two distinct populations that diverged 10000 years ago: the European flies which colonized the US with the European settlers and the African flies which were introduced into the Caribbean islands via the trans-Atlantic slave trade. Previous studies have already established the existence of clines in several pre-zygotic/pre-mating reproductive characteristics in flies out of this area. We have phenotyped post-mating traits in female flies including re-mating rates, egg laying rates, and post-mating lifespan and have found evidence that some of these traits may be post-mating reproductive barriers. We also have evidence that partial temporal isolation might be occurring in this cline. Genome-wide signatures of selection especially in genes relating to pre- and post-mating reproductive barriers will be examined by re-sequencing 23 isofemale lines from this area of the world.

575B

Patterns of divergence reveal genomic "islands of speciation" in young semispecies of *Drosophila athabasca*. Karen M. Wong Miller[1], Michael B. Eisen[2,3], Doris Bachtrog[1]. 1) Department of Integrative Biology, University of California, Berkeley, CA; 2) Department of Molecular and Cell Biology, University of California, Berkeley, CA; 3) Howard Hughes Medical Institute, University of California, Berkeley, CA.

Previous studies utilizing *Drosophila* have contributed significantly to our understanding of speciation and particularly postzygotic reproductive isolation. However, the difficulty in teasing apart the factors that are important to the actual process of speciation, rather than those that have secondarily accumulated with time has been widely noted. By examining very recently diverged systems, we are more likely to identify the molecular mechanisms responsible for speciation. We use next-generation sequencing technologies to collect whole-genome resequencing data for 28 individuals of *Drosophila athabasca*, a North American species complex composed of three semispecies. The semispecies are morphologically identical, have overlapping ranges, and show no evidence of postzygotic isolation, however they exhibit behavioral isolation in the form of semispecies-specific male courtship song. With estimated divergence times ranging from ~5-20 kya, the semispecies of *D. athabasca* are among the youngest incipient species known within *Drosophila*. We quantified levels of genome-wide diversity

and differentiation within and between semispecies and find ~2 Mb of variable sites within *D. athabasca*, with only 1% of variable sites being private and fixed within semispecies. Furthermore, we find divergence is not evenly distributed across the genome, with the X-chromosome exhibiting increased levels of divergence compared with autosomes, and genome-wide scans showing evidence for "islands of speciation" between semispecies. The likely presence of variants driving reproductive isolation in these regions, with relatively low levels of divergence throughout the rest of the genome, establishes *D. athabasca* as an excellent model to study not only the genetic mechanisms driving prezygotic isolation, but also how genomes diverge during incipient speciation.

576C

The hybrid incompatibility gene Lhr represses repetitive elements. Satyaki P. Rajavasireddy, Shuqing Ji, Daniel A Barbash. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Heterochromatin keeps in check selfish elements such as transposable elements and satellites that will otherwise increase their copy number and potentially reduce host fitness. These selfish elements have been proposed to be in a continuous arms race with heterochromatin proteins, driving their sequence divergence and causing interspecific hybrid incompatibility (HI). However, direct evidence linking HI with genetic conflicts between selfish DNA and heterochromatin is limited. *Lhr* encodes a rapidly evolving, HP1a interacting, heterochromatin protein that causes hybrid lethality between *D. melanogaster* and *D. simulans*. To determine whether *Lhr* evolved to suppress genetic conflicts requires understanding its intraspecific function . We therefore knocked-out *Lhr* via homologous recombination in *D. melanogaster*and discovered that *Lhr* mutant (Lhr^{KO}) females have reduced fertility. mRNA-seq analysis shows that *Lhr* loss leads to widespread increase in the abundance of transcripts from transposable elements (TEs) and satellite DNAs but has little effect on protein-coding genes. ChIP and reporter gene studies suggest that this increased abundance of TE transcripts is due to a defect in post-transcriptional TE repression. We further found that Lhr^{KO} flies have long telomeres, which we propose is due to derepression of telomeric TEs as well as to LHR protein being required at the telomere cap in the wild type. We have also performed an RNA-seq analysis of a *D. simulans Lhr* mutant strain, providing a rare opportunity to compare the function of an adaptively evolving heterochromatin protein between two species. We are currently analyzing these data to determine if the *Lhr*-dependent regulation of specific TEs or satellite DNAs has changed between *D. melanogaster* and *D. simulans*. Our data strongly suggest that selfish elements are a direct selection pressure driving the divergence of *Lhr* and are thus consistent with the arms race model.

577A

Transgenerational effects on *Drosophila melanogaster* populations detected after specific environmental changes. Patricia Ramos-Morales, Adriana MuÑoz, Blanca R. Hernandez, Hugo Rivas, Armando MuÑoz. FACULTAD DE CIENCIAS, UNAM, MEXICO.

Introduction. The change in the inherited characteristics of biological populations occurring through successive generations leads to the emergence of new varieties and species of organisms. How much time is required for the changes to be measurable? How long the changes in environmental conditions continue to affect populations? Hydroxyurea (HxU) is an antimetabolite, which is cytostatic by inhibiting ribonucleoside reductase, and is widely used to treat some metabolic diseases. Metabolic activation of HxU increases its potential for genetic damage; being hydrogen peroxide a metabolic product of HxU. Aim: to determine the effect of HxU on the reproductive activity of exposed flies and monitoring the impact through successive generations. Methods: From 32 mM HxU, eighteen concentrations mixed on the food were assayed. Third instar larvae were fed chronically and the effects were analyzed on the adult flies. Toxicity was determined as: experimental flies/control flies; and the Sex rate as the proportion of females (or males)/total of flies into each vial. The transgenerational effect of a single exposure was established using wild type flies (wt) and insecticide resistant flies which differ in their capacity to transform xenobiotics (ORR). For the reprotoxic effect, 10 couples of treated flies were put individually into vials to recover the F1. From F2 to F4 generations, two couples from five different families were randomly chosen and transferred to fresh media to produce the next generation. Results: In the first generation, progeny from wt and ORR exposed flies was more numerous than that from unexposed flies. For F2-F4 generations, progeny from wt exposed flies trend was similar to that from control flies or larger but the reverse effect was found for ORR flies. Discussion: Hx Treatment modified the reproductive function on exposed flies and their descendents, which suggest an epigenetic potential for HxU. Acknowledgment: to the Genetic and Environmental Toxicology Program'Students.

578B

JAK/Stat signaling in the *D. melanogaster* cellular immune response. Susanna E. Brantley, Nathan Mortimer, Todd Schlenke. Biology, Emory University, Atlanta, GA.

The JAK/Stat pathway is a conserved signaling cascade involved in development and cell proliferation in *Drosophila* and other lineages, including vertebrates. Recent reviews cite the important role of JAK/Stat signaling in vertebrate innate immunity, autoimmunity, leukemias, and lymphomas. Our lab studies the immune response of *Drosophila* after attack by parasitic wasps, a cellular immune response that may depend on JAK/Stat activity. In this response, hematopoiesis and hemocyte differentiation lead to increased numbers of blood cells called lamellocytes, which are actively involved in the encapsulation of wasp eggs. Gain-of-function mutations in JAK/Stat pathway genes have been shown to induce lamellocyte differentiation in unattacked flies. Here we use two fly strains to delineate the role of JAK/Stat in the encapsulation response more closely: a StatGFP reporter and a fly with a gain of function mutation in *Drosophila* JAK (hopscotch). We show that

JAK/Stat signaling is activated in specific temporal and spatial patterns in response to infection by avirulent wasps. Furthermore, we show that some virulent wasps upset lamellocyte differentiation signaling via the JAK/Stat pathway as a virulence strategy.

579C

Variation in fly transcriptional responses after infection by diverse endoparasitoid wasp species. Lindsey C Fallis, Todd A Schlenke. Biology Department, Emory University, Atlanta, GA.

Endoparasitoid wasps inject eggs into host hemocoels and flies attempt to encapsulate and kill the eggs by forming a multicellular hemocytic capsule. However, wasps inject venom cocktails with their eggs to suppress host immune function. Previous work showed tremendous variation in the fly transcriptional response to infection by two virulent Figitid wasp species suggesting closely related wasps can use vastly different immune suppressive virulence strategies. In this study I used *Drosophila melanogaster* as a common host to uncover variation in the ability of 10 different Figitid wasp species to suppress particular components of the fly transcriptional response to infection. Specifically, I used RNA-seq to assay the fly immune response to each wasp at three time points post-infection (0-4, 4-8, 8-12 hours). Using these data, I will identify the immune pathway targets of specific wasp venoms and identify associations between venom content, virulence effects, and wasp natural history.

580A

A role for nematocytes in the cellular immune response of the Drosophilid *Zaprionus indianus*. Balint Z. Kacsoh, Julianna Bozler, Todd A. Schlenke. Biology, Emory University, Atlanta, GA.

The fruit fly *Drosophila melanogaster* has served as a model system for understanding innate immune responses such as the humoral induction of antimicrobial peptides by the Toll and Imd pathways. However, the cellular melanotic encapsulation response mounted by *D. melanogaster* against macroparasites, which is based on hemocyte binding to the foreign object, is poorly characterized and appears to be variable across insect lineages. The genus Zaprionus is a diverse clade of flies imbedded within the genus Drosophila. I have characterized the immune response of *Z. indianus* against endoparasitoid wasp eggs, which elicit the melanotic encapsulation response in *D. melanogaster*. I found that *Z. indianus* is highly resistant to a diverse panel of wasps. Although *Z. indianus* mounts the canonical melanotic encapsulation response against some wasp species, it can also fight off wasp infection using two other mechanisms *D. melanogaster* is not known to possess: encapsulation without melanization and non-cellular killing potentially based on wasp egg chorion disruption. *Z. indianus* produces a large number of hemocytes including nematocytes, which are large fusiform hemocytes absent in *D. melanogaster*, but which I found in several other species in the subgenus Drosophila. Several lines of evidence suggest these nematocytes are involved in anti-wasp immunity in *Z. indianus* and in particular in the encapsulation of wasp eggs. Altogether, my data show that the canonical anti-wasp immune response and hemocyte makeup of the model organism *D. melanogaster* vary across the genus Drosophila.

581B

Extracellular matrix-modulated FGF signaling in *Drosophila* blood progenitors regulates their differentiation via a ras/ETS/FOG pathway and TORC1 function

. Julian A. Martinez-Agosto, Michelle Dragojlovic-Munther. Department of Human Genetics, University of California Los Angeles, Los Angeles, CA.

Maintenance of hematopoietic progenitors ensures a continuous supply of blood cells during the lifespan of an organism. In particular, these progenitors are required for the cellular immune response against foreign pathogens and tissue injury. A large pool of undifferentiated blood progenitors are maintained in the *Drosophila* hematopoietic organ, the larval lymph gland (LG), by a complex network of signaling pathways that are mediated by niche-, progenitor-, or differentiated hemocyte-derived signals. We have examined the function of the *Drosophila* Fibroblast Growth Factor receptor (FGFR), Heartless (Htl), a critical regulator of early LG progenitor specification in the late embryo, during later hematopoiesis. Activation of Htl signaling in hemocyte progenitors by its two ligands, Pyramus (Pyr) and Thisbe (Ths), is both required and sufficient to induce progenitor differentiation and formation of the plasmatocyte-rich LG cortical zone. We identify two transcriptional regulators that function downstream of Htl signaling in LG progenitors, the ETS protein, Pointed, and the Friend-of-GATA (FOG) protein, U-Shaped, which are required downstream of Ras signaling for this Htl-induced differentiation response. Furthermore, cross-talk of FGFR and Target of Rapamycin (TOR) signaling in hemocyte progenitors is required for lamellocyte differentiation downstream of Ths-mediated Htl activation. Finally, we identify the *Drosophila*heparan sulfate proteoglycan (HSPG), Trol, as a crucial negative regulator of FGF signaling in the LG. Trol knockdown promotes precocious progenitor differentiation, and this phenotype is rescued by downregulation of Pyr or Ths, demonstrating the interaction between Trol and FGF ligands. These findings demonstrate that sequestration of differentiation signals by the extracellular matrix is a unique mechanism employed in blood progenitor maintenance that is of potential relevance to the cellular immune response and many other stem cell niches.

582C

Immune self recognition in Drosophila: *tuSz* mutants exhibit an aberrant self-directed immune response. Nathan T. Mortimer, Todd A. Schlenke. Department of Biology, Emory University, Atlanta, GA.

Drosophila melanogaster larvae are commonly infected by parasitoid wasps and mount a robust cellular immune response following parasitization. This immune response culminates in the melanotic encapsulation of the wasp egg, however, the molecular mechanisms by which fly immune cells recognize the wasp egg as foreign are unknown. Flies mount a similar encapsulation response against xenografts or abiotic objects, suggesting that rather than non-self recognition, the melanotic encapsulation response may be initiated by 'missing-self' recognition. In this process, immune cells recognize a distinctive mark on self tissues, and the absence of this mark is sufficient to direct an immune response against a foreign object. The identity of the *D. melanogaster* self signal is unknown but mutations have been isolated in which larvae mount a self-directed cellular immune response, presumably due to the absence of, or the inability to recognize, such a signal. To gain insight into the mechanisms underlying this system, I have chosen to characterize one such mutant, *tumor(1)Suzuki* (*tuSz*), in which caudal fat body tissue is targeted for self-encapsulation. I found that the phenotype genetically maps to two closely linked loci. One mutation maps to *hopscotch*, the D. melanogaster JAK homolog, and causes ectopic activation of the cellular immune response. The mutation in the second locus disrupts protein N-glycosylation, and I found that both mutations are required for the self-encapsulation phenotype. These findings suggest that the *D. melanogaster* self mark is dependent on protein N-glycosylation, and that this mark is recognized by immune cells following activation of the cellular immune response.

583A

The role of hemocytes in the control of viral replication and disease in vivo. Javier Robalino, Louisa Wu. Institute for Bioscience and Biotechnology Research, Dept. of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742.

Several pathways have been implicated in the innate antiviral response of flies, including RNAi, autophagy, JAK/STAT, Toll, and Imd signaling. Apart from RNAi, for which the mechanisms of action are well understood, the ways in which these other immune pathways participate in the control of viral replication remain largely unknown. Moreover, in flies and other invertebrates, the contributions of specific cell types to the antiviral response at an organismal level have remained mostly unexplored. We show that plasmatocytes, the major hemocyte type in the adult fly, effectively and preferentially internalize Drosophila X Virus (DXV) particles introduced into the hemolymph, and their role in this context is to restrict the replication of the virus. In spite of avidly capturing virus from circulation, hemocytes are not a major site of viral replication in vivo, and decreasing the number of viable hemocytes by targeted cell ablation leads to increased susceptibility to viral infection. This organismal level, hemocyte-mediated antiviral protection, critically requires the beta subunit of the Signal Recognition Particle Receptor (SRPRB). Down-regulation of SRPRB leads to uncontrolled DXV replication within hemocytes, and this is sufficient for a dramatic increase in the susceptibility of the fly to infection with this virus. Surprisingly, down-regulation in hemocytes of several of the subunits of the Signal Recognition Particle (SRP) and of the Sec61 translocon, which act upstream and downstream, respectively, of the SRP receptor during protein translocation into the ER, do not cause increased viral replication. We hypothesize a unique role for SRPRB in the ability of hemocytes to restrict viral replication, a role that is likely independent of its canonical function in co-translational targeting of membrane and secreted proteins.

584B

RpS6 is a substrate of PALLBEARER and a negative regulator of apoptotic cell clearance in Drosophila. Hui Xiao, Nathalie Franc. The Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA.

The swift removal of apoptotic cells by phagocytes is a critical event during development of all multi-cellular organisms. Yet, little is known about the molecular mechanisms regulating phagocytosis of apoptotic cells. We previously demonstrated a role for the ubiquitin-proteasome pathway in promoting efficient phagocytosis of apoptotic cells via the PALL-SCF complex composed of SkpA, dCullin-1 and the F-box protein PALLBEARER (PALL). In these complexes, the F-Box protein provides the substrate-binding function and specificity. Identifying PALL substrates is important to further our understanding of how the ubiquitin-proteasome promotes phagocytosis. We have identified the ribosomal protein RpS6 as a putative substrate for PALL by co-immunoprecipitation and mass spectrometry. RpS6 plays a role in immune cells, as RpS6 mutants have melanotic tumors, the sign of an aberrant immune response, as well as enlarged hemocytes. RpS6 knock-down enhances apoptotic cell clearance in S2 cells. Furthermore, overexpression of RpS6 in embryonic macrophages results in decreased phagocytosis in vivo. In addition, RpS6 genetically interacts with the pall null allele, which we recently generated by homologous recombination, further showing that RpS6 acts in the same pathway as pall and that RpS6 can suppress the pall mutant phenotype. We propose that RpS6 is a substrate of PALL that negative regulates phagocytosis of apoptotic cells, and are now investigating the detailed mechanisms of action of RpS6 in phagocytosis.

585C

Production and Verification of *Drosophila melanogaster* Nora virus Monospecific Antisera. Morgan Caron Abert, Brad L. Ericson, Darby J. Carlson, Kimberly A. Carlson. Biology, University of Nebraska at Kearney, Kearney, NE.

Nora virus is a picorna-like virus that has four open reading frames (ORFs), as opposed to the one long ORF found in most members of this group. The coding potentials of the ORFS are not fully characterized, but ORF1, ORF3, and ORF4 are believed to code capsid proteins. There are three viral proteins identified in ORF4 that are of interest, VP4A, VP4B, and VP4C. The purpose of this study was to produce monospecific antisera to purified whole Nora virus and purified recombinant VP4B, and to evaluate the specificity of both via Western blot analysis. Nora virus was purified from infected*Drosophila melanogaster* on CsCl gradients and His-tagged recombinant Nora virus VP4B protein was purified on Ni^{+2} columns. Both whole virus and VP4B

were injected into mice to make polyclonal antibodies. The resulting monospecific antisera were evaluated in Western Blot assays. The results showed that a majority of the predicted Nora virus structural proteins were detected using whole virus antiserum. Monospecific antiserum against VP4B detected two proteins in purified virus. The production and validation of monospecific antisera is a useful tool to investigate other aspects of Nora virus such as replication sites in host flies and the location of the various structural proteins in the virion. This work was partially made possible by Grant Number P20GM103427 from the National Institute for General Medical Science, a component of the National Institutes of Health.

586A

The Larval Clot and Immune Defense. Clara I. Bajzek, Mitchell S. Dushay. BCHS, Illinois Institute of Technology, Chicago, Il.

Since the identification of larval clotting factors in *Drosophila*, experiments have been performed to study how they interact to form the clot and to understand the clot's physiological and immune functions. Loss of the major clotting factor Hemolectin abolishes coagulation *in vitro*, but *hml* mutant larvae show surprisingly small decreases in wound survival. This indicated the presence of other, still-poorly understood hemostatic mechanisms, and the limited value of wound survival as an *in vivo* clotting assay. We developed a method using microcapillary tubes to measure how much injured larvae bleed. The capillary assay revealed a greater difference between *hml* and control larvae than shown by wound survival. To further reduce coagulation, we tested *fon*; *hml* double mutants. To our surprise, these larvae survived wounding as well as *hml* alone. Loss of *fon* altered the clot's physical properties, but apparently did not reduce coagulation. Fondue is a major substrate for transglutaminase - the only clotting factor shared by *Drosophila* and vertebrates (factor XIIIA). Transglutaminase RNAi knockdown reduced coagulation by *in vitro* assay and these larvae were more susceptible to infection. Yet *hml* mutant larvae were not consistently more prone to infection, making it difficult to evaluate the importance of the clot in immune defense. These seemingly conflicting results were obtained using different bacterial challenges and different protocols. We have tested feeding and wandering stage third instar larvae bearing combinations of mutations in *hml, fon, tiggrin, Eig71Ee*, and transglutaminase RNAi knockdown by *in vitro*and *in vivo* clot assays and by needle puncture infection to learn more about how Hemolectin and Transglutaminase work together, the nature of the clot, and its involvement in immune defense.

587B

Route of Nora virus transmission in *Drosophila melanogaster*. Justin L. Buchanan, Brad L. Ericson, Darby J. Carlson, Kimberly A. Carlson. Biology Department, University of Nebraska at Kearney, Kearney, NE.

Nora virus was recently discovered in four species of *Drosophila* and one species of *Nasonia*. This virus is classified in the *Picornaviridae*, which includes human pathogenic viruses such as Hepatitis C virus, Poliovirus, and Rhinoviruses. The mode for transmission of Nora virus has yet to be conclusively shown. The proposed modes of transmission in *D. melanogaster* are fecal-oral and vertical (gamete transmission). To examine the mode of transmission and site of replication, we used infected female *D. melanogaster*, removed their digestive tracts and ovaries, and tested each separately for the presence of Nora virus. Reverse transcription-polymerase chain reaction with Nora virus specific primers, and Western blot analysis were used to detect the virus. The results showed that Nora virus was primarily localized to the gut (trace amounts were found associated with the ovaries). Nora virus associated with the ovaries was able to be washed away, suggesting that the virus was a surface contaminant. Therefore, the mode of transmission appears not to be vertical. Previous work using dechorionated eggs from infected flies showed that flies could be cured of the virus via this method. These observations are all consistent with a fecal-oral route of transmission and not a vertical transmission rout e for Nora virus. This work was made possible by Grant Number P20GM103427 from the National Institute for General Medical Science, a component of the National Institutes of Health.

588C

RNA-Seq analysis of the *Drosophila* transcriptome in response to infection by entomopathogenic nematodes and their mutualistic bacteria. Julio C. Castillo, Ioannis Eleftherianos. The George Washington University, Department of Biological Sciences, Washington, DC 20052.

The *Drosophila* immune system is capable of activating a variety of responses against microbial infections. However, how the fly responds to parasitic infections is currently unknown. The nematode*Heterorhabditis bacteriophora* is an insect parasite that forms a mutualistic relationship with the gram-negative bacterium *Photorhabdus luminescens*. Following infection, the nematodes release*Photorhabdus* bacteria that quickly multiply within the insect and produce several toxins that eventually kill the host. Although we currently know that the insect immune system interacts with *Photorhabdus*, information on similar interaction with the nematode vector is lacking. The objective of the current study is to identify the number and nature of *Drosophila* genes that are regulated upon infection with the nematode and its bacteria. We have used next generation RNA-sequencing to analyze the transcriptional profile of adult flies infected by axenic *Heterorhabditis* nematodes (lacking *Photorhabdus* bacteria), symbiotic *Heterorhabditis* nematodes (carrying *Photorhabdus* bacteria), and *Photorhabdus* alone. In total, we have obtained around 54 million reads from the different infection types. Preliminary analysis shows that *Photorhabdus* infection induces several genes encoding recognition and antibacterial effector molecules in *Drosophila*. Interestingly, *Heterorhabditis* infection leads to regulation of genes that are involved in lipid homeostasis and metabolism. Our data provide valuable information on what *Drosophila* genes are activated or repressed following infection with the two pathogens, either separately or together. This study provides exciting clues on the molecular immune events that may take place in *Drosophila* upon infection with a potent entomopathogenic nematode-bacteria complex. Such large-scale

transcriptomics studies set the stage for future functional studies aimed at identifying the exact role of key factors in the *Drosophila* anti-nematode immune response.

589A

Severity of chronic infections depends on the amount of dietary sugar. Moria C. Chambers, Chloe Ota, Ilana Porges, Brian P. Lazzaro. Entomology, Cornell University, Ithaca, NY.

Metabolic state impacts response to infection and vice versa. Discerning the patterns of metabolic-immune interaction will help us make better predictions about how manipulating host metabolism could improve patient outcomes. We infected *Drosophila melanogaster* with pathogens binned into two kinetic classes, acute and chronic, and tested whether dietary sugar content affected adult flies' defense against each class of infection in a predictable manner. We defined acute infection as infection that kills flies within a few days, while chronic infection produces a median time to death of greater than 5 days while sustaining high bacterial loads. During acute infections, amount of dietary sugar had no significant effect. In contrast, feeding flies a high sugar diet during chronic infections resulted in higher bacterial loads and reduced survival as compared to flies fed on a low sugar diet. Our interpretation is that metabolic or physiological alteration influences the severity of chronic infection by altering the host "environment" from the perspective of the pathogen, but that acute infections are comparatively unconstrained so subtle host physiological differences have no effect. We determined the importance of diet and metabolic regulation as related to the timing of infection using diet switch experiments and drug inducible RNAi knockdown of metabolic regulators. To the extent that infection kinetics associate with metabolic response and sensitivity, we can harness kinetic data to propose metabolic interventions to limit infection and manage symptoms.

590B

Neural primordium as a target of Spiroplasma-induced male killing in Drosophila melanogaster. Trisha N Chong, Jennifer C Martin, Patrick M Ferree. W. M. Keck Science Department, The Claremont Colleges, Claremont, CA.

Male killing bacteria are widespread pathogens of the arthropods and can have dramatic effects on the population dynamics of these host organisms. However, the specific molecular and cellular targets are currently unknown. We have performed developmental analyses of D. melanogaster males that are targeted by the male killing bacterium, Spiroplasma poulsonii. Previous studies in another host species, D. nebulosa, suggested that male killing occurs during two phases: embryonic and larval. We used sex-specific fluorescence probes and the yellow1 visible marker in order to distinguish between infected D. melanogaster males and females at all developmental stages so that we could carefully analyze the development of both sexes. We found that over 99% of infected male embryos fail to hatch into first instar larvae. Rare escaper males that developed to the third instar stage exhibited only marginally reduced body size, salivary gland size, brain size, and brain cell mitotic index. Thus, the primary lethal phase in this host-pathogen system is embryonic. Confocal microscopic analyses of dying male embryos revealed that early cleavage stages progressed normally. However, stage 12-13 embryos showed dramatic morphological defects that mapped to the neural primordium. Using an antibody against Neuroglian to visualize the neural tissues, we observed that the neural primordium became increasingly disorganized during later stages, with severely broken glanglia and irregular ventral nerve cord. In contrast, mesoderm and endoderm-derived tissues appeared normal during stage 12-13 and also at earlier stages. These results suggest that the male-killing effect originates specifically in the neural tissues. Additionally, our studies revealed that ~40% of females die as embryos. However, female embryos show normal neural morphology. Thus, Spiroplasma kills males specifically by targeting the neural tissues but also kills female embryos in an alternate manner. These findings will greatly facilitate ongoing studies aimed at identifying the exact molecular target(s) of male killing.

591C

Regulation of energetic metabolism by adenosine during parasitic wasp infection. Tomas Dolezal[1,2], Adam Bajgar[2], Katerina Kucerova[2]. 1) Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA; 2) Department of Molecular Biology, Faculty of Science, University of South Bohemia in Ceske Budejovice, Czech Republic.

Immune response is energetically demanding process and precise regulation is crucial since both over- and under-stimulation of energy release may have deadly consequences. Although there is a lot of information about the changes of metabolism itself during immune response, we know very little about how these changes are regulated. We are using parasitic wasp infection of Drosophila larvae as a model to study effects of extracellular adenosine during infection. Our results show that the adenosine signaling is required for a circulating glucose increase during infection and that a blocking of this effect results in a decrease of immune cells proliferation and differentiation which in turn affects the outcomes of immune response against the parasite. We are further using this model to dissect mechanisms of the adenosine production and signaling during immune response. Our model has a potential to demonstrate for the first time that the extracellular adenosine is perfectly suitable signal for very dynamic and fine-tuned regulation of energetic metabolism during immune response.

592A

Sex-specific immune response against bacterial infection. David Duneau, Brian Lazzaro. Entomology, Cornell university, Ithaca, NY.

Host allocations of resources into immunity are of a prime importance for parasites. Variations in resource allocation strategies among hosts are expected to influence parasite success and therefore influence parasite evolution. Males and

females are often strikingly dimorphic for many traits. We hypothesize that sex differences in immune allocation could effectively make males and females into distinct "types" of hosts, offering different challenges and opportunities to their parasites. We therefore are examining differences in immune responses of male and female *Drosophila melanogaster* facing a bacterial infection. We infected both sexes of *Drosophila melanogaster* with its natural Gram-negative bacteria *Providencia rettgeri*. We determined that bacterial load is higher in males than females at 24h after infection, despite their smaller body size. We used qPCR to measure the constitutive and dynamic post-infection expression of five immune-related genes (antimicrobial peptides; AMPs). The constitutive expression of these AMP is very low in both sexes but female hosts show faster induction after infection. This and other measures of immunological performance indicate a true difference between the sexes in immunological capacity, which can influence host-pathogen coevolution and the epidemiology of disease. Such sex differences will be particularly important to the epidemiology of infection in populations of animals with sex-biased social structures.

593B

Characterization of an anti-wasp response gene in Drosophila. Erin S. Keebaugh, Todd A. Schlenke. Dept Biol, Emory Univ, Altanta, GA.

Parasitic wasps lay eggs in Drosophila larvae that hatch, consume larval tissues, and eclose from the fly pupal case, killing the fly in the process. In addition to eggs, wasps also inject venom proteins that serve to aid in wasp success. Drosophila larvae can respond to wasp infection by melanotic encapsulation, where fly hemocytes form a capsule around and kill the entrapped wasp egg. Microarray analysis of Drosophila larvae post-wasp infection identified several promising candidate anti-wasp genes including an extracellular C-type lectin, *lectin-24A*. Expression analysis of *lectin-24A* shows enriched expression in the larval fat body, regulation by the Imd/JNK pathway, and a wasp-specific regulatory response. Flies mutant for *lectin-24A* are less successful in encapsulating wasp eggs and surviving wasp attack. I have generated an anti-Lectin-24a antibody to determine whether Lectin-24a binds to wasp eggs or host tissues in infected flies. I have also generated a *lectin-24a* reporter construct to further dissect its temporal, spatial, and genetic regulation and to determine whether venoms of certain wasp species suppress induction of *lectin-24a* as part of their virulence mechanism. If so, I will analyze venom fractions from these wasps to determine the venom components necessary for the inhibitory effect.

594C

Evaluation of a Yeast Expression System to Direct Assembly of the *Drosophila melanogaster* Nora virus. Kellie D. Licking-Murray, Brad L. Ericson, Darby J. Carlson, Kimberly A. Carlson. Biology, University of Nebraska at Kearney, Kearney, NE.

Nora virus is a recently discovered RNA picorna-like virus that produces a persistent infection in *Drosophila melanogaster*. This virus is of interest because it is similar to the human picornaviruses that are responsible for human diseases, such as polio, hepatitis A, foot and mouth disease, and the common cold. The Nora virus RNA genome is approximately 12,000 bases long and is split up into four open reading frames (ORF1, -2, -3 and -4). ORF4 is most likely expressed as a polyprotein that is cleaved into three polypeptides by the viral-encoded protease, and these are designated as viral protein (VP) 4A, VP4B, and VP4C. These three viral proteins are thought to be the major capsid components of the virus, making ORF4 of particular interest in how this virus assembles. For this study, a yeast protein expression system was transformed and the genes from ORF1, -3, and -4 were cloned into the yeast system to express these genes of interest. Additionally, ORF1 and ORF3, and all three ORF4 proteins, were combined into one yeast clone to determine if virus-like particles can be assembled. The Nora virus assembly pathway is not yet known, but an understanding of this could lead to a deeper comprehension of how picornaviruses, in general, undergo assembly. This work was made possible by Grant Number P20GM103427 from the National Institute for General Medical Science, a component of the National Institutes of Health.

595A

Identification of *Drosophila* genes involved in recovery from infection using an inducible RNAi system. Karla L. Lightfield, David S. Schneider. Microgiology and Immunology, Stanford University, Stanford, CA.

The ability of a host to survive infection is dependent upon a variety of host (and microbial) physiologies and responses, however current research tends to focus more on immune effectors and the destruction of the invading pathogen. The host must also cope with the damage that is caused by the pathogen and the resultant immune response in addition to returning host physiologies to normal (healthy) levels during the recovery process. The goal of this project is to identify genes involved in regulating recovery from infection. To this end we use an inducible RNAi system that allows us to knock down gene expression during the recovery process while limiting developmental influences. We are currently screening a variety of genes by infecting RNAi mutant *Drosophila* with *Listeria monocytogenes*, allowing the infection to proceed as normal for 48 hours, then treating the flies with ampicillin, which in normal flies cures the infection. Flies that are not able to clear the infection after ampicillin treatment will be studied further.

596B

Identification of recovery mechanisms from infection. Alexander Louie, David Schneider. Microbiology and Immunology, Stanford University, Stanford, CA.

During an effective immune response, pathogens are eliminated and host physiologies return to baseline. When we discuss

infection we do a good job of describing immune effectors but seldom look at recovery. Our goal is to identify the mechanisms that drive recovery from infection. We developed a model system with two criteria. 1) The flies get sick and then recover from infection. 2) We can monitor the effects of microbe levels on pathology. Using this system, we tracked global gene expression changes across the full course of infection in recovering, moribund, and control files. When we plot gene expression levels against microbe load in phase space for all genes, we find that the phase plots group into eight categories: positive and negative linear correlations, clockwise and counter-clockwise loops, positive and negative switches, complex and no change. Phase space analysis puts genes into groups that make biological sense. For example, many of the known immunity genes group together in the positive linear correlations. Phase space is a simple framework for taking apart biological systems. Furthermore, we identified a group of genes that spike as microbe numbers decline. One of these genes is wntD. When we infect and treat wntD mutants they fail to fully recover from infection despite retaining the ability to clear bacteria. wntD expression is required for recovery from infection.

597C

Antiviral Autophagy in *Drosophila*. Jerome M Molleston, Ryan H Moy, Beth Gold, Sara Cherry. Microbiology, Penn Genome Frontiers Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA.

Autophagy is a highly conserved pathway for degradation of intracellular components and plays a role in immune defense. Our previous studies found that Vesicular Stomatitis virus, an insect-borne virus transmitted to vertebrates, is recognized by a pattern recognition receptor, Toll-7, to induce an antiviral autophagy program in cells and adult flies. Indeed, mutations in Toll-7 or core autophagy components reverts a non-pathogenic infection into a lethal one. This has led us to explore the role of autophagy against additional viruses including Sindbis virus, West Nile virus, and Rift Valley Fever Virus, which represent viruses from the three major families of human arthropod-borne viruses. We are exploring this using genetic and biochemical approaches. We will discuss our findings on the induction of autophagy upon infection and whether autophagy is necessary for control of these viruses. In addition, for those viruses that are restricted by autophagy in insects we are extending our studies to mammalian systems where we are examining whether autophagy may underlie defense against viral infections in across hosts spanning from arthropods to mammals.

598A

Role of Nora virus VP3 protein in *Drosophila melanogaster* infection. Sajna Anand Sadanandan[1*], Jens-Ola EkstrÖm[1], Dan Hultmark[1,2]. 1) DEPARTMENT OF MOLECULAR BIOLOGY, UMEÅ UNIVERSITY, UMEÅ, SWEDEN; 2) INSTITUE OF BIOMEDICAL TECHNOLOGY, UNIVERSITY OF TAMPERE, TAMPERE, FINLAND.

Nora virus is a picorna-like virus causing persistent, non-pathological infection in *Drosophila melanogaster*. Nora virus genome consists of four open reading frames (ORF), where the C-terminus of the ORF1-encoded VP1 protein is an RNAi inhibitor, ORF2 encodes the replicative cassette and ORF4 encodes the major capsid proteins. The function of the ORF3-encoded VP3 protein is under investigation. On gel electrophoresis of viral proteins from purified virus particles, VP3 appears with the VP4 capsid proteins, suggesting that it is involved with the viral capsid. To further study the role of VP3 we constructed mutants 1) where we introduced stop codons such that expression of VP3 is eliminated 2) where we removed 54 nucleotides within the predicted coiled-coil domain of VP3. The effect of these mutations was tested in a wild-type fly stock. The first mutant, wherein VP3 is not produced, affects the transmission of the virus. Viral genome replication could be detected in animals injected with an infective cDNA construct, but not in their offspring. On analyzing for presence of virus particles from injected animals and their feces, we could detect virus particles only in the whole flies. These results suggest that the absence of VP3 makes Nora virus incapable of transmission via the fecal-oral route.

599B

Identification of *Pseudomonas aeruginosa* virulence factors in a *Drosophila melanogaster* intestinal infection model. Haller Samantha, Ferrandon Dominique. UPR9022 , CNRS, Université de Strasbourg, IBMC, Strasbourg, France.

Pseudomonas aeruginosa is an opportunistic pathogen able to infect plants, *Drosophila melanogaster* and humans. *P. aeruginosa* is the third most common pathogen at the origin of nosocomial infection and is multi-resistant against antibiotics. Therefore, there is a need to find novel therapeutic targets. Although *P. aeruginosa* pathogenicity has been extensively studied, mostly in vitro, the interactions between the *Drosophila* immune response of the host, especially the cellular immune response, and *P. aeruginosa* remain poorly understood.

Previously, we have shown that some orally ingested *P. aeruginosa* (PA14) bacteria escape the intestinal barrier and proliferate in the hemolymph until the flies succumb to systemic infection, *i.e.*, bacteremia (Limmer *et al*., 2011). Moreover, we found that the quorum sensing transcription factor RhlR plays a key role in circumventing the cellular immune response against PA14. The same intestinal infection model was used to identify important virulence factors of PA14 and genes potentially mediating *rhlR*'s phenotype. We have screened in wild-type *Drosophila* 348 mutants PA14 that displayed an attenuated virulence in an intestinal infection model in *C. elegans* (Feinbaum *et al*., 2012). 20 PA14 mutants exhibited a reproducible attenuated virulence phenotype. Among these bacterial mutants, we found mutants for genes implicated in peptidoglycan remodeling, transcription and regulation of gene transcription, type two secretion system, exotoxins, metabolism, and some known virulence factors. Five of these bacterial mutants behave like *rhlR* mutants in phagocytosis-blocked *Drosophila*, that is, they recover full virulence. Thus they represent candidates for genes that are regulated by RhlR. Unexpectedly, we discovered that RhlR's function in circumventing the cellular immune response is likely independent from

its role in quorum sensing (perception of the concentration of a C4-homoserine lactone), a situation never described before.

600C

Elucidating the mechanistic basis for the trade-off between reproduction and immunity in female *D. melanogaster*. Robin A. Schwenke, Brian P. Lazzaro. Cornell University, Ithaca, NY.

Immune defense and reproduction are critical components of fitness and have been documented to trade-off in numerous biological systems. Although these trade-offs are pervasive, the underlying physiological mechanisms remain poorly defined. Female *Drosophila melanogaster* become more susceptible to infection after mating, suggesting a physiological trade-off between reproduction and immunity. Additionally, prior results have shown that females are genetically variable for the severity of this trade-off suggesting the potential for an evolutionary trade-off. We are interested in unraveling the mechanistic basis for this trade-off in order to understand the function and evolution of the traits involved. Using a series of egg development mutants, we found that the effect of mating on immunity is erased in the absence of a germline, but that the trade-off persists if egg production is halted downstream of germline formation. Furthermore, we have found that mated females remain immunosuppressed even after egg production returns to virgin levels due to sperm depletion. This finding suggests that post-mating immunosuppression is driven by a 'permanent' physiological shift away from virgin homeostasis. We are currently investigating the role endocrine signaling in regulating this shift toward decreased immune defenses in mated females.

601A

Effects of host diet on the tradeoff between mating and immunity in *Drosophila melanogaster*. Parvin Shahrestani, Brian Lazzaro. Entomology, Cornell University, Ithaca, NY.

Female *Drosophila melanogaster* experience post-mating depression of immune defense, suggesting a trade-off between mating or reproduction and immune defense. Both reproduction and immune defense are physiologically demanding and may therefore be mutually limiting as a function of finite energetic reserves in the host. Therefore, the severity of the post-mating immune depression may depend on the condition of the host, which in turn may depend on the diet of the host. I tested the severity of the post-mating immune depression on numerous diets comprised of varying amounts and relative abundances of glucose and protein. The severity of the post-mating immune depression was diet specific, and also depended on the timing of the administration of each diet. Dietary protein seems to be of particular importance, perhaps because of the major amino acid requirements of both egg provisioning and induction of the immune response.

602B

Role of Thioester-containing proteins in the immune response of *Drosophila* against entomopathogenic nematodes and their mutualistic bacteria. Upasana Shokal, Ioannis Eletherianos. Department of Biological Sciences, George Washington University, Washington, DC.

Drosophila melanogaster is an outstanding model to understand the intricacies of signaling during host-pathogen interactions and innate immune defense mechanisms. The fly employs different immune responses according to the type of pathogen it encounters. While most *Drosophila* studies have so far concentrated on immune responses to bacteria, fungi and viruses, little is known about how eukaryotic parasites such as insect pathogenic nematodes interact with the fly immune system. Recent studies on the entomopathogenic nematode *Heterorhabditis bacteriophora* and its mutualistic bacteria*Photorhabdus luminescens* have started to investigate the fly response to nematodes carrying the bacteria (axenic worms), nematode lacking the bacteria (symbiotic worms) and the bacteria alone. The advantage of using this model is that the immune response of the fly can be studied against each partner of the mutualistic interaction that allows comparative studies between antibacterial and anti-nematode defenses. Thioester-containing proteins (TEPs) are conserved throughout the animal kingdom. In mammals they are involved in complement system activation or exhibit a protease inhibitor activity whereas in insects they act as opsonins, binding to parasites and promoting their phagocytosis or encapsulation. Recent studies in *Drosophila* have shown that TEPs are not involved in the immune defense against certain bacterial and fungal pathogens. Here we present data on the potential role of TEPs in the immune response of *Drosophila* against infection by *Heterorhabditis* nematodes and their *Photorhabdus* bacteria (separately or together). Such studies will provide additional information on the functional significance of *Drosophila* immune factors in anti-nematode reactions, which is of potential importance in both medicine and agriculture.

603C

Identification and characterization of the novel antiviral gene rogue in Drosophila melanogaster. Jessica Tang[1,2], Anne Macgregor[1,3], Louisa Wu[1,3]. 1) Institute for Bioscience and Biotechnology Research,; 2) Molecular and Cell Biology Graduate Program,; 3) Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD.

Drosophila melanogaster has a robust and efficient antiviral innate immune system. For example, RNA interference (RNAi) is a general antiviral immune response. The three evolutionarily conserved signaling pathways: Toll, Imd and JAK-STAT are shown to defend against certain viruses. Autophagy is shown to play a role in immune response in Drosophila as well. Given the complexity of antiviral immunity in Drosophila, additional factors are likely to be involved. With the goal to discover novel antiviral genes, a pilot screen for mutants with increased susceptibility to a dsRNA virus Drosophila X Virus (DXV) was done, and four mutants were identified. One of the mutants, rogue, was mapped to an uncharacterized novel gene (rogue).

Knockdown of rogue by RNAi in the whole animal or in specific tissues (hemocytes or fat-body) results in higher mortality after DXV infection compared to wildtype. Characterization of these rogue knockdown flies has revealed that although they are able to activate the Toll, Imd and JAK-STAT pathways, the transcript levels of some immune related genes are altered in the whole flies. Taken together, our results indicate that rogue is vital for defense against DXV and may be a novel factor that regulates the immune system in Drosophila. Identification and characterization of the antiviral gene rogue may provide new insight into how the immune system responds to viral infection.

604A

Rapid spread of *Spiroplasma* defensive endosymbionts in *Drosophila hydei* under high parasitoid wasp pressure. Jialei Xie, Lauryn Winter, Caitlyn Winter, Mariana Mateos. Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX.

Maternally transmitted endosymbionts of insects are ubiquitous in nature and play diverse roles in the ecology and evolution of their hosts. To persist in host lineages, many of these symbionts manipulate host reproduction to their advantage (e.g. Cytoplasmic incompatibility and male-killing), or confer fitness benefits to their hosts. Among the benefits conferred, a growing number of studies report symbiont-mediated defense against natural enemies of their hosts. Recent studies suggest that two non-male-killing bacteria strains (genus *Spiroplasma*) protect their host fly against natural enemies. *Spiroplasma* strain hy1, which is naturally harbored by *Drosophila hydei*, confers partial protection to its host against mortality induced by the parasitoid wasp, *Leptopilina heterotoma*. The *Spiroplasma*-conferred protection is partial and flies surviving a wasp attack have reduced adult longevity and fecundity. Therefore, it is unclear whether protection against wasps alone can counter *Spiroplasma* loss by imperfect maternal transmission and any possible fitness costs to harboring *Spiroplasma*. To address this question, we conducted a population cage study comparing *Spiroplasma* frequencies over time (host generations) under conditions of high wasp pressure and no wasp pressure. Our preliminary results indicate that *Spiroplasma* frequencies increase over time under high parasitoid pressure, whereas *Spiroplasma* frequencies fluctuate slightly in the absence of wasps. The prevalence of *Spiroplasma* reaches approximately 97% after three consecutive generations from 50% of starting frequency, and the population dynamics of both fly and wasp were largely influenced by the presence of *Spiroplasma*. The rapid spread of *Spiroplasma* under high parasitoid-pressure confirmed its strong of host-protection effects and may explain the high prevalence of *Spiroplasma* hy1 in natural *D. hydei* populations.

605B

Regulation of *Drosophila* innate immune signaling by amyloids and phospholipids. Anni Kleino[1], Jixi Li[2], Johanna Napetschnig[2], Lucy Chai[1], Kingsley Essien[1], Hao Wu[2], Neal Silverman[1]. 1) Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA, USA; 2) Program in Cellular and Molecular Medicine, Children's Hospital Boston, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA.

In *Drosophila*, innate immune responses against Gram-negative bacteria are mainly mediated *via* the IMD signaling pathway. The transmembrane receptor PGRP-LC, and the intracellular receptor PGRP-LE are required for the recognition of DAP-type peptidoglycan, and they both utilize RIP-homotypic interaction motif (RHIM)-like motifs to drive signal transduction. In mammals, the RHIM-motifs of RIP1 and RIP3 are involved in TNF-induced programmed necrosis, and were recently reported to form amyloid fibrils upon death signaling. Our aim is to clarify the mechanism of the PGRP-LC/LE RHIM-like motifs in the IMD signaling and to investigate the role of lipid-protein interactions in the regulation of the IMD signaling. We find that RHIM-like motifs from PGRP-LC and PGRP-LE, as well as their interaction partners IMD and Pirk, form amyloids *in vitro*. Furthermore, the treatment of S2* cells with a known inhibitor of amyloid formation, Thioflavin T, blocked the IMD pathway activity, suggesting that amyloid formation is required for immune signaling. However, Toll signaling was unaffected by Thioflavin T. Additionally, we find that the receptor-proximal adaptor protein IMD and the negative regulator Pirk bind negatively charged phospholipids, indicating association with cellular membranes. Further experiments are required to elucidate the mechanism by which amyloid fibrils and lipid-interactions regulate the IMD signaling pathway.

606C

***Drosophila* as a model organism to understand infection tolerance mechanisms.** Victoria Allen, Reed O'Connor, Clarice Zhou, Vanessa Hill, Elizabeth Stone-Jacobs, Thomas McCord, Michelle Shirasu-Hiza. Genetics and Development, Columbia University Medical Center, New York, NY.

The rise of antibiotic-resistant strains of bacteria has led to a modern crisis in treatment of severe bacterial infection (sepsis). There are two ways the host can fight infection: resistance and infection tolerance. Resistance mechanisms limit bacterial growth; infection tolerance mechanisms help the host to endure the pathogenic effects of infection. Here we use Drosophila as a model organism to understand infection tolerance mechanisms. Our lab focuses on identifying circadian-regulated physiologies that contribute to survival of infection. We found that circadian mutants are less resistant to several types of bacterial infections and more tolerant of others. Circadian mutants have altered feeding habits; we found that short-term differences in the flies' diet affect tolerance of infection. We are currently identifying specific nutrients and signaling pathways that impact infection tolerance. Our work will provide insights into the underlying mechanisms of infection tolerance and potentially new therapeutic approaches to the treatment of sepsis.

607A

***Big bang* and septate junctions modulates gut immune tolerance in *Drosophila*.** François Bonnay[1], Eva Cohen-Berros[1], Gabrielle L. Boulianne[2], Jules A. Hoffmann[1], Nicolas Matt[1], Jean-Marc Reichhart[1]. 1) UPR9022, CNRS, Université de Strasbourg, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France; 2) Programme in Developmental Biology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.

Chronic inflammation of the intestine is detrimental to mammals. Similarly, constant activation of the immune response in the gut by the endogenous flora is suspected to be harmful to Drosophila. Therefore, the innate immune response in the gut of *Drosophila melanogaster* is tightly balanced in order to simultaneously prevent infections by pathogenic microorganisms and tolerate the endogenous flora. To understand the molecular mechanism underlying this selective immune response in the gut, we undertook a pilot screen that identified the *big bang* (*bbg*) gene.

We show that in the adult Drosophila midgut, BBG is present at the level of the septate junctions, on the apical side of the enterocytes. In the absence of BBG, these junctions become loose enabling the intestinal flora to trigger a constitutive activation of the anterior midgut immune response. This chronic epithelial inflammation leads to a reduced lifespan of *bbg* mutant flies. Clearing the commensal flora by antibiotics prevents the abnormal activation of the gut immune response and restores a normal lifespan. We provide genetic evidence that Drosophila septate junctions are part of the gut immune barrier, a function that is evolutionary conserved in mammals.

Collectively, our data suggest that septate junctions are required to maintain the subtle balance between immune tolerance and immune response in the Drosophila gut, which represents a new powerful model to study inflammatory bowel diseases.

608B

Investigating the allelic determinants of immunological natural variation in Drosophila melanogaster. Alejandra Guzman, David Schneider. Microbiology and Immunology, Stanford University, Stanford, CA.

Immunity consists of at least two branches: resistance, the host's ability to control pathogen number, and tolerance, the host's ability to neutralize the infection's pathology. Immune natural variation increases the fitness of a population because, as a whole, the population can better respond to a wider range of pathogens and environmental conditions. In 2004, Lazzaro et. al showed that in D. melanogaster there are polymorphisms in the regions surrounding previously described immunity genes. Our work used an unbiased approach to discover alleles responsible for the natural variation in D. melanogaster immunity. Here, we screened 115 of the 192 fully sequenced inbred D. melanogaster lines derived from a single population in North Carolina called the Drosophila Genetic Reference Panel (DGRP). We infected these homozygous lines with Listeria monocytogenes and measured changes in fly survival and bacterial growth. Using ANOVAs we found polymorphisms that significantly ($p < 10^{-6}$) correlated with changes in immunocompetence. These polymorphisms were in close proximity to 153 genes. Roughly 10% of these genes are ion channels; we are currently investigating the role ion-channels during infection.

609C

Infection Susceptibility in a TPI Deficiency Model. Natasha C Hardina[1], Carolyn Steglich[1], Stacy L Hrizo[1,2]. 1) Biology , Slippery Rock University, Slippery Rock, PA; 2) Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Triosephosphate Isomerase (TPI) is a glycolytic enzyme. Mutations in this enzyme are associated with a neurodegenerative disorder called TPI Deficiency. Individuals with TPI Deficiency also exhibit increased susceptibility to infection due to reduce immune system function. We can model TPI deficiency using a Drosophila melanogaster strain with a mutation in the TPI gene that causes an M80T transition in the amino acid sequence. This mutation is called "sugarkill". These sugarkill flies exhibit similar phenotypes to humans with the disorder such as reduced lifespan and neurodegeneration. However, it has not been determined if sugarkill animals have reduced immune function. Therefore we have examined mutant TPI flies and wildtype flies to determine if they model this characteristic of the human disease. We hypothesized that the immune response in the sugarkill flies should be impaired compared to wild type Drosophila melanogaster.

610A

Diedel, induced by NF-kB pathway, regulates tolerance during Sindbis infection. Olivier Lamiable[1], Cordula Kemp[1], Friedemann Weber[2], Laurent Troxler[1], Nadege Pelte[3,4], Michael Boutros[4], Charles Hetru[1], Jean-Luc Imler[1]. 1) Institut de Biologie Moléculaire et Cellulaire CNRS UPR9022, Strasbourg, France; 2) Institut für Medizinische Mikrobiologie und Hygiene, Freiburg, Germany; 3) Donnelly Centre for Cellular and Biomolecular research, Toronto, Canada; 4) Department of Cell and Molecular Biology, Heidelberg University, Heidelberg, Germany.

Infection of *Drosophila* by viruses leads to an innate immune response, which involves different facets. On one hand, the small interfering (si) RNA pathway contributes to the recognition and degradation of the viral RNAs. On the other hand, an inducible response contributes to the host defense, but the regulation of this response and the role of the induced effectors molecules are still poorly understood.Using genome-wide microarrays, we identified the gene *Diedel* (*CG11501*) that is strongly induced during Sindbis (SINV) infection. Diedel is an early response gene, which is also strongly induced by another arbovirus, Vesicular Stomatitis Virus (VSV). The gene encodes a 12 kDa circulating protein, secreted by the fat body upon infection. Induction of Diedel expression does not depend on the Jak/STAT pathway, or the NF-kB related transcription factor Relish, but is completely abolished in flies mutant for the gene encoding *Dif*, another NF-kB related transcription factor. *In vitro* and *ex vivo* analyses do not support the hypothesis that Diedel acts as an antiviral molecule. *In vivo* analyses of Diedel null mutant flies revealed a dual role for Diedel, first in viability during developmental and adult stages, and second in the

tolerance to SINV infection. Indeed, Diedel mutant flies have similar viral loads than wild-type controls, but succumb more rapidly to SINV infection. Microarray analysis of infected mutant flies pointed to a strong increase of the inducible response to infection, suggesting that *Diedel* encodes a cytokine acting in the maintenance of homeostasis during viral infection.

611B

Ingestion of *Pseudomonas fluorescens* Pf-5 by *Drosophila melanogaster* causes larval immune response dependent on bacterial media type. Kristin L. Latham, Amy Nicholson, Jenna Schneider, Elizabeth Mason. Biology, Western Oregon Univ, Monmouth, OR.

While immune system response to pathogens has been well-studied in Drosophila adults, little is known about response to bacterial exposure during early larval stages. We have exposed first- and second-instar larvae to *Pesudomonas fluorescens* strain Pf-5 by a non-invasive feeding procedure. Larvae fed *P. fluorescens* show dose-dependent differences in time to metamorphosis and survival. Interestingly, larvae fed Pf-5 cultured in different types of bacterial media show distinct differences in growth rate and immune phenotype, suggesting that this bacterial strain produces metabolites that vary with culture media components, leading to varied larval immune response. We are currently investigating more deeply whether the larval response is cellular and/or humoral. These data suggest that immune response in Drosophila larvae elicited by Pf-5 bacteria is dependent not just on bacterial dose but also on which bacterial metabolites are present.

612C

Analysis of a novel antibacterial protein, Noduler that is conserved in insects and mammals. Asha Minz, Javaregowda Nagaraju. Laboratory of Molecular Genetics, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, Andhra Pradesh, India.

In the absence of adaptive immunity, insects have acquired a powerful innate immune mechanism. Innate immune system acts as a primary defense mechanism in detecting microorganisms that aid in eliciting a plethora of antimicrobial responses in an organism. This phenomenon is marked by cellular and humoral responses leading to synthesis of effector molecules as antimicrobial peptides. A striking similarity between insect and mammalian innate immunity being constantly supported by several reported investigations. In our study, we have identified a novel antimicrobial peptide Noduler in the wild silkmoth that belong to one of the largest orders of insect, Lepidoptera, which includes various economically important insects. In silico predictions led to the finding of its orthologues in other insects and mammals. Several infection experiments and expression studies were carried out in Bombyx mori larval fat body, Drosophila adult flies and THP-1 macrophagic cells. The results suggest that the orthologues of Noduler are upregulated upon microbial challenge in a time course manner. The knockdown of these orthologues results in rendering these organisms highly susceptible to infection, suggesting the gene's role in host defence in insects and mammals. These results approve the evolutionary conservation of innate immune mechanisms among species.

613A

Identification of Transcriptionally Induced Antiviral Effectors in Drosophila. Gregory Osborn[1*], Jie Xu[1], Ari Yasunaga[1], Ian Lamborn[2], Beth Gordesky-Gold[1], Sara Cherry[1]. 1) Department of Microbiology, University of Pennsylvania, Philadelphia, PA; 2) Department of Immunology, University of Pennsylvania, Philadelphia, PA.

The innate immune system is characterized by the precise regulation of gene expression programs to restrict pathogens. We recently identified a virally-induced gene expression program that is rapidly activated by diverse viral infections. We found that the transcriptional pausing pathway is antiviral and controls this response, as over half of this gene induction program is genetically-dependent on pausing and has pausing-associated biochemical chromatin features. Transcriptional pausing and this rapidly induced program plays an essential antiviral role in vivo, as NELF and P-TEFb restrict viral replication in adult flies and in vector mosquito cells. Furthermore, this program included components of all known antiviral pathways including RNA silencing, autophagy, JAK/STAT, Toll, and Imd signaling along with a subset of Toll receptors. This suggests that additional genes within this gene set may have antiviral effector function. Utilizing a directed RNAi screen to these transcriptionally induced genes, we have identified a subset with previously unknown roles in Drosophila immunity against multiple viruses.

614B

Cr(VI) induced suppression of Drosophila cellular immune response: protection by sod overexpression. Pragya Prakash[1*], Arvind Shukla[1], M.Z. Abdin[2], Debapratim Kar Chowdhuri[1]. 1) Embryotoxicology, CSIR-Indian Institute of Toxicology Research, Lucknow, Uttar Pradesh, India; 2) Department of Biotechnology, Jamia Hamdard, Hamdard Nagar, New Delhi 110 062.

The immune system is the first line of defense mechanism in all metazoans. A number of anthropogenic activities release thousands of chemicals into the environment which exert adverse effects on immune system. In the present study, in vivo effect of Cr(VI) on Drosophila cellular immune response was evaluated and subsequent protection by sod overexpression was examined. The immunosuppressive potential of Cr(VI) was demonstrated by a significant reduction in total hemocyte count in the exposed organism. A significant increase in annexin V-FITC positive cells, DEVDase activity and Reactive Oxygen Species (ROS) generation along with deterioration of antioxidant defense system in hemocytes of the exposed organism was also observed. This indicates that Cr(VI) exposure generates ROS mediated oxidative stress in the hemocytes. Further, Cr(VI)

induced immunosuppressive effect was shown to be significantly reduced by the overexpression of one of the antioxidant genes, sod, in Drosophila hemocytes. This study demonstrates the applicability of Drosophila melanogaster to examine the possible immunotoxic effects of environmental chemicals along with its amelioration by sod overexpression. Overall, it has been suggested that overexpression of sod benefits the organism from Cr(VI) induced immunosuppressive effect on cellular immune response.

615C

Characterisation of lipid-mediated inflammatory pathways in Drosophila. Mark A Watson[1], Karen Massey[2], Soyeon Kwon[1], Anna Nicolaou[2], Paul Badenhorst[1]. 1) Institute of Biomedical Research, University of Birmingham, Edgbaston B15 2TT, UK; 2) Bradford School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK.

Oxygenated metabolites of polyunsaturated fatty acids (PUFAs) are key modulators of inflammatory responses, and are therapeutic targets for intervention in inflammatory diseases such as rheumatoid arthritis. PUFAs are categorized as omega-6 (n-6) or omega-3 (n-3) and are broadly considered pro-inflammatory or anti-inflammatory respectively. The best- studied example of these bioactive lipid mediators are prostaglandins - eicosanoid (C20: n-6) derivatives of arachidonic acid. The cyclooxygenase enzymes that generate prostaglandins are currently the main targets of therapeutic intervention in inflammatory disease. Recent reports have indicated the existence of cyclooxygenase activity in Drosophila, suggesting that these compounds may also modulate Drosophila inflammatory pathways. Using an inflammatory model based on the inflammatory tumor-promoting hopTum mutation, we have investigated lipid mediator-signaling pathways in Drosophila. Dietary supplementation with fatty acids was able to modulate inflammation, with omega-6 fatty acids increasing, and omega-3 fatty acids reducing, the severity of the hopTum inflammatory phenotype. While cyclooxygenase inhibitors were able to reduce inflammation, LC-MS analysis of Drosophila extracts failed to detect the existence of prostaglandins or the arachidonic acid (C20) precursor. Interestingly, however, we have been able to detect C18 oxygenated metabolites known as HODEs (Hydroxy-octadecadienoic acids). These can be generated by enzymatic oxidation of the C18: n-6 fatty acid linoleic acid by myeloperoxidase (MPO) enzymes. MPO inhibitors were able to reduce the inflammatory phenotype and we have identified candidate Drosophila MPO homologues. Using the inflammatory tumor assay we have been able to distinguish 3 that may be required to generate HODE inflammatory lipid mediators. Functional characterization of these enzymes in Drosophila inflammatory responses is presented.

616A

Bacterial diversity associated with Drosophila in the laboratory and in the natural environment. Fabian Staubach[1], John Baines[2], Sven Kuenzel[2], Elisabeth Bik[3], Dmitri Petrov[1]. 1) Biology, Stanford University, Stanford, CA; 2) Max Planck Institute for Evolutionary Biology, Plön, Germany,; 3) Department of Microbiology & Immunology, Stanford School of Medicine, Stanford, California, United States of America.

All higher organisms are associated with bacterial communities. Bacteria have a range of effects on their metazoan hosts from being indispensable for survival to being lethal pathogens. Because bacteria have phenotypic effects on their hosts, they can also be involved in host adaptation to the environment. The fruit fly Drosophila is a classic model organism to study adaptation as well as the relationship between genetic variation and phenotypes. Recently, Drosophila has received attention in immunology and studies of host-microbe interaction. Although bacterial communities associated with Drosophila might be important for many aspects of Drosophila biology, little is known about their diversity and composition or the factors shaping these communities. We used 454-based sequencing of a variable region of the bacterial 16S ribosomal gene to characterize the bacterial communities associated with wild and laboratory Drosophila isolates. In order to specifically investigate effects of food source and host species on bacterial communities, we analyzed samples from wild Drosophila melanogaster and D. simulans flies collected from a variety of natural substrates, as well as from adults and larvae of nine laboratory-reared Drosophila species. We find substantial variation of bacterial communities within and between laboratories that could interfere with phenotype studies. We show that bacterial communities associated with wild-caught Drosophila contain more bacterial species than laboratory-raised flies, but that they are on average less diverse than vertebrate communities. The natural Drosophila-associated microbiota appears to be predominantly shaped by food substrate with an additional but smaller effect of host species identity. .

617B

Mechanisms of Wolbachia intracellular accumulation in somatic cells of the Drosophila ovary. Ajit Kamath, Eva Fast, Michelle Toomey, Horacio Frydman. Biology, Boston University, Boston, MA.

Wolbachia are obligatory intracellular bacteria infecting up to 70% of insect species. Many of these insects are vectors for the transmission of devastating tropical diseases. Wolbachia mainly propagates by vertical transmission via the female oocyte. They are under selective pressure to reach the female germline and infect the next generation. However the Wolbachia infection is not limited just to the germline cells, but also several somatic cell populations have been shown to have higher Wolbachia loads compared to surrounding tissue, including stem cell niches, neurons, trachea and malpighian tubes. The different mechanisms underlying the higher intracellular loads are unknown. In terms of stem cell niche tropism, we have shown that targeting of different niches is determined by Wolbachia intrinsic factors. However, another possible means for a specific cell type to display higher Wolbachia levels is through lower mitotic rates compared to the surrounding cells. Here we show that within the follicular epithelium, the polar cells (PC) have higher levels of intracellular Wolbachia accumulation.

After PC specification, the surrounding follicular epithelium retains mitotic activity until stage 6 of oogenesis. Using this system we can follow all the stages of the polar cell development allowing us to investigate the kinetics of the intracellular accumulation of Wolbachia in the polar cells and the surrounding follicular epithelia. This analysis will provide us mechanistic insights into the preferential targeting of Wolbachia to the polar cells in the Drosophila ovary.

618C

Molecular mechanisms for Wolbachia hub tropism in Drosophila melanogaster. Rama Krishna Simhadri, Michelle Toomey, Parthena Mantis, Ajit Kamath, Horacio Frydman. Biology, Boston University, Boston, MA.

Wolbachia are symbiotic intracellular bacteria that infect reproductive organs of a wide range of insects and worms, many of which have medical relevance. The bacteria are maternally transmitted analogous to mitochondria and have been shown to confer the host a reproductive advantage and increased resistance to many viruses that cause Dengue, West Nile and Chikungunya, and parasites like Plasmodium. Recently several field trials have been successfully conducted to spread Wolbachia in wild mosquito populations to reduce the spread of these diseases. Understanding the molecular and cellular basis of Wolbachia transmission and increase of insect resistance against human pathogens are highly relevant towards developing these Wolbachia-based approaches for disease control. Working in this direction we have found that Wolbachia displays tissue tropism which is dependent on the strain of Wolbachia. Specifically we are looking at the tropism of two strains of Wolbachia - wMel and wMelPop, which are native to Drosophila melanogaster. The two strains differ considerably in their infection levels in the niche harboring the stem cells in the testis under some conditions. To understand the molecular details of this strain specific phenotype, we have performed transcriptional analysis on the apical tips of the testis containing the niche, associated stem cells and primary spermatocytes. This analysis revealed several genes that are deferentially expressed between the three groups - uninfected, wMel infected and wMelPop infected tissue, and most of them are involved in processes related to Metabolism, including protelolysis, lipid metabolism, transport, oxidation-reduction and cell cycle. In agreement with previous work indicating that Wolbachia depends on host amino acid pool as an energy source, our data indicates greater proteolytic activity in the Wolbachia strain with higher niche tropism - wMelPop. We are currently validating these results and by utilizing the genetic tools available in Drosophila, we will test the functional significance of the candidate genes for tissue tropism.

619A

Does stem cell niche tropism favor the evolutionary success of specific Wolbachia strains? Michelle E. Toomey, Mark Deehan, Kanchana Panaram, Horacio Frydman. Biology, Boston University, Boston, MA.

The intracellular bacteria Wolbachia infect most insect species, including the vectors of devastating infectious diseases such as Dengue and malaria. Although Wolbachia is being targeted as a novel control mechanism for the spread of these diseases, very little is known about the cellular and molecular mechanisms of the host-microbe interactions. Wolbachia are mainly vertically transmitted, however there is also evidence of extensive horizontal transmission. In order to successfully invade a new host species, once Wolbachia crosses the species barrier, it must avoid the host's innate immune system and colonize the germline. We hypothesize that targeting of stem cell niches in the gonad offers a facilitated route to access the germline, aiding subsequent vertical transmission after invasion of a new host species. If this hypothesis is true, related Wolbachia strains that infect diverse host species (grouped by sequence type clonal complexes) will be efficient at colonizing stem cell niches. To address this hypothesis, we first looked at clonal complex ST-13, which has the highest number of diverse host species infected by closely relate Wolbachia strains. Many strains of Wolbachia infecting the Drosophila genus are represented in ST-13. We show that tropism for the stem cell niches in the ovary across the Drosophila genus is evolutionarily conserved in all Wolbachia strains investigated, and that this tropism may promote germline transmission. We also began looking at species that are infected with Wolbachia not belonging to the ST-13 complex, i.e. Culex pipiens pipiens infected with Wolbachia wPip. The limitation to this analysis is that the stem cells and associated niches in the gonads are not unequivocally identified in many other insect species. We have, however, identified the stem cells and niches in the ovary of Cx. pipiens, and there is no Wolbachia tropism present. These observations support our hypothesis that niche tropism, present in the Wolbachia strains in the ST-13 clonal complex, may promote successful spreading of Wolbachia in nature.

620B

Bayesian Analysis of Genetic Variation in Complex Social Group Behaviour. Brad R. Foley[1], Julia B. Saltz[1], Paul Marjoram[1,2], Sergey Nuzhdin[1]. 1) Mol Comp Bio, Univ Southern California, Los Angeles, CA; 2) Preventative Medicine, Keck School of Medicine, USC, Los Angeles, CA.

Social behavior is an example of a complex system. Complex systems are typically characterized by multiple interacting components, commonly resulting in nonlinear outcomes. These systems are challenging to analyse using standard linear approaches, but are naturally modeled using Agent Based Models. ABMs are increasingly common in behavioral studies, but principled methods for fitting models to empirical data are needed. Here, we fit behavioral genetic parameters to empirical results of complex group formation in *Drosophila melanogaster*.

621C

An Analysis of the Genetic Architecture of Aggression in *Drosophila melanogaster*. John R. Shorter[1,3], Charlene Couch[2,3], Robert R. H. Anholt[2,3], Trudy F. C. Mackay[1,3]. 1) Genetics, North Carolina State, Raleigh, NC; 2) Biology, North Carolina State,

Raleigh, NC; 3) W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC.

Aggression is a complex trait, with variation in populations due to both genetic and environmental factors. We investigated the natural genetic variation of aggression using the *D. melanogaster* Genetic Reference Panel (DGRP), a collection of 192 inbred lines with fully sequenced genomes, and identified 244 SNPs associated with aggression. Additionally, we performed an independent experiment to replicate causal candidate SNPs by creating an outbred population from these inbred lines, identified epistatically interacting loci, and confirmed candidates by using RNAi knockdown lines. These results provide insight into the genetic architecture of aggression and identify novel genetic variants responsible for naturally occurring variation in this complex trait. We identify potential gene targets that can be modified to reduce aggression.

622A

Evaluation of the functional roles of painless and dTRPA1 in chemical nociception in Drosophila. Samantha J Mandel, Madison L Shoaf, Pam A Fazio, Jason T Braco, Wayne L Silver, Erik C Johnson. Wake Forest University, Winston-Salem, NC.

The detection of harmful chemical irritants is vital for the evasion of potential life threatening compounds. Among vertebrates, the trigeminal nerve is an important site of chemical nociception in its direct response to an array of chemical compounds. One of the many targets for multiple trigeminal stimulates is the TRPA1 channel. Drosophila possess multiple homologs of mammalian TRPA1 channels, two of which are painless and dTRPA1. However, there is disagreement regarding the role of painless in the behavioral aversion to allyl isothiocyanate (AITC). Previous studies showed that heterologous expression of painless failed to confer AITC-elicited changes, supporting the hypothesis that painless may not have a role in mediating aversion to chemical irritants. We have analyzed the behavioral phenotypes of painless and dTRPA1 mutants using a two-choice feeding assay and proboscis extension reflex (PER) assay, both of which pointed to the requirement for each channel in the aversion to AITC. Concerning the PER assay, we are standardizing the assay in order to determine whether responses are due to averse behaviors, starvation sensitivity, or sugar preference. Preliminary results support the conclusion that both pain and dTRPA1 are critical for averse behaviors. We have evaluated the expression patterns of painless and dTRPA1 to determine if there is any colocalization, and so far, there has been none observed in central or peripheral populations. To evaluate painless and dTRPA1 cell excitability we employed the GCaMP transgene to observe fluctuations of calcium levels. Both painless and dTRPA1- expressing cells showed significant changes in fluorescence following application of AITC. Collectively, our results provide further insight into the contribution of each of these channels in chemical nociception. Application of future directions includes investigation into pyrexia, another homologue to the mammalian TRPA1 channel, and whether it has a role in chemical nociception as well as examination of the neural circuits and how these channels interconnect with one another.

623B

Mutational analysis suggests that circadian period-altering mutations of DBT affect Interactions of DBT with other circadian Proteins. Anandakrishnan Venkatesan[1], Michael Muskus[2], Ed Bjes[1], Jeffrey Price[1]. 1) University of Missouri Kansas City, Kansas City, MO; 2) Washington University, St Louis, MO.

Mutational analysis of DBT is addressing if its effects on circadian period are determined in part by interactions with other proteins. The period-altering mutations of DBT possess lower kinase activity but have different effects on period. We hypothesized that if these mutations alter period by affecting interaction of DBT with the other circadian partners then these effects should persist in a kinase-inactive background ($DBT^{K/R}$ - dominant negative). To test this hypothesis we made double mutants combining both $dbt^{K/R}$ and each of the period altering mutants in cis. All three double mutants reduce the long period of the $dbt^{K/R}$;dbt^{WT} genotype and partially restore oscillations of PER phosphorylation. The period-shortening of the by these mutations suggests that they affect the period by a mechanism in part independent of kinase activity (e.g. interaction with other clock components). The TAU mutation producing a short period (21 hrs) has been proposed to be party of a triad that binds phospho-amino acids, thereby targeting DBT to sites that have already been phosphorylated. We hypothesized that the amino acids around the TAU mutation might also be involved in this process or in producing other protein-protein interactions. We made mutations in amino acids that were closer to this triad as well as those and that were away from it. The mutations that were close to the triad produced a short period (~16 - 22 hrs) and the mutations that were farther away produced a longer period. The shortening of period by some mutations suggests that they affect a TAU-like domain involved in a protein-protein interaction that lengthens period. The identification of the interacting partners is underway.

624C

Drosophila cryptochrome achieves high effective light sensitivity by integrating photon information over extreme time periods. Pooja G Vinayak, Jamie Coupar, S. Emile Hughes, Preeya Fozdar, Jack Kilby, Jay Hirsh. University of Virginia, Department of Biology, Charlottesville, VA.

Drosophila melanogaster show extreme dim light sensitivity for entrainment to 12:12 hour light/dark schedules (Hirsh et al, 2010). To better understand this light sensitivity, we use a related paradigm, shifting circadian phase by administration of dim light pulses in the subjective night. Here we show that flies show graded responses to varying intensities of dim light pulses. However, light sensitivity shows a surprising increase with pulse duration up to durations of 360 minutes, implying that photic integration is occurring over a time scale of hours. To explicitly test for photic integration, we exposed flies to light pulses containing equal numbers of photons given over time intervals between 0.1 and 100 minutes. To account for minimum and maximum possible phase shifts, these pulses were given in separate experiments during both the early and late subjective

night respectively. Flies respond to late-night pulses with increasing phase advance amplitude as time intervals increase, showing a surprising intensity/duration relationship. However, early-night pulses result in phase delays that are characterized by increased amplitude and light sensitivity relative to phase advances. Furthermore, phase delay amplitude remains constant with increasing time intervals for light pulses of equal photon numbers, implying that the process of temporal integration is different during the early and late subjective night. The large amplitude phase advances that result from this temporal integration depend critically on the circadian photopigment cryptochrome, with little input from visual photoreceptors in the eyes. These results show that cryptochrome has inherently low photic sensitivity, but achieves high effective sensitivity by integrating photon information over extremely long times. Our findings provide a general mechanism by which a non-image forming photopigment can achieve or exceed the light sensitivity of image forming visual photopigments.

625A

The RHO1 signaling pathway acts in circadian clock neurons to control behavioral rhythms. Herman Wijnen[1,2,3], Neethi Rao[3], Rachel Siegmund[3], Laura Thomason[3], Ariel Talts[3], Emmanuel Anyetei-Anum[3]. 1) Centre for Biological Sciences, University of Southampton, Southampton, United Kingdom; 2) Institute for Life Sciences, University of Southampton, Southampton, United Kingdom; 3) Deparment of Biology, University of Virginia, Charlottesville, VA.

Circadian clocks in animals involve feedback loops of gene expression that organize daily rhythms in physiology and behavior. It remains largely unclear, however, what signaling mechanisms link circadian gene expression to rhythmic behavior. As a result of a systematic genetic screen we discovered that components of the RHO signaling pathway that is known to regulate the actin cytoskeleton in many eukaryotes affect *Drosophila* circadian behavior . Although a functional RHO1 pathway is essential for basic cellular functions, we found that the observed circadian phenotypes were not generally associated with cell death, abnormal morphology, or developmental defects, but appeared to specifically affect the molecular clock circuits. Specifically, RHO1 signaling in the adult small ventral lateral (s-LN_v) clock neurons controls daily locomotor activity rhythms. Knockdown of the downstream effector ROK (RHO-KINASE) slows rhythms in the accumulation of the clock components TIMELESS (TIM) and PAR-DOMAIN PROTEIN 1 (PDP1) and selectively delays nuclear entry of PERIOD (PER) and TIM. ROK could act on the molecular circadian oscillator in the s-LN_v clock neurons cell-autonomously or via synaptic or peptidergic intercellular signaling. Experiments addressing the latter possibilities revealed that regulation of molecular oscillations by ROK in clock neurons does not require active neurotransmission or the neuropeptide PIGMENT-DISPERSING FACTOR (PDF). In addition, rhythms in the morphology of the s-LN_v dorsal projections are unaffected by rok knockdown. Taken together, these results suggest that conserved RHO-GTPase pathways acts in s-LN_v circadian pacemaker cells to link modulation of the actin cytoskeleton to molecular and behavioral circadian rhythms.

626B

Quantification of post-mating feeding behavior in *Drosophila* females. Jennifer Apger, Mariana Wolfner. Cornell University, Ithaca, NY.

In *Drosophila*, seminal fluid proteins transferred from the male during mating elicit physiological and behavioral changes in the female that are important for fertility. One of these changes is an increase in feeding by mated females. A male seminal protein, sex peptide (SP), is known to induce the post-mating increase in feeding (Carvalho et al., 2006), in addition to other effects, such as decreasing receptivity to remating and increasing ovulation and egg-laying. Some of SP's effects are transient, but others, such as its effects on egg-laying and receptivity, persist for up to 2 weeks. Persistent SP effects are due to the peptide's binding to, and slow release from, sperm in the female. It was unknown whether SP's effect on feeding also showed such long-term characteristics. To determine whether SP effects on feeding were part of its long-term response we assayed food intake by female flies, by an assay (Cognigni et al., 2011) that measures fecal output. We found that SP affects feeding over several days. By mating females to either males that don't produce sperm, or males that produce a form of SP that cannot be cleaved from sperm, we showed that the gradual cleavage of sperm-bound SP is necessary for the increase in feeding to persist for at least 72 hours after mating. Additionally, consistent with previous studies linking egg production and feeding behavior, we found that SP indirectly increases feeding behavior by increasing egg production over several days.

627C

Male-specific isoforms of Drosophila *fruitless* have different transcriptional regulatory roles conferred by their distinct DNA binding domains. Michelle Arbeitman[1], Justin Dalton[1], Justin Fear[2], Simon Knott[4], Bruce Baker[3], Lauren McIntyre[2]. 1) College of Medicine, Biomedical Sciences, Florida State Univ, Tallahassee, FL; 2) Genetics Institute, University of Florida, Gainesville, FL 32610-3610; 3) Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, 20147; 4) Cold Spring Harbor Laboratory, One Bungtown Road Cold Spring Harbor, NY 11724.

fruitless (*fru*), a gene in the sex hierarchy that is crucial for male courtship, encodes multiple male-specific isoforms (Fru^M) that vary in their DNA binding domains. We have examined the distinct roles of three Fru^M isoforms, by individually overexpressing each isoform in *fru*-expressing neurons and assaying gene expression in males and females. We find that each isoform has different regulatory activities. We identify many genes regulated downstream of Fru^M isoforms, including those with known neuronal functions. Examination of the genes induced by over-expression of Fru^M demonstrates that Fru^M functions differently in male *fru P1*-expressing neurons as compared to females, suggesting that Fru^M function is dependent on the sex of the cell it is produced within. In addition, expression analysis on RNA derived from Fru^M mutant males

showed that many of the genes differentially expressed in the Fru^M over-expression experiment are identified in the more physiologically relevant loss-of-function experiment. Furthermore, we demonstrate that each Fru^M isoform has a different DNA binding specificity, by determining the binding sites using SELEX. Genome wide analysis of these binding sites finds a significant enrichment of binding sites within and proximal to genes that are induced, but not repressed by Fru^M-overexpression. Chromosomal distribution of genes regulated by Fru^M showed that those that are induced and repressed as a consequence of Fru^M over-expression are highly enriched and depleted, respectively, on the X chromosome in males, but not in females when Fru^M is over-expressed. These results suggest that the X chromosome in males may have unique properties with respect to gene expression downstream of Fru^M.

628A

Towards a molecular and functional analysis of the Drosophila mating plug. Frank W. Avila, Fatima S. Ameerudeen, Mariana F. Wolfner. Dept Mol Biol & Gen, Cornell Univ, Ithaca, NY.

In many animals, a mating plug is formed in the female reproductive tract during and/or after mating. Mating plugs are composed of male seminal fluid proteins (SFPs), and have been shown to have a wide range of functions across numerous species. In insects, the structure has been shown to be required for the storage of sperm, acting as a physical barrier to re-mating, and decreasing female receptivity to re-mating (Avila et al., 2011). In the house mouse, proper mating plug formation is required for fertility, as malformation of the structure decreases the probability that sperm will reach the uterus (Murer et al., 2001). In D. melanogaster, a mating plug is formed within minutes after the start of mating and is comprised of two distinct parts: a posterior region (posterior mating plug, PMP) composed of proteins from the male ejaculatory bulb, and an anterior region (AMP) composed of proteins from the male accessory gland. In D. melanogaster, a handful of mating plug proteins have been identified to date (Peb, PebII, and Acp36DE), and at least one of them (PebII) has been reported to be necessary for a short-term decrease in sexual receptivity. To fully assess the structure and role of the mating plug in Drosophila reproduction, we used mass spectrometry to identify the most abundant proteins of mating plugs dissected from females at 1 hour post-mating. This analysis revealed >60 proteins. Using RNAi knockdown we removed individual mating plug candidates from the male ejaculate and examined for a role in mating plug formation and subsequent post-mating processes. Of the 10 candidates screened thus far, we have found two that reduce female fertility.

629B

***jim lovell* (*lov*) regulates behavior through roles in both the PNS and CNS.** Kathleen M. Beckingham, Sonia Bjorum, Rebecca Simonette, Raul Alanis, Michael Trejo, Keith Hanson. Dept Biochem & Cell Biol, Rice Univ, Houston, TX.

jim lovell (*lov*) encodes a putative transcription factor of the BTB/POZ domain class. The gene is expressed in many neurons in the larval and adult nervous systems. An analysis of embryonic expression indicates roles for *lov* in the terminal differentiation of subsets of both central and sensory neurons. The lov^{47} mutation affects expression of the major *lov* neural transcript and results in behavioral defects in larvae and adults. Larval locomotion is regulated by a CNS central pattern generator (CPG) that coordinates waves of contraction along the abdominal walls. lov^{47} larvae show locomotor defects characteristic of loss of input to the CPG from the peripheral sense organs, particularly from the chordotonal (CH) class of neurons. In lov^{47} Lov expression in a subset of CH neurons is lost, suggesting that the aberrant locomotion reflects loss of CH input to the CPG. In adults, lov^{47} strongly affects male courtship behavior. However, in this case, Gal4 directed expression of *lov* RNAi to different neuron types indicates that the defects originate in the CNS rather than the periphery. Use of a *fruitless* (*fru*) Gal4 driver indicates that some of the affected neurons also express *fru*.

630C

To Sing or to Fly: Role of Muscle Proteins in *Drosophila* Song and Flight Behaviors. Samya Chakravorty, Bertrand Tanner, Matthew Rosenthal, Jim Vigoreaux. University of Vermont.

Complex behaviors using wings have facilitated the evolutionary success and diversification of insects. *Drosophila* uses indirect flight muscles (IFM) to power their wings for flight, a behavior subject to natural selection. *Drosophila* IFM also gets neurally activated to generate sound by wing vibrations for species-specific male courtship song, a behavior subject to sexual selection. To gain insight into how competing selection regimes are manifested at the molecular level, we investigated the effect on flight and mating behaviors of mutations affecting two contractile proteins essential for IFM function, flightin (FLN) and myosin regulatory light chain (MLC2). A deletion of 62 N-terminal amino acids of FLN ($fln^{\Delta N62}$), the faster evolving region of the protein (dN/dS= 0.4 vs 0.08 for rest of protein), results in myofilament lattice disorder and reduced flight ability (flight score: 2.8±0.1 vs 4.2±0.4 for fln^{+} rescued control) despite a normal wing beat frequency. $fln^{\Delta N62}$ males sing with an abnormal interpulse interval (IPI, 56±2.5 vs 37±0.7 ms for fln^{+}) and a reduced pulse duty cycle (PDC, 2.6±0.2 vs 7.3±0.2 % for fln^{+}), suggesting that FLN N-terminal region fine-tunes sexually selected song parameters in *D. melanogaster*. Unlike FLN, mutations of the highly conserved MLC2 (N-terminal 46 amino acid deletion and mutations of myosin light chain kinase phosphorylation sites) result in flight impairments through their effect on actin-myosin contractile kinetics and subtle but significant changes in myofilament lattice spacing. The MLC2 mutations do not affect sexually selected song parameters (IPI and PDC). Our data suggest that the highly conserved amino acid regions of FLN and MLC2 are under purifying selection to support the IFM's myofilament lattice structure and contractile function necessary for flight, whereas the fast evolving FLN N-terminal region is under positive selection to optimize IFM's biological performance in flight and species-specific song.

631A

An RNAi screening for genes involved in female mate choice in *Drosophila melanogaster*. Youngmin Chu, Rui Sousa Neves. Department of Biology, Case Western Reserve University, Cleveland, OH.

The purpose of this study is to identify genes that are involved in female mating choice. To that end we selected three closely related species of *Drosophila* (*D. melanogaster, D. simulans, and D.sechellia*) that prefer different types of males. We then made pair-wise comparisons of each gene in the genome to isolate those that diverge the most in all three species. We then systematically knocked down the expression of these genes by RNAi in *D. melanogaster* using an ubiquitous Gal4 driver. The adult female flies expressing RNAi were tested for their ability to accept wild type *D.melanogaster*males by video analyses. From this screening, we tested 35 of such lines and identified 7 genes that significantly reduce mating. In a second phase, we asked whether these genes act in the central nervous system or in other body parts. Here we will present the results of these experiments.

632B

Investigation of how the presence of a female germline and the receipt of sperm during mating influences gene expression changes in adult female head tissues after mating. Nicole R Newell[1], Justin E Dalton[1], Peter L Chang[2], Sergey V Nuzhdin[2], Michelle N Arbeitman[1]. 1) Biomedical Science, Florida State University, Tallahassee, FL; 2) Molecular & Computational Biology, University of Southern California, Los Angeles, CA.

Drosophila melanogaster females, once mated, undergo both physiological and behavioral changes that last about a week. This post-mating response includes increased immunity and egg laying, changes in metabolic activity, and decreased receptivity to courtship. These changes are initiated by the receipt of the male accessory gland proteins (Acps) and sperm. One Acp, Sex Peptide (SP) immediately circulates systemically eliciting a short-term post-mating response, whereas another portion is bound to the sperm tail and is gradually released by cleavage. It is this gradual release of SP from sperm that maintains the long-term post-mating response. We have previously shown that different sets of genes are significantly differentially expressed in female head tissues at four different time points we assayed post-mating, as compared to age-matched virgin females. However, what is still unknown is the contribution of the presence of a germline, egg production and egg laying in females on these gene expression changes. Furthermore, the effect of receipt of sperm on gene expression changes in female adult head tissues is not understood. This is especially interesting considering the long-term effect on female behavior from the gradual release of SP from sperm. Illumina RNA-seq libraries were made from mRNA derived from adult heads of: (1) wild type females that had been mated to males with or without a germline, (2) females with or without a germline that had been mated to wild type males, and (3) virgin females of the same genotypes. We examined gene expression one and three days post-mating, to see the early and the sustained gene expression responses to mating. The analysis of the Illumina RNA-Seq data we obtained on gene and transcript-isoform expression will be presented.

633C

Sexually experienced *Drosophila melanogaster* males are better at courting and competing for mates. Sehresh Saleem, Ginger E. Carney. Texas A&M University, College Station, TX.

Competition for mates is a widespread phenomenon that affects reproductive success. Gaining a mating advantage over competitors is therefore a priority for increasing individual fitness. The ability of animals to adjust their behaviors in response to changing social environment is important and well documented. *Drosophila melanogaster* males compete with one another for copulations with females and vary their reproductive behaviors based upon prior social interactions. However, it remains to be determined how male social experience that culminates in mating with a female impacts subsequent male reproductive behaviors. In this study we quantified the effects of prior mating experience on subsequent *D. melanogaster* male courtship behavior and mating success. Males with previous sexual experience performed less courtship but extended their wings and attempted to mount the females more often compared to sexually naïve males. When a sexually experienced and a naïve rival competed for mating with a female, sexually experienced males won more often by increasing the effort they directed towards component courtship behaviors. Interestingly, males with only courtship experience or with incomplete copulations did not out-compete naïve males; therefore courtship experience alone was not sufficient in providing this competitive advantage, indicating that copulation plays a role. Our results demonstrate the ability of previously mated males to learn from their sexual experience and modify their behaviors to gain a mating advantage. These experienced-based changes in behavior reveal learned strategies that animals likely use to increase their fecundity in natural competitive environments.

634A

Characterization of novel genes affecting male courtship and mating behavior in *Drosophila melanogaster*. Janna Schultzhaus, Ginger Carney. Biology, Texas A&M, College Station, TX.

Drosophila melanogaster males perform stereotypical courtship behaviors to attract female mates. While the roles of many genes involved in these mating behaviors have been characterized and the basic genetic hierarchy underlying male mating behavior is well understood, there are undoubtedly numerous other components that contribute to the expression of this dynamic, complex trait. To identify additional genes that may contribute to male courtship behavior, a list of candidate genes involved in courtship was generated from microarray analyses on the heads of males that had courted females. We tested these genes for effects on courtship and mating behaviors by decreasing adult expression via RNAi. Reducing expression of two genes, *hairy* and *CG1416*, increased mating latency, indicating that the males are either less attractive to females or less

efficient at courting. Reducing expression of two other genes, *Drop* and *Tdc1*, decreased copulation duration, signifying an inability of males to mate for longer periods or an unwillingness to invest more energy into a single mating. We have identified and characterized how these genes, which had previously not been implicated to play a role in male courtship behavior, can shape male-female interactions.

635B

The ontogeny of feeding behavior. Maria A. Carvalho[1], Beryl Jones[2], Christen K. Mirth[1]. 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Janelia Farm Research Campus, Ashburn VA, USA.

In the wild, the choices an organism makes can be both crucial to its development and molded by its developmental stage. New insights in environmentally-regulated development and the characterization of some of the neurons involved in interpreting the chemical environment and regulating food choice in Drosophila provide an exciting opportunity to investigate how foraging choices in defined ecological contexts depend on developmental processes. We address this question in two ways, 1) By defining how development shapes the foraging preferences of larvae and 2) By identifying how development acts on the neural circuits that regulate foraging behavior to influence the stage-dependent food choices. Our results show that in the third larval instar, an environmentally sensitive developmental event called critical weight shapes foraging preferences. We observe larvae have different food preferences before and after this developmental milestone. Pre-critical weight larvae are more careful in their food choices and forage for longer periods until they burrow in the food substrate than their post-critical weight siblings. Additionally, we screened a Gal4 collection selected to have sparse patterns of expression in the central nervous system. Using a neuronal inhibition approach, we have identified neurons that are strong candidates to be part of the circuit regulating foraging behavior and food preferences throughout development. We predict these neurons either show different morphologies or different activities before and after critical weight, molding, in that way, larval feeding behavior according to its development.

636C

Increased dopamine induces lethal foraging in Drosophila. Wanhao Chi, Cristi Frazier, Liwen Xu, Jeff Beeler, Xiaoxi Zhuang. Neurobiology, University of Chicago,IL.

Foraging strategies reflect economic decision-making. When food is scarce, it is essential for survival to balance the energy gained from potential food acquisition against the energetic cost of finding food. The effectiveness of different strategies, such as exploiting a food source versus expending energy on exploration, will be dependent on food availability in the environment. Despite an abundance of studies on neural circuits encoding value and economic decision-making, and the significance of such studies is based on the assumption that neural mechanisms underlying adaptive economic choice ultimately promote survival. However, studies rarely tested this most fundamental aspect of neuroeconomics directly. Dopamine is implicated in reward and economic decisions. Our recent study in mice reported that elevated dopamine via reduced dopamine transporter (DAT) reduced coupling between reward history and action choice. As a consequence, mutant mice expressed diminished exploitation of readily available food, increased energy expenditure unrelated to procurement of food and impaired energetic thriftiness. In order to test if such a phenotype can affect survival when food source is limited, we turned to Drosophila. The fumin mutant has a truncated DAT protein and severely reduced dopamine reuptake. We set up a foraging paradigm, in which a thin capillary is the sole food source inside a cylinder compartment. Compared to wild-types, activities of fumin flies were much less directed toward food, and their consumption and survival severely impaired. Such a phenotype can be rescued genetically by re-expressing DAT. Dopamine may play a critical role in modulating the balance between exploration and exploitation. Higher dopaminergic activity promotes exploration at the expense of energetic thrift, decreasing foraging efficiency and survival when food source is limited.

637A

Direct comparison of *Drosophila* food intake assays. Sonali A Deshpande, Ariadna Amador, Sany Hoxha, Angela M Phillips, William W Ja. Department of Metabolism and Aging, The Scripps Research Institute, Jupiter, FL.

Metabolic diseases like type-2 diabetes and obesity are associated with disorders in food consumption. Although *Drosophila* is widely used as a model organism to study feeding and metabolic disease, assays to accurately measure food consumption remain poorly characterized. Due to the difficulty in measuring small volumes consumed by flies, existing food intake assays are argued to be unreliable, imprecise and inaccurate. Poor food intake measurements can result in erroneous interpretations of studies in metabolism, nutrition, behavior, and disease. Here, we compare four popular food intake assays: the Capillary Feeder (CAFE), food-labeling with a radioactive tracer or a colorimetric dye, and observations of proboscis extension. We measure the resolving power of each technique under typical experimental conditions and directly compare them by performing combinations of assays simultaneously on the same cohort. While each approach has distinct advantages, we show that the CAFE and radio-labeling assays stand out by resolving differences in feeding that dye-labeling and behavioral measurements fail to distinguish. Understanding the strengths and limitations of the various food intake assays greatly facilitates all studies where feeding behavior plays an important role.

638B

Regulation of *Drosophila* feeding, growth, and development: linking neural precursor identity to functional significance. Amy L. Gresser, Brian Gebelein. Division of Developmental Biology, Cincinnati Children's Hospital Medical

Center, Cincinnati, OH.

The regulation of animal growth requires the interaction of neuronal, hormonal, and metabolic pathways. One key factor in characterizing growth regulation is the ability to link the development of discrete cell populations to their later functional roles. In this study, we show that an enhancer of the *rhomboid* serine protease, a catalyst for epidermal growth factor secretion, acts in bilaterally symmetric cell clusters in the *Drosophila* embryonic head. Lineage tracing reveals that these cells give rise to a subset of deutocerebral CNS neurons as well as to the hypopharyngeal sensory organ, a largely uncharacterized neural structure of unknown function. Targeted ablation of the cells results in impaired larval growth, delayed pupation, and pupal lethality. To better ascertain the nature of the growth defect, we used a variety of feeding assays to show that ablated larvae are capable of locating, ingesting, and clearing food. Importantly, however, we observed that while feeding rates for starved control and ablated larvae are initially similar, ablated larvae subsequently exhibit a rapid decrease in feeding that that could suggest an abnormal sense of satiety. In total, our studies reveal that this *rhomboid* enhancer serves as a valuable tool for labeling and ablating the precursors of two distinct neural structures and that at least one of these structures plays an essential role in the regulation of feeding behavior, animal growth, and viability.

639C

Decision-making neurons for feeding behavior revealed by thermogenetic activation in *Drosophila*. Shinya Iguchi, Michael Gorczyca, Motojiro Yoshihara. Neurobiology, UMASS Med, Worcester, MA.

The decision of when to eat is a complex function of both environmental variables and internal physiological state. How these external and internal determinants are integrated by the nervous system is largely unknown, and the neural substrates of the feeding decision remain poorly characterized. Work from our lab, in collaboration with B. White and K. Ito, has identified a single pair of neurons in the *Drosophila*brain, Fdg(feeding)-neurons, which command the entire sequence of feeding behavior (in preparation). They were identified from a screen of Gal4 lines established by the NP consortium[1] by activation of a neuron subset expressing a heat-activated cation channel, TrpA1. We used infrared laser light from a 2-photon microscope to spatially restrict heat to the cell body of either the left or right Fdg-neuron expressing TrpA1, which led to asymmetric proboscis extension in the same direction as the illuminated side and also triggered pharyngeal pump movement. Ablation of an Fdg-neuron cell body on one side by strong laser illumination made sucrose-induced proboscis extension response asymmetric, extending in the opposite direction. Furthermore, ablation of Fdg-neurons on both sides led to complete suppression of feeding behavior, indicating a pivotal role for Fdg-neurons in the feeding circuit. Here we tested various gustatory modalities that might be integrated by the feeding behavior circuit. Taking advantage of our newly devised method to record calcium signals while simultaneously observing feeding behavior[2], we tested the effect of satiety or bitter signals on Fdg-neuron activity correlated with feeding behavior. Fdg-neurons were activated in response to food presentation and this coincided with a proboscis extension, but only in the starved state. Fdg-neuron activation by sugar stimulus was suppressed by the bitter compound, caffeine. These results support our hypothesis that Fdg-neurons function as a decision making center, integrating multiple kinds of information. 1)Yoshihara and Ito (2000) Dros. Inf. Ser. 83:199. 2)Yoshihara (2012) JoVE, 62, e3625, DOI: 10.3791/3625.

640A

Dissecting the Dopaminergic Circuitry Underlying Feeding Behavior in *Drosophila*. Lay Kodama[1], Qili Liu[1], Mark Wu[1,2]. 1) Department of Neurology, Johns Hopkins University, Baltimore, MD; 2) Department of Neuroscience, Johns Hopkins University, Baltimore, MD.

Obesity is a major problem in modern society, and there is great interest in understanding the mechanisms mediating hunger and appetite. In animals, these hunger signals are essential for food seeking behaviors and can be modulated by various neural circuits. Dopamine (DA) is a key neuromodulator that has been shown to regulate a wide variety of behaviors in mammals, including feeding. In *Drosophila*, recent studies have suggested that DA increases the probability of the proboscis extension response (PER) by modulating the sensitivity of gustatory neurons to sucrose. Here, we investigated the role of DA and specific DA circuits in regulating feeding. To identify DA neurons involved in feeding, we activated distinct subsets of DA neurons and measured food intake after a mild-starvation protocol. These data suggest that a specific subset of DA neurons, distinct from those that promote arousal, is involved in promoting feeding. Using the PER assay, we also found that activation of these DA neurons promotes an increase in sensitivity to sweet-tasting substances, caloric (but tasteless) substances, and water, suggesting a general enhancement of appetitive responses. Using various intersectional approaches, we are further dissecting the specific DA neurons involved in these processes. In addition, we are working to identify the specific DA receptor and downstream target cells required for these behaviors.

641B

Acid taste detection in *Drosophila*. Sandhya Charlu[1], Zev Wisotsky[2], Adriana Medina[3], Anupama Dahanukar[1,2,3]. 1) Biomedical Sciences Graduate Program, UC Riverside; 2) Interdepartmental Neuroscience Program, UC RIverside; 3) Department of Entomology, IIGB, UC Riverside.

The taste system acts as the first line of defense against ingestion of harmful substances. Stimuli that could cause damage to internal organs are usually signaled as aversive by the taste system. Acids, which are often present in spoilt or unpalatable foods, are perceived as sour by humans but little is understood about their detection by the Drosophila taste system. Here we identify the behavioral and cellular responses of the fly to acid tastants. Using two independent behavioral assays we find that

acid tastants inhibit behavioral responses to sucrose, suggesting that they are recognized as deterrents. Recordings from individual taste hairs in the labellum reveal that a subset of bitter taste neurons is activated by acidic pH. Correspondingly, flies in which bitter taste neurons are genetically silenced show reduced aversion to mixtures of acids with sucrose. Nevertheless, such flies still retain the ability to reject high concentrations of acids. We therefore investigated the effect of acids on the activity of sweet taste neurons, and found that sucrose response is inhibited in a pH-dependent manner. Sucrose response inhibition occurs directly on sweet taste neurons and does not rely on the presence of functional bitter taste neurons. The inhibitory effect can be alleviated by an increase in the concentration of sucrose, suggesting that sweet taste neurons can calibrate information about the sugar content as well as the pH of a food substrate. Together, our results reveal two independent cellular pathways for sensing and responding to acid tastants. Currently, we are investigating the molecular mechanisms that underlie acid detection by taste neurons.

642C

Gustatory perception regulates the behavioral response to starvation. Nancy J. Linford, Scott D. Pletcher. Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI.

Starvation stress rapidly leads to death. Upon starvation, a behavioral switch is initiated that promotes sleep loss. This behavioral change facilitates a shift in resources away from homeostasis and toward activities that promote survival or reproduction. When feeding is re-initiated, the information indicating nutritional success must be relayed to neuronal sleep regulatory centers to promote the return to normal sleep homeostasis. Little is known about how this information is relayed. We were interested in determining the role of sensory perception in regulating starvation-induced sleep loss. We have determined that gustatory perception is sufficient to promote normal sleep homeostasis in the absence of nutrition. Furthermore, under conditions of low nutrient availability, gustatory perception is also required to suppress starvation-induced sleep loss. However, under conditions of high nutrient availability, gustatory-independent nutrient perception is capable of terminating starvation-induced sleep loss. This work establishes the gustatory system as a crucial component of behavioral regulation under conditions of nutrient stress. It also delineates a concentration-dependent redundant role for gustatory-independent perception in regulating nutrient-dependent behavior. These findings provide insight into potential mechanisms for all organisms, including humans, to overcome behavioral side effects during periods of short-term nutrient stress.

643A

Modularity of Function among Rickets-expressing neurons in the Wing Expansion Network of Drosophila. Feici Diao[1], Fengqiu Diao[1], Chun-yuan Ting[2], Chi-hong Lee[2], Benjamin White[1]. 1) NIMH, NIH,Bethesda,MD; 2) NICHD, NIH,Bethesda,MD.

We have previously shown that wing expansion can be induced in Drosophila by stimulating a single pair of neurons located in the subesophageal ganglion that express the hormone bursicon. These bursicon-expressing neurons trigger not only the behavioral and somatic changes that support wing expansion, but also suppress the inhibitory effects of unfavorable environments. To understand how these diverse functions are coordinated, we have identified the downstream targets of the bursicon-expressing neurons using the T2A-GIFF technique, generating a Gal4 line that expresses in the same pattern as the bursicon receptor encoded by the rickets gene. Expression of a UAS-RK transgene under the control of the rk-Gal4 driver fully rescues the wing expansion deficits of rk4 null mutants. Further, wing expansion can be induced by activating RK-expressing neurons using UAS-dtrpA1. By varying the period of activation between 0 - 10 min, we are able to distinguish two distinct effects: 1) immediate induction of wing expansion behaviors, and 2) suppression of environmental inhibition. To determine whether distinct subsets of RK-expressing neurons mediate these motor and modulatory effects, we coupled rk-Gal4 to the Split LexA system and used 179 VP16AD enhancer-trap lines to drive LexAop-dTrpA1 expression in different subsets of RK-expressing neurons. Consistent with our initial observations, we identified two distinct functional groups of flies, one showing an immediate motor response, but no suppression of environmental inhibition, and the other showing suppression of environmental inhibition, but no immediate motor response. We conclude that at least two distinct groups of RK-expressing neurons act within the wing expansion network, one directly transducing the bursion signal to motor outputs, and the other acting via a positive feedback loop to disinhibit bursicon-expressing neurons. The latter group is likely to help mediate the decision to expand the wings.

644B

The *Drosophila* fat body modulates sexually dimorphic responses to stress. Wendi S. Neckameyer, Kathryn J. Argue. Dept Pharmac & Physiol Sci, St Louis Univ School Med, St Louis, MO.

As for mammals, the stress response in *Drosophila melanogaster* is sexually dimorphic. However, unlike mammals, the sex determination pathway in *Drosophila* is well established, making this an ideal system to identify factors involved in the modulation of sexually dimorphic behavioral responses to stress. In this study, we show that the fat body, which has been shown to be important for body size, energy homeostasis, and sex determination, is a dynamic tissue that changes in response to stress in a sexually and temporally dimorphic manner. Manipulation of the sex determination pathway in the fat body via targeted expression of the transcription factors *transformer* and *transformer2*, was able to change physiological output in response to starvation and oxidative stress to that of the opposite sex. These data suggest that the fat body in head tissue may serve as the functional analogue of the mammalian pituitary gland. Additionally, our data uncover the possibility of additional downstream targets for transformer that are separate from the sex determination pathway that can influence behavioral

responses.

645C

Neurotransmitter receptors regulate ecdysteroid biosynthesis and developmental transition in Drosophila. Yuko Shimada[1], Yosuke Umei[1], Jevgenija Maramzina[1], Ryusuke Niwa[1,2]. 1) Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan; 2) PRESTO, JST, Japan.

The Insect principal steroid hormones, ecdysteroids, play crucial roles in many aspects of development. During larval stages, ecdysone is synthesized from dietary cholesterol in the specialized endocrine organ called the prothoracic gland (PG). Ecdysteroid biosynthesis is influenced by several external conditions, such as nutrition, temperature, and photoperiod, which lead to flexible alterations of developmental timing accompanied with molting and metamorphosis. However, it remains unclear how external information is transmitted to the PG to adaptively control the ecdysteroidogenic enzymes. To uncover genes involved in controlling the ecdysteroid biosynthesis in the PG, we conducted a transgenic RNAi screen using the PG-specific GAL4 drivers and UAS-RNAi strains. We found that developmental arrests were caused by RNAi for genes encoding some neurotransmitter receptors. These phenotypes were rescued when the RNAi animals were fed 20-hydroxyecdysone. Moreover, the ecdysteroid titer and the expression levels of ecdysteroidogenic enzyme genes significantly decreased in the RNAi animals. These results suggest that these neurotransmitter receptors are essential for ecdysteroid biosynthesis. Interestingly, a subset of neurons, which was different from the previously-identified PTTH neurons, directly innervated to the PG. The dendrites of these neurons extended toward the subesophagal ganglion, known as the insect feeding center, implying that these neurons receive signals related to food. Consistently, the projection of the neurons to the PG was affected by nutrient condition. Moreover, genetic manipulations inhibiting the projection into the PG resulted in a delayed pupariation. Considering that neurotransmitters modulate neuronal activities in response to external stimuli, we currently hypothesize that the neurotransmitters and their receptors play important roles in reflecting environmental conditions on ecdysteroid biosynthesis in the PG.

646A

Deciphering how general anesthetics work: the role of ion channels. Joel P. Goodman[1], Trevor Batty[1], Winnie Cheung[1], J. Ryan Jackson[1], Michael J. Murray[2], Gerald B. Call[1]. 1) Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ; 2) Department of Anesthesiology, Mayo Clinic, Scottsdale, AZ.

For more than 160 years, volatile anesthetics (VAs) have been used, yet the action of VAs upon the central nervous system is unknown. Recent evidence suggests that VA effects occur through multiple targets as opposed to a single common mechanism. Previous studies have indicated that ion channels may be VA targets. Our previous study was the first comprehensive analysis of ion channels in the response to isoflurane. We tested 359 ion channel genes within *Drosophila melanogaster* in an RNA interference (RNAi) screen to determine potential candidates for VA action. In the previous study, a small halothane screen was performed for data verification on a few ion channels; however, this revealed differences in their responses to the two VAs. As isoflurane and halothane have overall similar chemical structures, their responses were expected to be similar. This study explores the effects of halothane on ion channels and compares them with the isoflurane data. RNAi constructs were used for gene-specific silencing of ion channels throughout the genome. An inebriometer was used to quantitatively study the effects of halothane on the flies. The data were analyzed and compared with the isoflurane data. Of the 343 genes tested, 38.8% exhibited resistance which is very similar to the 39.7% resistant to isoflurane. Of all the genes that showed resistance to VAs, 69 were resistant to halothane only, 78 had resistance to isoflurane only, and 64 showed resistance to both VAs. In this shared resistance category, we found that 11 genes were either resistant or strongly resistant to both VAs. A more detailed analysis will be presented at the meeting. Analysis of the data revealed that there are significant differences between the actions of isoflurane and halothane on ion channels. These differences indicate that there are many potential pathways for VA action, even among those with similar chemical structures.

647B

The ion channel *seizure* regulates Adipokinetic hormone cell excitability. Rebecca J. Perry, Jason T. Braco, Erik C. Johnson. Department of Biology, Wake Forest University, Winston-Salem, NC.

The mechanisms of how organisms maintain metabolic homeostasis in light of dynamic nutrient availability is not completely understood. In *Drosophila*, the adipokinetic hormone (AKH) is a principal hormone that functions in this process. AKH signaling regulates energy levels, through the direct mobilization of trehalose during low hemolymph sugar. Adipokinetic hormone is required for starvation-induced hyperactivity, an adaptive behavior that assists in foraging. In order to better understand AKH signaling, we are conducting a genome-wide RNAi based screen targeting different ion channels that regulate AKH cell physiology. We evaluated the consequences of RNAi expression in AKH cells on AKH related phenotypes, specifically lifespan and locomotion during starvation. From this initial behavioral screen, we identified the channel encoding the *seizure* potassium channel as a candidate AKH regulatory element. Expression of the *seizure* RNAi in AKH cells leads to lengthened lifespan during starvation. Additionally, there were observable changes in starvation-induced hyperactivity. We confirmed *seizure* expression in AKH neuroendocrine cells through analysis of the transcriptome on this cells type with RNAseq. We will also report preliminary experiments on AKH cell activation in a *seizure* mutant background and report other findings from the genome-wide RNAi screen and expression data.

648C

Genetic variation in associative learning ability of Drosophila melanogaster larvae. Seana Lymer, Julia Saltz, Sergey Nuzhdin. University of Southern California, Los Angeles, CA.

Social learning requires the integration of associative learning, memory and social behavior (LMS), and these vary considerably among individuals within a species. Genetic differences cause alterations in neural functions that ultimately cause variability in social learning, but the genes and neural circuits that play a role in LMS are not fully understood. Here, we use Drosophila melanogaster to find the range of LMS variation in a number of natural populations. Third-instar Drosophila larvae were screened for learning and memory ability using associative conditioning. Pools of larvae were exposed simultaneously to a mild electric shock and a specific smell, training the larvae to avoid that smell. Memory for avoidance behavior was tested at short-, medium-, and long-term delays, as different neural substrates may be responsible for these types of memory. In our heterozygous populations, the larvae show genetic variation in associative learning for all memory types. While some genotypes show a robust memory in all conditions, other genotypes show memory decay over time. By combining phenotypic screens representing natural variation in larval learning ability with molecular-genetic techniques examining the mushroom body, we can identify how genetic variation is functionally responsible for individual differences in LMS.

649A

Mapping Glial Circuits Underlying Neuronal Function and Behavior in *Drosophila*. Taylor R. Fore[1], Camille Milton*[1], Alexander Nasr*[1], Kody McKay*[2], Jered Stowers[1], Hong Bao[1], Bing Zhang[1]. 1) Department of Biology, University of Oklahoma, Norman, OK; 2) Department of Biological Sciences, Southwestern Oklahoma State University, Weatherford, OK.

Behavior is carried out directly by motoneurons but it is highly influenced by a large network of cells, including sensory cells, interneurons, modulatory neurons, and muscles. Glial cells are an often-overlooked brain cell type in this process. Despite increasing interest in glia cells, their roles in the nervous system are still to be explored. Here, we offer a new approach to study the role of glial cells in neuronal function and behavior. Instead of focusing on specific genes and the consequence of their mutations, we undertake an unbiased genetic approach to screen for the critical subsets of glial cells that regulate behaviors in fruit flies. Once many different subsets of glial cells are identified, we will be able to identify specific glial partner neurons, probe glia-neuron connectivity, and examine the effect of neighboring glial cells on neuronal function and behavior. To achieve this objective, we express UAS-MJD:PolyQ^{78} to perturb glial function and observe the effect on locomotion. Pan-glial expression of PolyQ^{78} results in significant age-dependent defects in climbing and walking. At the cellular level, these behavioral defects are marked by a significant change in the morphology of both glia and neuronal circuits, as well as a dramatic change in NMJ physiology, underscoring the importance of glia in neuronal health. Utilizing the FINGR method (Bohm et al. 2010) enables us to unbiasedly refine 'glial circuits' into smaller units and correlate behavioral changes to these circuits. Specifically, flies containing Tub>Gal80>; UAS-PolyQ; Repo-Gal4, UAS-GFP are crossed to a collection of enhancer-trap Flippase (FLPx2) lines. When FLPx2 expression overlaps with Repo-Gal4 positive cells, Gal80 is flipped out, resulting in selective activity in subsets of glial. This forward genetic screen has identified a number of positive FLPx2 lines, demonstrating the feasibility and success of mapping glial circuits involved in locomotion.

650B

Individual leg tracking reveals the basic building blocks of behavior. James S. Kain[1], Chris Stokes[1], Quentin Gaudry[2], Xiangzhi Song[1,3], James Foley[1], Rachel Wilson[2], Benjamin de Bivort[1,4]. 1) The Rowland Institute at Harvard, Cambridge, MA; 2) Department of Neurobiology, Harvard Medical School, Boston, MA; 3) College of Chemistry & Chemical Engineering, Central South University, Changsha, China; 4) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

All animals have evolved unique sets of behavior, but how did these behaviors evolve? To examine this question we engineered a high-resolution technique to track, for the first time, all six legs of a fruit fly behaving spontaneously while tethered to a floating ball. We developed custom software to automatically identify and categorize all the behaviors of an individual walking fly. Our sensitive methods easily identified walking, turning and postural adjustments, and can discriminate several distinct types of grooming. With this setup we are exploring several important aspects of basic locomotor behavior: 1) Recording the behavioral fingerprints of flies revealed that individuals possess unique behavioral personalities that persist between trials. 2) We can probe circuit-level properties of locomotor behavior since all of our leg tracking was designed to be compatible with electrophysiology, optophysiology, two-photon and fluorescent microscopy. Additionally, we can present visual stimuli to the fly to study more complex locomotor behavior (e.g. phototaxis or optomotor responses). Using moving bar displays, we found flies exhibited less variation in their behavior under closed-loop conditions relative to open-loop. 3) Finally, we developed methods to uncover the basic behavioral building blocks. We suspect these motifs or atoms of behavior are the fundamental units that the animal strings together to generate complex behaviors such as phototaxis or a head groom. We found that there were approximately 20 motifs underlying locomotor behavior. Equipped with these techniques, we can now ask whether differences in behavior across age, ecological conditions, sex, and species have corresponding changes in the types of motifs or in the way the motifs are arranged? This knowledge will be vital to understanding the evolution of behavior.

651C

Sensory modalities relevant for the walking behavior of adult Drosophila melanogaster. Cesar S. Mendes[1], Imre Bartos[2],

Turgay Akay[3], Szabolcs Marka[2], Richard Mann[1]. 1) Columbia University, Dept. Biochemistry and Molecular Biochemistry, , New York, NY; 2) Columbia University Dept. of Physics New York , NY; 3) Columbia University Dept. of Neurological Surgery, New York, NY.

Coordinated walking in vertebrates and multi-legged invertebrates such as the fruit fly Drosophila melanogaster requires a complex neural network. This network is comprised of motor neurons, Central Pattern Generators and sensory neurons. CPGs produce rhythmic outbursts, without input from the central brain that target leg motor neurons. Critical to this network, sensory neurons constantly report the position and load of each of the leg segments and the terrain conditions. The fruitfly somatic system bears distinct classes of sensory neurons that report distinct proprioceptive parameters and environmental cues. Identify the components and understand the relevance of each one of these sensory components is critical to unravel the circuit regulating the walking behavior. To detect disturbances in the walking circuit of the fruit fly, we developed a high-speed optical imaging system that allows us to track footprints and the fly's body as it walks freely on a flat surface. A custom analysis software allows us to quantify many parameters exhibited by walking flies, such as step timings, footprint positions and left-right coordination. With this method as readout, we used a combinatorial expression system to perform loss and gain of function experiments targeting different classes of leg sensory neurons. Surprisingly, we find that inactivation of proprioceptive feedback in the leg led to deficient step precision, but interleg coordination and the ability to execute a tripod gait were unaffected. Moreover, different sensory modalities display speed-dependent requirements indicating that CPGs generate the primary set of instructions required for walking. Finally, we investigate how external sensory cues influence walking. Interestingly, antenna removal affected several gait parameters, possibly through gravity-sensitive neurons.

652A

Increasing Tip60 HAT levels rescues axonal transport defects and associated behavioral phenotypes in a Drosophila Alzheimer's disease model. Ashley A. Zervos, William Reube, Felice Elefant. Dept Biol, Drexel Univ, Philadelphia, PA.

Axonal transport defects and axonopathy are prominent in early pre-clinical stages of Alzheimer's disease (AD), often preceding known disease-related pathology by over a year. As epigenetic transcriptional regulatory mechanisms such as histone acetylation are critical for neurogenesis, it is postulated that their misregulation might be linked to early pathophysiological mechanisms that contribute to AD. The histone acetyltransferase (HAT) Tip60 epigenetically regulates genes enriched for neuronal functions and is implicated in AD via its formation of a transcriptional regulatory complex with the amyloid precursor protein (APP) intracellular domain. Disruption of APP function is associated with axonal transport defects, raising the possibility that an epigenetic role for Tip60 might also be involved. Here, we examine whether Tip60 HAT activity functions in axonal transport using Drosophila CNS motor neurons as a well characterized transport model. We show that reduction of Tip60 HAT activity in the nervous system causes axonopathy and transport defects associated with misregulation of certain axonal transport linked genes. Functional consequences of these defects are evidenced by reduced locomotion activity of the mutant Tip60 larvae and these phenotypes can be partially rescued with the HDAC inhibitor ms-275. Finally, we demonstrate that Tip60 function in axonal transport is mediated by APP and that remarkably, excess Tip60 exerts a neuroprotective role in APP induced axonal transport and functional locomotion defects. Our observations highlight a novel functional interactive role between Tip60 HAT activity and APP in axonal transport and provide insight into the importance of specific HAT modulators for the treatment of cognitive disorders.

653B

Mechanisms of force generation and auditory amplification in auditory neurons of *Drosophila melanogaster*. Somdatta Karak[1], Julia Jacobs[2], Maurice Kernan[3], Daniel Eberl[2], Martin Goepfert[1]. 1) Schwann Schleiden Forschungszentrum, Univ of Goettingen, Goettingen, Niedersachsen, Germany; 2) 269BB, Dept. of Biology, Univ of Iowa, Iowa, IA 52242, USA; 3) Dept. of Neurobiology and Behavior, SUNY, New York, USA.

Hearing in both vertebrates and invertebrates employ an amplifier to enhance sensitivity of auditory neurons, enabling detection of low intensity sounds. Prestin in vertebrate hair cells changes conformation of the hair cells in a voltage dependent way and thus confers electromotility and amplification (Zheng *et al*, 2000). Though it is known that auditory neurons of *Drosophila melanogaster* are motile, the molecular mechanism of this process yet remains to be elucidated. Though prestin homologs are reported in fly auditory system experiments have failed to show its role as a molecular motor to facilitate amplification. The gating spring model predicts a positive interplay between ion channels and adaptation motors that leads to both auditory amplification as well as adaptation (Nadrowski *et al*, 2008). The dynein arms in the axoneme of the ciliated dendrites of auditory neurons hint at the role of axonemal dyneins in force generation and ciliary motility. Using laser Doppler vibrometry we analyzed roles of axonemal dynein mutants in auditory amplification. We show that axonemal dynein intermediate chain dmDNAI2 is required for auditory amplification and generation of sound-evoked action potentials. On the other hand we demonstrate that axonemal dynein heavy chain dmDNAH3 is dispensable for amplification but is required for generation of action potentials suggesting its probable role in electrical signal propagation along the length of the cilium. Epistatic analyses suggest that both the axonemal dyneins act downstream to TRPV channels present along the length of the cilia to facilitate amplification. Thus, our results hint towards at least two different roles of axonemal dyneins regulated by TRPV channels, as a direct force generator and a modulator of force generation in auditory neurons.

654C

Dopamine and ecdysone acutely modulate AKH signaling during physiological stress in Drosophila. Jason T. Braco,

Greg E. Alberto, Emily L. Gillespie, Erik C. Johnson. Biology, Wake Forest University, Winston-Salem, NC.

The link between dopamine (DA) and stress response has been well established in both vertebrates and invertebrates. However, the precise mechanisms of how dopamine is modulating behavioral and physiological responses to stress are unknown. In Drosophila, we show that genetic and pharmacological manipulations that increase levels of dopamine cause elevated locomotion during physiological stress and a concomitant decrease in survivorship. Conversely, manipulations that decrease dopamine signaling cause blunted locomotor responses and longer survival during physiological stress. To identify underlying mechanisms of dopamine action, we performed these manipulations in different mutant backgrounds. Notably, manipulations of dopamine levels in flies with altered Adipokinetic Hormone (AKH) signaling implicate epistatic interactions. AKH is required for stress- induced hyperactivity, and the mobilization of energy from stores, and we have previously evaluated the role of AKH as a stress hormone. We next tested whether DA exerts its effects directly on AKH cells, through employing the fluorescent Ca2+ reporter, GCaMP, to examine AKH cell activation. Application of dopamine leads to heightened calcium levels, in an energy-independent manner. To identify the dopamine receptor subtypes that mediate DA responses, we selectively introduced RNAi elements targeting the four different DA receptors present in Drosophila. RNAi elements targeting the DopEcR result in behavioral phenotypes consistent with DA activation and we confirmed the expression of this receptor with RNAseq. Notably, this receptor is inhibited by ecdysone, and co-application of DA and ecdysone results in a loss of GCaMP responsiveness. These results suggest a complex neuroendocrine circuit involving DA and ecdysone convergence that regulate AKH signaling during physiological stress.

655A

The temporal pattern of neural activity underlying ecdysis behavior is regulated by neuropeptides downstream of Ecdysis Triggering Hormone. John Ewer, Wilson Mena. Centro Interdisciplinario de Neurociencias, Universidad de Valparaiso, Valparaiso, Valparaiso, Chile.

The insect molt culminates with ecdysis, an innate and vital behavior that is used to shed the remains of the old cuticle. Ecdysis includes several behavioral subroutines that are expressed sequentially to loosen and then shed the old cuticle, then expand and harden the new one. Ecdysis is triggered by the neuropeptide, Ecdysis-Triggering Hormone (ETH), which activates sequentially a number of peptidergic neurons, all of which express the A isoform of the ETH receptor (ETHR). Current models propose that each class of peptidergic neurons then activates or modulates the different phases of the ecdysis motor programs. We examined ecdysis behavior and used the calcium sensitive GFP, GCaMP, to monitor the activation of direct ETH targets, as well as that of motoneurons, which provided an *in vitro* readout of the resulting behavior. We determined the pattern of GCaMP activation in wild-type animals and also in animals in which ETHR was disabled using RNAi or were mutant for specific neuropeptides. All these manipulations affected ecdysis behavior. However, whereas decreasing ETHR expression using RNAi caused a quantitative reduction in the neural response to ETH, eliminating neuropeptides downstream of ETH caused qualitative changes to the pattern of neural activity induced by this triggering hormone. Thus, unlike the model in which neuropeptides downstream of ETH are the outputs that are sequentially activated to then turn on specific ecdysial subroutines, our results suggest that these neuropeptides configure the network's response to ETH, which then controls the ensuing behaviors. In addition to contributing to the further understanding of how this critical insect behavior is regulated, our results provide insights for understanding how multiple peptides regulate complex physiological and behavioral responses.

656B

Neuropeptide signaling is required for tissue damage-induced nociceptive sensitization in *Drosophila* larvae. Seol-Hee Im[1], Daniel Babcock[1], Felona Gunawan[2], Michael Galko[1]. 1) Biochemistry and Molecular Biology, University of Texas MD Anderson Cancer Center, Houston, TX; 2) Department of Biochemistry Cell and Molecular Biology, Rice University, Houston, TX.

Nociception is the sensory perception of noxious stimuli. It serves the adaptive function of protecting organisms from potential tissue damage. When tissue is damaged, organisms display a subsequent reduction in their detection threshold for noxious stimuli, a phenomenon called nociceptive sensitization or allodynia. To understand the genetic basis of nociceptive sensitization, we combined an assay for tissue damage-induced thermal allodynia with a tissue specific *in vivo* RNAi screen for genes required within nociceptive sensory neurons for the development of thermal allodynia. We found that knock-down of the *Drosophila tachykinin receptor* (*DTKR*) inhibited UV-induced thermal allodynia. *DTKR* encodes the *Drosophila* homolog of the mammalian G-Protein-Coupled Receptor (Neurokinin receptor) for Substance P, a prominent nociceptive peptide neurotransmitter. Mutant alleles of *DTKR* also exhibit strong sensitization defects suggesting that the *in vivo* RNAi results are on-target. Pan-neuronal, but not nociceptive sensory neuron-specific RNAi targeting *tachykinin* (*dTk*), which encodes the neuropeptide ligands for DTKR, also resulted in a decrease in thermal allodynia. Importantly, no *DKTR* or *dTk*knockdowns or mutants gave defects in baseline nociception, suggesting a specific function in damage-induced sensitization. Furthermore, overexpression of *DTKR* in the nociceptive sensory neurons induced an ectopic sensitization in the absence of tissue damage. Genetic epistasis analysis using larvae with hyperactivated neuropeptide signaling suggested that Tachykinin signaling is upstream of Hedgehog signaling but independent of TNF signaling (two other nociceptive modulators) in pain sensitization. Our study suggests that Tachykinin, a *Drosophila* homolog of Substance P, plays a conserved role in nociceptive sensitization that will be amenable to genetic dissection using the powerful genetics and nociception assays available in *Drosophila*.

657C

Control of Body Size by TGF-β Signaling. Lindsay Moss-Taylor, Michael O'Connor. University of Minnesota, Minneapolis, MN.

Body size is tightly regulated during development to maximize adult fitness. In *Drosophila*, final body size is mainly determined by growth rate and duration of growth during juvenile stages; once maturation occurs, body size is set. The rate of growth is determined by insulin-like peptide (dilp) signaling, while the duration of growth is regulated by the neuropeptide prothoraciotropic hormone (PTTH). Manipulation of these pathways alters final body size by either accelerating or delaying ecdysone production and the onset of metamorphosis. Under normal growth conditions, termination of *Drosophila* juvenile development is triggered when third instar larvae achieve "critical weight," a size after which starvation no longer delays the time to metamorphosis. How larvae sense critical weight and the relationship of critical weight to dilp and PTTH signaling remains largely unknown. We are investigating the newly identified role that TGF-β ligand Activinβ plays in regulating *Drosophila* body size and timing of metamorphosis. We have found that mutations in dActivinβ (dActβ) cause accelerated larval development and smaller final body size. Our preliminary results show that dActβ is expressed in the Insulin Producing Cells (IPCs) in the central brain. Since increased insulin signaling in the IPCs advances metamorphosis, we will test the hypothesis that this TGF-β ligand is affecting body size and developmental timing by regulating insulin production and/or release.

658A

Dopamine Can Regulate Period of the *Drosophila* Circadian Clock. Karol Cichewicz[1], Emma Garren[1], Magali Iché-Torres[2], Serge Birman[2], Jay Hirsh[1]. 1) Biology, University of Virginia, Charlottesville, VA; 2) CNRS, ESPCI, Paris.

Dopamine (DA) is important for many physiological functions including locomotor activity, learning and memory and light sensitivity. We have developed a new genetic background lacking DA in the CNS, which allows us to study complex DA modulation by rescuing its expression in specific neurons. Here we show that spatial imbalance of DA expression results in altered period of the circadian clock.*Drosophila* tyrosine hydroxylase (DTH, *ple*), encoding the rate limiting enzyme in DA biosynthesis, expresses in the CNS and hypoderm, with tissue-specific alternative splice forms. A recent study from our laboratories (Riemensperger et al, 2010) shows that a modified DTHg FS+/- gene that selectively expresses in the hypoderm rescues the lethality of a DTH null mutation (*ple*^{2}), generating healthy flies with normal lifespan. These flies were constructed with the GAL4:UAS binary expression system, such that further genetic manipulations are difficult. Using the general approach from the previous study, we generated a genetic background lacking DA in the CNS without using binary expression tools. The DTH FS+/- mutations were recombineered into a genomic BAC, which was integrated site-specifically into a 3rd chromosome, and then recombined onto a *ple*^{2} mutant background. BAC plasmids containing the wild type 20kb DTH gene or the DTH FS+/- rescued *ple*^{2} lethality, showing that all DTH cis-regulatory elements are contained within this segment. This DTH FS+/- *ple*^{2} genetic background allows the study of DA signaling by restoring its expression in a spatial and temporal manner using Gal4 and LexA drivers. Restoration of DTH expression in the DA neurons labeled by TH-Gal4 produced a 27h period of locomotor activity in constant darkness, whereas total loss of DA, or expression driven by both TH-Gal4 and DDC-Gal4 produced a normal 24h rhythm. These results indicate an important circadian role for DA in the non-overlapping TH-Gal4 and DDC-Gal4 neurons. Since TH-GAL4 prominently lacks expression in PAM DA neurons, these are candidates for this role. We are studying the mechanism by which this occurs.

659B

Functional DTH expression from undriven UAS-DTHg. Emma J. Garren, Karol Cichewicz, Jay Hirsh. Biology, University of Virginia, Charlottesville, VA.

Dopamine (DA) biosynthesis is regulated by the rate limiting enzyme tyrosine hydroxylase (DTH, *ple*). Our lab has created a Drosophila genetic background lacking DA in the CNS. The approach uses a large genomic BAC clone containing a DTH gene modified to selectively express in the hypoderm, rescuing *ple*^{2} lethality but not CNS DA (see Cichewicz et al. poster). This background allows for further genetic manipulations using the Gal4-UAS binary expression system to study the role of DA modulation in circadian behavior. The intended approach relies on cell-specific rescue of DA using selective Gal4 drivers to express UAS-DTHg, genomic DTH. Unexpectedly, undriven UAS-DTHg is sufficient for DTH expression in a pattern similar to that observed with TH-Gal4. Immunostaining revealed DTH expression at a level much lower than expression driven by TH-Gal4 or in wild type. However, HPLC measurement of DA in whole brain extracts shows normal levels of DA, indicating that post-translational regulation of DTH can compensate for reduced DTH protein expression. Furthermore, circadian period phenotypes of the undriven UAS-DTHg in the DA-deficient background are the same as TH-Gal4-driven (see Cichewicz et al. poster). We are testing DTH derivatives lacking putative regulatory elements to allow our initial approach to succeed.

660C

The Effect of Peripheral and Central Histamine Deficiency on Courtship Behavior in *Drosophila melanogaster*. Judith A. Ingles[1], Anthony Hage[1], Shelby Lemke[1,3], Martin G. Burg[1,2]. 1) Biomedical Sciences, Grand Valley State University, Allendale, MI; 2) Cell & Molecular Biology, Grand Valley State University, Allendale, MI; 3) Univ. of Michigan Med. School, Univ. of Michigan, Ann Arbor, MI.

Histamine is a biogenic amine synthesized from L-histidine via a decarboxylation step by the enzyme histidine decarboxylase (HDC). Mutations in the *Hdc* gene, which disrupt HDC function, have been used to identify the effects of histamine deficiency on a number of behaviors in *Drosophila* including visual, mechanosensory, and temperature preference behaviors. Histamine

has been localized to peripheral sensory receptor cells (photoreceptor and mechanosensory receptor cells) and a small number of central brain neurons. Thus far, it has not been possible to separate the function of the histaminergic neurons in the CNS from that of the PNS, as *Hdc* mutations eliminate or severely reduce histamine levels in all cells through reducing *Hdc* transcript levels. Recently, we have characterized an *Hdc* transgene P[*gHdc*$^{+}$;*w*$^{+}$] in an *Hdc* null mutant background that rescues the *Hdc* mutant phenotype completely, restoring histamine in all cells and developmental stages. A set of deletions in the 5' noncoding region of the P[*gHdc*$^{+}$;*w*$^{+}$] transgene were made, some of which eliminate *Hdc* expression in adult central brain neurons. We are now using these *gHdc* transgene deletions to determine whether histamine deficiency in the central brain or peripheral tissues could disrupt a complex behavior, such as courtship. Virgin male and female flies were introduced into a small chamber to observe courtship behavior, and the time after introduction at which the various steps of courtship were exhibited was recorded. Results indicate that a total lack of histamine has a profound effect on the ability of flies to exhibit a normal courtship behavioral repertoire. Preliminary results also indicate that flies with only a CNS histamine deficiency appear to be disrupted in some aspects of courtship, although the precise effects are still being investigated.

661A

Virtual Fly Brain. Cahir J O'Kane[1], David Osumi-Sutherland[1], Marta Costa[1], Nestor Milyaev[2], Gregory Jefferis[3], J. Douglas Armstrong[2]. 1) Department of Genetics, University of Cambridge, Cambridge, UK; 2) Institute for Adaptive and Neural Computation, University of Edinburgh, Edinburgh, UK; 3) MRC Laboratory of Molecular Biology, Cambridge, UK.

This is an exciting time for research into the function of neural circuits in *Drosophila*. We can now control neuronal activity and gene expression in single classes of neuron over specific time windows and to assay the consequences for behaviour and for neuronal activity. At the same time, advances in bulk data generation and image analysis are producing huge new bulk-data sets including large collections of single neuron images, lineage data, synaptic connectivity data and transgene expression data. This is on top of a large and ever growing literature. To exploit the full potential inherent in all this new data, researchers need to be able to rapidly search and query it to find what, if anything, is known about the neurons they identify, find potential circuit partners and track down transgenes that specifically target neurons of interest. Virtual Fly Brain (VFB) is the only project dedicated to providing this query functionality across multiple bulk data sets and the literature. VFB includes referenced descriptions of hundreds of neuron classes curated from the literature, along with query-able details of their innervation patterns, and thousands of query-able transgene expression patterns and phenotypes linked to FlyBase. Integrated bulk data-sets include expression patterns and images for over 3000 GAL4 lines from HHMI Janelia farm. They also include over 16000 3D single neuron images from FlyCircuit, many mapped to published neuron classes, and all clustered by similarity using a neuron blast system (developed by G.Jefferis). These clusters, which may represent novel neuron classes, are all viewable as rotatable 3D images. In the near future we will add query-able neuron lineage data and expand data on our site to cover the entire adult and larval nervous systems. In summary, Virtual Fly Brain is well on the way to becoming the major data-integration hub for Drosophila neuroanatomy.

662B

Neurochemical Analysis of Drosophila Syndecan Mutants. LaPortia Pierce[1], Marleshia Hall[2], Olugbenga Doherty[2], Maria Deluca[3], Janis O'Donnell[2]. 1) Department of Natural Sciences, Stillman College, Tuscaloosa, AL; 2) Department of Biological Sciences, University of Alabama, Tuscaloosa, AL; 3) Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL.

Syndecans are transmembrane proteins that function as co-receptors that regulate signaling pathways with functions as diverse as cell adhesion, neuronal development, and inflammation. While mammals have four syndecan proteins encoded by four different genes, Drosophila have only one functionally conserved syndecan gene. Previous quantitative genetic studies identified Drosophila syndecan as a potential candidate gene affecting variation in fat storage. These studies along with human studies also uncovered a role for syndecans in sleep traits. Since dopamine pathways are reported to regulate sleep and arousal, and high-throughput yeast two-hybrid screens identified Drosophila syndecan as an interactor of tyrosine hydroxylase, the rate-limiting enzyme in dopamine biosynthesis, we hypothesized that mutations in the syndecan gene would result in alterations of the neurochemistry of the Drosophila brain, specifically dopamine, and possibly other monoamines as well. High performance liquid chromatography (HPLC) was used to quantify monoamines in the heads of adult flies in three homozygous syndecan mutant strains relative to wild type controls. These analyses revealed alterations in dopamine pools. Interestingly, serotonin levels also were modified suggesting that syndecan may function in a parallel fashion in serotinergic pathways.

663C

Two odor receptors contribute distinct and complex signals in response to structurally similar odor molecules. Scott A. Kreher, Christine Nguyen, Abhiram Nagaraj, Jorge Gacharna, Lorien Menhennett, Raquel Robles, Michael Wesolowski. Department of Biological Sciences, Dominican University, River Forest, IL.

The first step in sensing an odor is the interaction of an odor molecule with a specific odor receptor (*Or*) protein. In previous research, we electrophysiologically characterized the entire repertoire of odor receptors in the fruit fly (*Drosophila melanogaster*) larva and characterized the behavior of larvae in response to these odors. In examination of the behavioral responses of mutants for either *Or42a* or *Or42b*to ethyl acetate, we found that each receptor contributed to the behavioral response in a predictable manner: mutants of the high-affinity receptor *O42b* were not attracted to low

concentrations of ethyl acetate and mutants of the low-affinity receptor *Or42a* were not attracted to high concentrations of ethyl acetate. We extended this analysis to examine how *Or42a* and *Or42b* mutants behaviorally responded to three odor molecules that are structurally similar to ethyl acetate. We found that the odor receptor mutations differentially affected behavioral responses to these three odors, and that the responses were not fully predictable from the electrophysiological data set. In two cases, although the odors elicited electrophysiological responses from both *Or42a* and *Or42b*, only one odor receptor mutant displayed altered behavioral responses, which were typically loss of attraction phenotypes. Using *Or* receptor GAL4 lines, we found that we could rescue the behavioral phenotypes of the *Or42a* and *Or42b* mutants using the appropriate UAS *Or* lines. We have found that ectopically expressing *Or42a* in the native *Or42b* sensory neuron can elevate behavioral responses to high concentrations of ethyl acetate. These data taken together suggest the importance of sensory neuron context in odor receptor function. A second non-mutually exclusive explanation is that other aspects of odor receptor response are the salient features for odor coding, such as response kinetics.

664A

A phospholipid flippase essential for olfactory neuron function in *Drosophila* . Coral G. Warr, Yu-Chi Liu, Takahiro Honda, Michelle Pearce, Marien de Bruyne. School of Biological Sciences, Monash University, Clayton, VIC, Australia.

In *Drosophila* the majority of general odorants are detected by a large family of 62 seven-transmembrane receptor proteins, the odorant receptor (Or) family. To identify new genes involved in Or and olfactory receptor neuron (ORN) function in *Drosophila* we used electro-antennogram recordings to screen 883 lines from the Zuker collection, a collection of EMS-treated homozygous viable lines1. We identified a recessive mutation that has greatly reduced electro-antennogram responses to all tested general odorants, but not to carbon dioxide. Olfactory sensillum and ORN morphology appear normal in the mutant. The mutation was deficiency mapped to a region at 86E-87B on chromosome III that contained 12 predicted genes. We performed whole genome re-sequencing on the mutant strain and identified a nonsense mutation in one of the 12 genes, CG14741. We also observed the mutant phenotype in a second independent mutant allele of CG14741, a line containing a piggybac transposable element insertion in the coding region, confirming this is the affected gene. Both mutations are predicted to affect all four known isoforms of the protein. RNA in situ hybridisation experiments showed expression of CG14741 in ORNs, and using RNA interference we have shown that this gene is specifically required in ORNs for their function. CG14741 has not previously been functionally characterised but encodes a phospholipid flippase, type 4 P-type ATPases that translocate phospholipids from the exoplasmic to the cytoplasmic leaflet of plasma and internal membranes, and is highly similar to the human flippases ATP8B1-4. We are currently performing further studies to determine how lack of this protein is affecting ORN function.

1. Koundakjian, E., et al. (2004).The Zuker Collection: A Resource for the Analysis of Autosomal Gene Function in *Drosophila melanogaster*. Genetics, 167: 203-206.

665B

Evaluating potential mechanisms underlying hormetic responses. Elizabeth J Ales, Erik C. Johnson. Biology, Wake Forest University, Winston-Salem, NC.

Hormesis is the adaptive response that raises the resistance of an organism to physiological stress following low-level exposure to repeated periods of stress. Although hormesis has been shown to be present in diverse organisms and for many different types of stress, precise cellular mechanisms are unclear. We are employing the genetics of Drosophila to discern potential cellular mechanisms. We observe that exposure to multiple periods of short starvation conditions (12 hours) for a period of 3-4 days significantly extends lifespan upon transitioning to complete starvation conditions. Specifically, we find an increase of approximately 20% in median survival from multiple wild-type strains under this experimental paradigm. We are extending these experiments to include genetic screens of candidate molecules, such as insulin, AKH, and AMPK to test whether these molecules are involved in the adaptive responses underlying hormesis. Furthermore, we will report on our efforts to identify genetic changes in animals displaying hermetic responses. Lastly, we will report on efforts to identify potential differences or similarities in potential hermetic mechanisms from homotypic stress compared to heterotypic stressors. We suspect that our results will offer insight into the mechanisms underlying adaptive responses to physiological stressors.

666C

JAABA: An interactive machine-learning tool for automatic annotation of animal behavior. Kristin Branson[1], Mayank Kabra[1], Alice A. Robie[1], Marta Rivera-Alba[1,2], Steven Branson[1,3]. 1) HHMI Janelia Farm Research Campus, Ashburn, VA; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Dept. of Computer Science and Engineering, UC San Diego, La Jolla, CA.

We present the Janelia Automatic Animal Behavior Annotator (JAABA), a new machine learning-based system to enable researchers to automatically compute interpretable, quantitative statistics describing video of behaving animals. Through our system, users encode their intuition about the structure of behavior by labeling the behavior of the animal, e.g. walking, grooming, or following, in a small set of video frames. JAABA uses machine learning techniques to convert these manual labels into behavior detectors that can then be used to automatically classify the behaviors of animals in large data sets with high throughput. JAABA combines an intuitive graphical user interface, a fast and powerful machine learning algorithm, and visualizations of the classifier into an interactive, usable system for creating automatic behavior detectors. We demonstrate that our system can be used by scientists without expertise in computer science to independently train accurate behavior

detectors. Our system is general purpose, and can be used to easily create a wide variety of accurate individual and social behavior detectors for both adult and larvae *Drosophila*. We also show that it can be used to create behavior classifiers robust enough to successfully be applied to a large, phenotypically diverse data set consisting of thousands of transgenic lines of *Drosophila melanogaster*. Statistics of the automatic behavior classifications such as the fraction of time spent performing a given behavior are powerful descriptions, and we show that these statistics can be used to understand the subtle behavioral differences between highly similar populations of wildtype flies, between flies starved for differing amounts of times, and between flies of different ages. Our system is complementary to video-based tracking methods, and we envision that it will facilitate extraction of detailed, scientifically meaningful measurements of the behavioral effects in large experiments.

667A

Dissecting the Mechanism of Parkinson's Disease Using Drosophila model. Dongsheng Chen. Metablic and Degenerative Disease Center, Institute of Molecular Medicine, Houston, TX.

Parkinson's disease is the second most common neurodegenerative disorder and is characterized by the degeneration of dopaminergic neurons in the substantia nigra. In order to dissect the mechanism of Parkinson's disease, we generated parkin-tagged transgenic flies and performed TAP (tandem affinity purification)-based isolation of dParkin-associated protein complex, from which we identified a group of putative dParkin-Interacting partners. Through co-IP and fly genetic assays, we further showed that one of the isolated candidates, which we named luke, can interacts with dParkin both physically and genetically. As an alternative approach to study parkin's cellular functions, we have also been characterizing darken mutant phenotypes in different fly tissues such as male gonads and female ovary. Results from the above studies will be discussed in the presentation.

668B

Dopamine neurons drive competing actions for alcohol preference in Drosophila. Karla R. Kaun, Reza Azanchi, Yoshinori Aso, Gerald M. Rubin, Ulrike Heberlein. Howard Hughes Medical Institute, Janelia Farm Research Campus, 19700 Helix Drive, Ashburn, VA 20147.

Neural circuits that mediate behavioral choice evaluate and integrate information from the environment with internal demands, then initiate an output response. Even circuits that support simple decisions remain poorly understood. Here we characterize the neural modulation underlying the simple choice to lay eggs on ethanol and show that distinct subsets of dopaminergic neurons compete to either enhance or inhibit egg-laying on ethanol. We propose a model where competing dopaminergic neurons modulate oviposition preference in order to adjust to changes in natural oviposition substrates. Moreover, we show that the circuits that mediate this innate choice overlap with the reward circuits responsible for evaluating the appetitive and aversive effects of intoxicating levels of ethanol. This suggests circuits that evolved to evaluate an oviposition substrate beneficial to the fitness of the flies, may be more generally required to evaluate appetitive and aversive stimuli.

669C

Neural dissection of active predator avoidance behavior in Drosophila. Claire J. Manson-Bishop, Gregg W. Roman. Biology and Biochemistry, University of Houston, Houston, TX.

The response of *Drosophila melanogaster* to predators has not only ethological relevance, but will contribute toward the establishment of an anxiety-like behavioral model within this organism. Such a model will enable the dissection of the molecules and neurocircuits involved in the modulation of anxiety. We have characterized the behavioral response of Drosophila to predators; it is the goal of these experiments to begin to dissect the neurochemicals implicated in these behaviors. For these experiments, we study Drosophila within the circular open-field paradigm using two predators, the Pantropical jumping spider (*Plexippus paykulli*) and the Carolina mantid (*Stagmomantis carolina*); both predators are capable of capturing and preying upon Drosophila in large arenas. Wild type Canton-S actively avoid a predator caged within the center of a circular open-field arena. Using mutant $norpA^{7};orco^{2}$ flies that are both blind and broadly anosmic, we show that the sensory modalities of sight and smell are together necessary for the detection and avoidance of predators. Toward the elucidation of the neurochemicals responsible for predator avoidance, we sought to determine whether serotonin plays a role using the UAS-gal4 binary system. Modulating the activity of serotonergic neurons did not alter predator avoidance, suggesting that serotonin does not affect this behavior.

670A

Behavioral contributions of the 12 neuron types in the fly lamina. Michael B. Reiser[1], John C. Tuthill[1,2], Aljoscha Nern[1], Gerry Rubin[1]. 1) Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA; 2) Department of Neurobiology, Harvard Medical School, 220 Longwood Avenue, Boston, MA.

Motion detection is a fundamental neural computation performed by many sensory systems. In the fly, local motion computation is thought to occur within the first two layers of the visual system, the lamina and medulla. We constructed specific genetic driver lines for each of the 12 neuron classes in the lamina. We then depolarized and hyperpolarized each neuron type, and quantified fly behavioral responses to a diverse set of motion stimuli. We found that only a small number of lamina output neurons are essential for motion detection, while most neurons serve to sculpt and enhance these feedforward pathways. Two classes of feedback neurons and two classes of lamina output neurons are required for normal detection of

directional motion. Our results reveal a prominent role for feedback and lateral interactions in motion processing, and demonstrate that motion-dependent behaviors rely on contributions from nearly all the neurons in the lamina.

671B

Elucidation of Drosophila melanogaster G protein coupled receptor interactions through heterodimerization and chimeric receptor studies. Michael J. Rizzo, Erik C. Johnson. Wake Forest University, Department of Biology, Winston Salem, NC 27109.

G protein coupled receptors (GPCR's) represent a well-characterized protein superfamily present in most Eukaryotic taxa. GPCR's are critically important to a multitude of physiological and behavioral processes, and currently represent a target for approximately 50% of all pharmaceutical drugs. Canonical GPCR signaling relies on conformational changes upon ligand binding to the receptor, which results in activation of a heterotrimeric G protein and liberation of a second messenger pathway. Additionally, activation of a GPCR results in the recruitment of specific kinases which phosphorylate the GPCR and cause the subsequent recruitment of arrestins, which lead to the termination of GPCR signaling and internalization. To identify the structural aspects that underlie ligand binding domains and specific G protein and arrestin interactions, we are generating a series of receptor chimeras focusing on the proctolin and FMRF receptor pairs. Such structural-functional studies have not been evaluated for Drosophila receptors. Furthermore, recent research indicates that many GPCR's exist as homodimers or heterodimers in the cell, and this dimerization has broad ramifications on signaling mechanisms, trafficking, expression, and internalization. To this date, no Drosophila GPCR's have been shown to assemble as heterodimers. To determine which GPCR's are able to form heterodimers, we are currently tagging the entire cohort of peptide and amine GPCRs with both a fluorophore (CFP/YFP) and Hemoagglutinin (HA) epitope tag and expressing these modified receptors in HEK-293 cells. Through FRET (Forster Resonance Energy Transfer) and co-immunoprecipitation (Co-IP) studies, we will evaluate a matrix of all Drosophila GPCR's to determine heterodimer pairs, and aim to determine the impact of such events on signaling. We suspect the results of both studies to dissect peptide receptor function will offer insight into the specific mechanisms underlying receptor signaling and inform future experiments into how GPCRs act as points of integration of different endocrine signals.

672C

Establishing Drosophila Behavioral Paradigms Analogous to Mammalian Anxiety and Depression Models. Lauren Stein, Kelly Hainz, Wendi Neckameyer. Pharmacology and Physiology, Saint Louis University School of Medicine, St. Louis, MO.

Exposure to a variety of aversive environmental and physiological stressors can result in a series of neurochemical and behavioral changes having negative effects on both physical and mental health of an individual. It is important to identify the neural substrates involved in the stress response as well as develop simple, high throughput assays to screen for contributing factors. The goal of our research is to develop diverse paradigms to assay adaptive and maladaptive responses to stress. These include the forced swim test, grooming behavior, and the response to and recovery from the sedative effects of ethanol. We have previously demonstrated that the stress response circuits differ between males and females as well as sexually immature and mature animals. We exposed these populations to four different stressors and evaluate the varying parameters as an output measurement for translational studies in an effort to develop this paradigm to use in our screen for neural substrates mediating responses to stress.

673A

Sexual Dimorphism in *Drosophila* exercise motivation. Alyson Sujkowski, Sara Ginzberg, Robert Wessells. Univ Michigan, Ann Arbor, MI.

Endurance exercise is a promising therapeutic intervention with substantial protective effects on multiple indices of healthspan, including muscle and neuronal function. Male *Drosophila* respond to a ramped daily program of exercise by inducing conserved physiological responses similar to those seen in mice and humans. Female flies, however, respond poorly to exercise induction and, as a result, do not induce the physiological changes seen in males, indicating a strong sexual dimorphism in response to exercise stimuli. Here, we demonstrate that poor female exercise response is behavioral, mediated by differences in neurons, not by differences in muscle. We also show that the sex specific "motivation" for exercise behavior is reversible even in adults, after development has been completed. Using tissue-autonomous sex determination constructs, we have sought to identify the minimum brain regions sufficient to govern adult exercise behavior. In addition, we find that sexually dimorphic exercise behavior varies between*Drosophila* species, suggesting that dimorphic exercise motivation may be specifically adaptive. Together, these findings indicate that, at least in flies, exercise motivation is a complex behavior controlled by the nervous system, and is independent of muscle. This model provides an important opportunity to further examine the specific changes in neural physiology that mediate behavioral motivation and plasticity.

674B

Investigating the cellular bases of cold nociception in *Drosophila* larvae. Luis Sullivan, Srividya C Iyer, Eswar P R Iyer, Kevin Armengol, Daniel N Cox. School of Systems Biol., Krasnow Inst. Adv. Study, George Mason University, Fairfax, VA.

Thermosensory nociception, particularly in poikilothermic organisms, is essential for survival and provides a mechanism for sensory perception of noxious thermal stimuli that alert the organism to potential environmental dangers coupled with pain sensation and complex behavioral responses to protect the organism from incipient damage. Moreover, acute and chronic pain may manifest as altered thermosensory nociception in neuropathic pain states. *Drosophila* has emerged as a powerful model

organism for dissecting the cellular and molecular mechanisms regulating both nociception and thermosensation, however, nothing is known regarding the bases of cold nociception. To address this knowledge gap, we have developed and implemented a novel behavioral assay to characterize responses of larvae to a declining thermal gradient. These analyses revealed a distinct and novel behavior we refer to as "cringing" which is characterized by a full body contraction along the anterior-posterior axis. Moreover, cringing behavior was uniquely observed only in the noxious cold range (≤10°C) and is the predominant behavioral response below 8°C. Using this behavioral platform, we demonstrate that synaptic transmission in a specific subset of dendritic arborization (da) sensory neurons is required for noxious cold thermosensation. Moreover, optogenetic-based activation of this da neuron subset is capable of phenocopying the noxious cold-induced cringing behavior. Conversely, optogenetic inhibition via halorhodopsin expression confirms our observations that this subset of da neurons is required for mediating the response to noxious cold in larvae. Finally, we demonstrate via GCaMP3 live imaging that these neurons actively respond to noxious cold stimulation. In summary, these studies provide insight into the cellular bases of noxious cold sensation in *Drosophila* larvae and the cellular mechanisms responsible for this sensory modality.

675C

A novel mutation in the *Drosophila slingshot* (*ssh*) gene identifies a requirement for its function in the maintenance of synapse morphology. Jason E. Duncan, Kayla Johnson. Department of Biology, Willamette University, Salem, OR.

Maintenance of the cytoskeleton is essential for normal function of nerve cells. We have identified a mutation in *slingshot* (*ssh*), a gene that encodes a phosphatase that dephosphorylates the<u>a</u>ctin-<u>d</u>epolymerizing <u>f</u>actor (ADF)/cofilin protein, which regulates microfilament dynamics. The ssh^{WU6} allele is the result of a missense mutation that converts the amino acid Glycine^{233} (G<u>G</u>A) to Glutamic Acid^{233} (G<u>A</u>A). Glycine^{233} is an amino acid that is invariant across the eumetazoa in the highly conserved cofilin binding domain of the ssh protein. Third instar ssh^{WU6} mutant larvae are uncoordinated and exhibit a tail-flip phenotype indicative of posterior paralysis and compromised axonal transport. Immunohistological analysis of axons of the peripheral nervous system of ssh^{WU6} larvae, however, fails to reveal focal swellings and accumulations of transported components, indicating that axonal transport is not disrupted. Given the role of ssh in the regulation of microfilaments, we examined the morphology of glutamatergic neuromuscular synapses in ssh^{WU6} larvae. Type Is and Ib synapses at ventral longitudinal muscles 6/7 from segments A2 through A5 were quantified on two metrics: ssh^{WU6} mutant synapses were morphologically larger than wildtype in both average area (μm^2) ($p<0.01$) and average number of boutons ($p<0.0001$). These results suggest a requirement for the cofilin binding region of ssh in normal phosphatase function and the maintenance of synapse morphology.

676A

Synaptic homeostasis is regulated by the kinesin motor protein Khc-73 in *Drosophila melanogaster*. Edward H. Liao, Kazuya Tsurudome, Wassim El Mounzer, Frances Wang, Fatima Elazzouzi, Pejmun Haghighi. Dept Physiology, McGill University, Montreal, QC, Canada.

Accumulating evidence suggests that homeostatic mechanisms participate in stabilizing function in neural circuits; however, we know little about the molecular mechanisms that control synaptic homeostasis. Previously, we have demonstrated that overexpression of a microRNA cluster that targets a member of the kinesin 3 family of motor proteins, Khc-73, suppresses the expression of a compensatory homeostatic synaptic response at the Drosophila larval neuromuscular junction (NMJ). Here we describe the characterization of a loss-of-function Khc-73 mutant. NMJ baseline electrophysiological properties as well as the number of synaptic boutons at NMJs in Khc-73 mutant larvae appear indistinguishable from wild type. However, consistent with our previous observations, the normal retrograde, homeostatic response at the NMJ is severely affected in these mutants. As normal synaptic compensation requires an enhancement of neurotransmitter release, we examined in more detail the accumulation and structure of active zones both at the level of light microscopy and electron microscopy. Our findings suggest that Khc-73 is required for the normal appearance of active zone protein Bruch pilot at the NMJ as well as the number of electron dense T-bars at active zones. Little experimental data is available that links the lack of normal homeostatic control to the overall behavioural outcome. We therefore tested the consequence of loss of Khc-73 on lifespan and locomotion in adult flies. While Khc-73 homozygous adults are viable, we observed a significant reduction in their lifespan. In addition, Khc-73 mutants showed reduced ability to climb and fly. We are currently using genetic approaches to identify the circuitry that is most affected as a result of loss of Khc-73.

679B

Talin autoinhibition is required for morphogenesis. Stephanie J. Ellis[1], Jenny Long[2], Michael J. Fairchild[1], Paolo Lobo[3], Stefan Czerniecki[1], Filip van Petegem[3], Frieder Schöck[2], Guy Tanentzapf[1]. 1) Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada; 2) Biology Department, McGill University, Montreal, PQ, Canada; 3) Department of Biochemistry, University of British Columbia, Vancouver, BC, Canada.

FERM (4.1/Ezrin/Radixin/Moesin) domain proteins modulate cell shape and adhesion during morphogenesis by linking the plasma membrane and the actin cytoskeleton. Talin, a FERM-domain containing protein, forms a direct link between integrin adhesion receptors and the actin cytoskeleton. Similar to other FERM proteins, talin makes an intramolecular interaction that could autoinhibit the activity of talin. However, the functional consequence of such an interaction has not been previously explored in vivo. Here, we demonstrate that targeted disruption of talin autoinhibition impinges on morphogenetic movements during fly development. Autoinhibition-impaired talin is more stable at sites of integrin-ECM attachment,

suggesting that talin autoinhibition is required to facilitate adhesion turnover during morphogenetic processes. Integrin is also stabilized at sites of adhesion when autoinhibition is blocked. Finally, we present evidence that talin autoinhibition is regulated by Rap1-dependent signaling. Based on our data we propose that talin autoinhibition provides a switch for modulating adhesion turnover and adhesion stability during morphogenesis.

680C

Septins regulate contractility of the actomyosin ring to enable adherens junction remodeling during cytokinesis of epithelial cells. Roland Le Borgne, Nabila Founounou, Nicolas Loyer. Institute of Genetics and Development of Rennes CNRS UMR 6290-Faculté de Médecine 2 av du Pr. Bernard 35000 Rennes FRANCE.

During cytokinesis, a contractile ring containing actin, myosin II and septins generates a furrow that divides one cell into two. How cytokinesis of epithelial cells making adhesive contacts with their neighbors is achieved is unknown. We report that, in Drosophila, septins are required for planar cell division, yet dispensable for orthogonal cell cytokinesis. During planar division, apicobasal integrity is preserved and furrowing is asymmetric with the contractile-ring displaced towards the adherens junction belt in a septin-independent manner. Local disengagement of adherens junctions between interphasic and mitotic cells is required to generate an adhesive interface between daughter cells. Loss of septins causes a two-fold reduction in the contractility of the actomyosin ring and prevents junction remodelling. Photo-ablation experiments reveal that septin-driven actomyosin contraction is needed to unzip E-cad contacts locally thereby allowing junction remodeling. Thus, septins exert a mechanical function needed to overcome the tension induced by adhesive contacts during cytokinesis.

681A

Region-specific activity of the Diego protein in Planar Cell Polarity. Simon Collier[1], Hugh Cahill[2]. 1) Dept Biological Sci, Marshall Univ, Huntington, WV; 2) School of Medicine, Marshall Univ, Huntington, WV.

The ankyrin-repeat protein Diego (Dgo) is a regulator of Frizzled (Fz) Planar Cell Polarity (PCP) signaling in Drosophila. Previous studies have shown that Dgo is required for normal ommatidial polarity in the eye, and hair polarity on the wing. In wing cells, Dgo is proposed to bind the Dishevelled (Dsh) protein at the distal end of the cell, prior to the distal initiation of a polarized cell hair. We have studied the effects of loss or gain of Dgo activity in a number of adult tissues. In the wing, both loss and gain of Dgo activity result in disruption of normal hair polarity, implying that there is a dose-specific requirement for Dgo in normal wing PCP. In contrast, neither loss nor gain of Dgo activity in the leg disrupt hair, bristle, or tarsal joint polarity, suggesting that the PCP specification in the leg is not sensitive to Dgo activity. In the thorax and abdomen, both loss and gain of Dgo activity affect hair and bristle polarity in the posterior compartment of each segment, whereas the anterior compartment remains unaffected. This leads to the surprising conclusion that different regions of the same tissue may have differing sensitivity to Dgo activity. We will present a model that proposes two distinct, region-specific, outcomes of Fz PCP signaling, only one of which is sensitive to Dgo activity.

682B

Fat2 controls planar microtubule alignment in the *Drosophila* follicle epithelium. Christian Dahmann, Ivana Viktorinova. Institute of Genetics, Dresden University of Technology, Dresden, Germany.

The polarization of cells in the plane of a tissue is an important characteristic of many epithelia, yet how this planar polarity is established is not fully understood. The *Drosophila* follicle epithelium has emerged as a useful system to study the mechanisms by which planar polarity is established. Planar polarity in the follicle epithelium is apparent by the planar polarized organization of actin filaments and extracellular matrix components. Establishment of proper planar polarity requires the atypical cadherin Fat2. Fat2 protein localization is planar polarized in follicle cells. Here we show that microtubules are planar polarized in the follicle epithelium. Microtubules are preferentially oriented perpendicular to the anteroposterior axis of the egg chamber. Microtubule orientation is randomized in *fat2* mutants. Moreover, microtubule destabilization results in a loss of planar polarization of the Fat2 protein. Our results suggest a feedback loop between Fat2 and microtubules in establishing planar polarity in the follicle epithelium.

683C

Analysis of Integrator 1 function in *Drosophila* epithelial cells. Timm Haack, Dan T. Bergstralh, Daniel St Johnston. The Gurdon Institute and the Department of Genetics, University of Cambridge, Cambridge, United Kingdom.

We use the follicle cell epithelium of the *Drosophila* egg chamber to study how epithelial cells establish and maintain polarization. In a forward genetic clonal screen for genes disrupting epithelial polarity and organization we identified a nonsense mutant allele of the conserved *Integrator 1* (*IntS1*) gene called $IntS1^{1\text{-}D15}$. Cells homozygous for $IntS1^{1\text{-}D15}$ leave the epithelial monolayer basally and form small wedge-shaped clusters with ectopic apical domains. This behavior of $IntS1^{1\text{-}D15}$ mutant cells is accompanied by a marked change in cytoskeletal organization characterized by increased microtubules and cortical actin. Mutant clones within the monolayer show altered cell shapes often with constricted apical membranes and partial mixing of apical and lateral membrane markers. $IntS1^{1\text{-}D15}$ also promotes changes in cell signaling that affect the cell cycle. Wildtype follicle cells switch from mitotic cycling to endocycling during mid-oogenesis in response to Notch signaling. In contrast, mutant $IntS1^{1\text{-}D15}$ clones in young egg chambers show premature loss of centrosomes and early expression of the transcription factor Hindsight indicating a deregulation of the Notch pathway. The IntS1 protein is part of the Integrator Complex, a large nuclear protein complex that has been linked to snRNA maturation. We aim to investigate whether the

change in epithelial cell behavior in *IntS1* mutants is a pleiotropic effect due to impaired spliceosomal activity as a consequence of snRNA misprocessing or whether it presents an unknown function of IntS1 independent of snRNA maturation.

684A

The role of Dachsous and Fat in regulating planar cell polarity across the embryonic epidermis. Kynan Lawlor, Stephen DiNardo. Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Directional cues are necessary to orient and coordinate tissue morphogenesis and patterning during development. The polarization of cells along the plane of a tissue, termed planar cell polarity, is one way in which these directional cues may be specified. Two groups of genes, the Dachsous (Ds) and Frizzled (Fz) systems, play key roles in the establishment, maintenance and propagation of planar cell polarity. While progress has been made in understanding the function of these two systems, unanswered questions remain. We have been studying the elaboration of polarity within the larval ventral epidermis to address several of these questions. Previously we showed that *dachsous* and *frizzled* contribute independently to polarity, and that they do so over spatially distinct domains. Here we examine how the atypical cadherins Dachsous and Fat regulate planar polarity across the denticle field within each segment of the epidermis. In this tissue, f-actin becomes enriched specifically on the posterior edge of aligned columns of cells. In *dachsous* mutants, sites of f-actin enrichment are not correctly positioned, with defects observed at a greater frequency within specific columns of each denticle field. Furthermore, over-activation of the pathway, by ectopic expression of extracellular Dachsous is able to re-orient f-actin positioning in adjacent cells. Using these assays, both in fixed tissue and in live-imaging, we will report on the role of key components of the Dachsous/Fat system in regulating planar cell polarity.

685B

***Tsp66E*, the *Drosophila KAI1* homologue, and *Tsp74F* function to regulate ovarian follicle cell and wing development by stabilizing integrin localization.** Soojin Lee, Seung Yeop Han, Minjung Lee, Kyoung Sang Cho. Department of Biological sciences, Kunkok university, Seoul, Seoul, South Korea.

The metastasis suppressor KAI1/CD82 has been implicated in various cellular processes; however, its function in development is not fully understood. Here, we generated and characterized mutants of Tsp66E and Tsp74F, which are Drosophila homologues of KAI1/CD82 and Tspan11, respectively. These mutants exhibited egg elongation defects along with disturbed integrin localization and actin polarity. Moreover, the defects were enhanced by mutation of inflated, an aPS2 integrin gene. Mutant ovaries had elevated aPS2 integrin levels and reduced endocytic trafficking. These results suggest that Drosophila KAI1/CD82 affects the polarized localization and the level of integrin, which may contribute to epithelial cell polarity.

686C

Moesin negatively regulates Crumbs at the marginal zone in Drosophila follicle cells. Kristin Sherrard, Richard Fehon. Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Epithelial polarity is crucial for both maintenance of organ function and for morphogenetic movements. The transmembrane protein Crumbs (Crb) has emerged as a key regular of apical and junctional polarity through PDZ-binding interactions with the apical Par complex and Stardust and PATJ. In addition, Crb can bind to FERM domain proteins such as Moesin (Moe), Expanded, and Yurt, though the function of this binding is not well understood. We have been investigating interactions between Moe and Crb in the Drosophila follicular epithelium. We have found that Crb and aPKC levels increase in Moe-depleted follicle cells, and also that Crb is essential for localization of Moe during a stage of oogenesis when the apical and junctional regions of follicle cells are undergoing rapid expansion. In cells expressing a mutant form of Crb unable to bind FERM proteins, phosphorylated Moe and Actin are absent from the marginal zone, a region of the lateral membrane just apical to the adherens junctions. Bazooka/Par3 and Ecadherin are similarly reduced and form discontinuous bands. In contrast, depleting Moe causes a number of effects, including a Rho-dependent decrease in cell size and upregulation of aPKC, Patj, and Crb. We are currently investigating the functional significance of Crb recruiting Moe to the MZ, including possible competition for binding between Moe and Par6. Ultimately we hope to gain a better understanding of how the organization of proteins in the marginal zone contributes to polarity and junctional stability, particularly during morphogenesis.

687A

Regulation of cell polarity and morphogenesis by Tousled-like kinase in Drosophila. Jenn-Yah Yu, Tsung-Han Yeh, Shu-Yu Huang, Gwo-Jen Liaw. Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan.

Tousled-like kinase (Tlk) is a conserved Serine/Threonin kinase. It is involved in DNA replication, chromatin assembly, and DNA repair. Interestingly, the expression profile of TLK2, a human tlk homolog, is similar to those of some apical-basal polarity-related genes during cancer progression. Therefore, we test if Tlk plays a role in cell polarity by using Drosophila follicle cells as a model. Forced-expression or knockdown of tlk led to morphological changes in the follicle cells at stage-9 to -10 egg chambers. Some tlk knockdown cells were detached from the apical side, suggesting that tlk is involved in cell polarity and morphogenesis. We further generated tlk mutant follicle cell clones by using the FLP/FRT system and examined the levels and localization of some cell polarity components. A key regulator, aPKC, was increased in tlk mutant cells. Adherens junction

proteins Armadillo and DE-cadherin were also increased in the mutant cells. Discs large, a basolateral complex protein, was re-distributed to the apical-lateral region. These results suggest a role of tlk in apical-basal cell polarity. In addition, morphology of the tlk mutant cells was changed from columnar to irregular shape and the filamentous actin was increased in the apical region. In conclusion, we found a novel role of Tlk in regulating epithelial cell polarity and morphogenesis.

688B

Proper ER Morphology in the Drosophila Syncytial Embryo Depends on Reticulon-like Proteins. Zane J Bergman, Justin D Mclaurin, Amanda Q Sims, Blake Riggs. Biology, San Francisco State University, San Francisco, CA.

Central to cell division is the correct and equal partitioning of genetic material to each daughter cell. Proper chromosome segregation requires the mitotic spindle, composed of microtubules and its associated proteins. Another important aspect of cellular division is the partitioning of the cytoplasm and organelles, which are needed for daughter cells to function. Unlike spindle events, the timing and mechanism of organelle segregation is not known. The endoplasmic reticulum (ER), an organelle essential for cellular function, exhibits a dramatic reorganization in mitosis and is also necessary for nuclear pore complex and nuclear envelope formation during mitotic exit. In the Drosophila melanogaster syncytial embryo, several rounds of rapid nuclear division occur without cytokinesis. Simultaneously, the ER is partitioned into discrete units that surround each nucleus. Recent evidence suggests that ER reorganization depends upon mitotic cyclin:CDK1 complex activity and a functioning APC/C, directly linking it to the same cell cycle events that control karyokinesis. The reticulon family of proteins has been shown in other organisms to be important for maintaining the morphology of ER structures. Here, we show that the reticulon-like protein 1 (Rtnl1) in Drosophila is responsible for correctly shaping the ER around the nucleus during mitotic events. Rtnl1 deficient embryos show defects in ER organization around the mitotic spindle and mitotic arrest with incorrectly shaped spindles. These mutations also negatively affect embryonic viability. Rtnl1 contains a Cdk1-consensus sequencethat may be the link between the cell cycle machinery and ER reorganization during mitosis. These data point to Rtnl1 being an important regulatory element that controls ER reorganization during mitosis.

689C

A Deficiency Screen to Identify Regions of the Third Chromosome that Genetically Interact with Activated Abl. Lacey Berry, Traci L. Stevens. Biology, Randolph-Macon College, Ashland, VA.

Abl nonreceptor tyrosine kinases are a highly conserved family of proteins that are essential in the development of multicellular organisms. Chronic myelogenous leukemia, a genetic disorder in humans, is linked to expression of Bcr-Abl, an activated form of Abl. Expression of the mutant Bcr-Abl in cell culture alters actin structures and cell migration. Studies in *Drosophila* have shown that the Abl protein plays a critical role in the ability of cells to migrate and communicate with neighboring cells *in vivo*. Expression of activated Abl in *Drosophila* disrupts dorsal closure and head involution, processes that require regulated cell migration. The pathways by which Abl receives signals from the cell surface and that link Abl to the actin cytoskeleton remain largely unknown, and the goal of our laboratory is to characterize proteins that work with Abl to regulate cell migration during development. In order to identify genes that function in Abl pathways, we are conducting a genetic screen to identify regions of the genome that contain genes that genetically interact with activated Abl expression during embryonic development. In this study, we identified ten deficiencies of the third chromosome that enhanced the phenotypes associated with Bcr-Abl expression when compared to the control. Three pairs of these interacting deficiencies overlap, and thus, each of these pairs is likely to represent a single interacting gene that falls in the region of overlap. Therefore, these ten interacting deficiencies represent seven independent genomic regions that are likely to contain genes that function in Abl signaling pathways. In the future, these interacting regions will be narrowed down to pinpoint single genes that encode proteins that work along with Abl in regulating cell migration.

690A

Determining the roles of Dock proteins in dorsal vessel development. Bridget H Biersmith, Erika R Geisbrecht. Division of Cell Biology & Biophysics, University of Missouri-Kansas City, Kansas City, MO.

Congenital heart defects (CHD) are one of the most common forms of birth abnormalities. Nearly 1.3 million Americans are affected by CHD today and have an increased risk of cardiovascular disease and stroke. Unfortunately, the underlying causes of CHD remain unclear. To address this issue, we have used the *Drosophila* heart, or dorsal vessel (DV), as a model tissue as aspects of early heart development are conserved throughout evolution. Specifically, two rows of heart cells form and then migrate to the midline where they undergo cell morphogenic events, including cell migration and adhesion, to make a muscular, beating, linear tube. Using this tissue, we are able to assess the roles of the highly conserved family of Dock proteins in heart development. Dock family members are guanine nucleotide exchange factors (GEFs) capable of activating the small GTPase Rac to modulate actin cytoskeletal movements, including cell migration and myoblast fusion. The *Drosophila* genome encodes two closely related Dock proteins, Myoblast city (Mbc or vertebrate Dock180) and Sponge (Spg or vertebrate Dock3/4), both of which are expressed in the DV. Previous studies have shown that removal of Mbc results in a complete lack of myoblast fusion in the embryonic musculature. Our preliminary data shows that Spg fails to substitute for Mbc in this fusion process, suggesting these two proteins are not functionally redundantly in this tissue. To address the specific roles Mbc and Spg are playing in heart development, we have examined the morphological changes that occur in dorsal vessel formation using 3-D reconstruction and histological thin-sections. Consistent with the idea that Mbc is required for cytoskeletal rearrangement, we failed to observe normal cell shape changes in embryos mutant for *mbc*. Data will be presented that

analyzes cardioblast cell shape changes in *mbc, spg* double mutants. Furthermore, we plan to do ultra-thin sections for EM, which will allow us to visualize adherens junctions to further determine the developmental roles of these Dock proteins in heart tube development.

691B

Cellular blebbing in the ventral furrow. Jonathan S Coravos, Adam Martin. Massachusetts Institute of Technology, Cambridge, MA.

Blebbing is a cellular process where the plasma membrane separates from the underlying actin cortex, inflates away from the point of separation, and finally retracts. Blebbing depends on actin cortex contraction by the molecular motor myosin II, which is thought to increase local hydrostatic pressure that causes blebs. While blebbing has been characterized in single cells, bleb function in a multicellular tissue has not yet been demonstrated. We investigate blebbing in the Drosophila ventral furrow (VF), a tissue invagination event at the onset of gastrulation. Because the VF blebs profusely, whereas neighboring epithelia do not, VF invagination is a promising place to identify roles for blebbing in multicellular tissues. Using 4-D confocal imaging with F-actin and membrane markers, we show that VF blebs have a similar cycle to those previously described in cultured cells. Similar to cell culture blebs, ventral furrow blebs exist for 30 to 60 seconds. Blebs coincide with local depletions in the actin and myosin cortex, expand as spherical actin-depleted bodies to about 3 μm in diameter, and then accumulate actin during membrane retraction. Injecting embryos with the F-actin stabilizing drug, phalloidin, increases the density of blebs in the VF. Together with the observation that bleb retraction coincides with actin accumulation in the bleb, the phalloidin result supports a model in which F-actin turnover is required for cortex assembly in the bleb, which is also required for bleb retraction. Finally, we show that blebs occur with greater probability in the space between the cell centroid and cell-cell junctions, suggesting a heterogeneous organization of the cell cortex. We are investigating potential regulators of this organization, such as Moesin, an actin-membrane crosslinker.

692C

Spatial control of F-actin dynamics during pupal eye morphogenesis. Steven J. DelSignore, Victor Hatini. Cell, Molecular & Developmental Biol, Tufts Univ Sackler Sch Biomed Sci, Boston, MA.

Coordinated cell shape change drives the morphogenesis of many epithelial tissues during organ development. These precise patterns of shape change require the dynamic regulation of adhesion between cells, as changes in adhesion can promote cell shape change by causing the expansion or contraction of particular cell borders. The strength of adhesion depends on the degree of association between cell junctions and the underlying F-actin cytoskeleton. This interaction is enhanced by the activity of the small GTPase Rac1, which itself is recruited to junctions in part by phosphatidylinositol 3-kinase (Pi3k). Though the role of Rac1 at junctions has been clearly demonstrated in vitro, it remains unclear whether Rac1 regulates adhesion differentially between expanding and contracting cell borders to promote cell shape change in vivo. We examined the effect of Rac1 on actin dynamics and cell shape change in the developing drosophila eye. The drosophila compound eye is composed of ~800 light sensing units called ommatidia, each of which are surrounded by pigment epithelial cells. During development, pigment cells undergo elaborate shape changes to form a hexagonal that surrounds each ommatidium. To determine how F-actin dynamics contribute to these cell shape changes, we characterized F-actin dynamics by live imaging of the F-actin binding peptide lifeact::ruby, and compared wild type dynamics to experimental eyes with altered Rac1 signaling. Further, we performed an RNAi-based screen to identify novel regulators of F-actin dynamics during morphogenesis. Together, these studies suggest mechanisms by which F-actin dynamics and adhesion are regulated spatially and temporally to control cell shape change during morphogenesis.

693A

Role of formins during Drosophila embryonic myogenesis. Su Deng[1], Ingo Bothe[2], Mary Baylies[2]. 1) Weill Cornell Medical College, New York, NY; 2) Memorial Sloan-Kettering Cancer Center.

Cell-cell fusion is a highly regulated process that is critical for various events, such as fertilization, bone remodeling, and the generation of skeletal muscle. In our lab we use Drosophila embryonic body wall muscle as a model system to study myoblast fusion. Current research has revealed that Arp2/3, which nucleates branched actin filaments, and its regulators, Scar/WAVE and WASp, are required for the actin rearrangements necessary for myoblast fusion. However, whether the generation of unbranched actin filaments is involved in cell fusion is unknown. Formins are a family of proteins that nucleate unbranched actin filaments. We screened known formin mutant alleles in Drosophila and assayed for muscle phenotypes during embryogenesis. We find that both loss and gain of function of the Drosophila formin, Diaphanous (Dia), generate muscle phenotypes, including fusion and muscle attachment defects. Consistent with the loss and gain of function phenotypes, Dia is present in myoblasts and is enriched at the fusion site. At later stages of development, Dia is expressed at muscle attachment sites. Together, these data suggest that Dia may be required for myoblast fusion and myotendinous junction formation and/or stability. Further studies of how Dia is involved during muscle differentiation are in progress.

694B

Group I PAKs Functions Downstream of Rac to Regulate Podosome Invasion During Myoblast Fusion *in vivo*. Rui Duan, Peng Jin, Fengbao Luo, Elizabeth Chen. Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD.

The p21-activated kinases (PAKs) regulate a wide variety of cellular activities including cell proliferation, apoptosis, polarity, migration and shape changes. Here, we have identified a novel function of group I PAKs in cell-cell fusion. We show that the two *Drosophila* group I PAKs, DPak3 and DPak1, play partially redundant roles in myoblast fusion *in vivo*. While DPak3 has a major function in myoblast fusion, DPak1 plays a minor role that could only be revealed in the absence of DPak3. During myoblast fusion, DPak3 is enriched at the sites of fusion, colocalizing with the F-actin focus within a podosome-like structure (PLS) in wild-type embryos, whereas DPak1 is recruited to the site of fusion in *dpak3* mutant embryos to compensate for the loss of DPak3. In embryos lacking DPak3 or both PAKs, the actin filaments within the PLS are disorganized and dispersed, resulting in defective PLS invasion and failure in fusion pore initiation. We further show that the small GTPase Rac regulates both the activation and localization of DPak3 during myoblast fusion, and that the kinase activity of DPak3 is required for its function *in vivo*. We propose a model whereby group I PAKs act downstream of Rac to organize the actin filaments into a dense focus within the PLS, which, in turn, effectively invades the adherent founder cell and promotes fusion pore initiation during myoblast fusion.

695C

Centrosomal and acentrosomal microtubules collaborate to direct the dorsal localisation of gurken mRNA in Drosophila oocyte. Rippei Hayashi, Mark Wainwright, Sophie Liddell, Sheena Pinchin, David Ish-Horowicz. Developmental Genetics Laboratory, London Research Institute, Cancer Research UK, London, London, United Kingdom.

Transport of gurken (grk) mRNA along microtubules establishes the two major body-axes of the Drosophila oocyte. To investigate how microtubules are nucleated and polarised in order to pattern the oocyte, we have screened chromosome 3L for EMS-induced mutations that disrupt localisation of fluorescently-labelled grk mRNA. Mapping the causative mutations by SNP recombinational mapping and deep genomic sequencing identified molecular lesions for mutations in 9 complementation groups, affecting grk mRNA localisation, dynein transport, piRNA biogenesis, and other aspects of oogenesis. These mutations include new null alleles of armitage affecting follicle cell development. Analysis of an induced kinesin-light-chain (klc) mutant shows that klc is required for the clustering and the cortex localisation of centrosomes. We find two sites of grk mRNA localisation in the mid stage klc mutant oocytes: near the mispositioned centrosomes and adjacent to the dorsal cortex, suggesting that grk mRNA is transported on two classes of microtubules, only one of which depends on centrosomes. We also discuss how these microtubule populations establish the dorsal localisation of grk mRNA.

696A

The love-hate relationship between APC2 and Diaphanous: Dissecting the mechanism of APC2-Diaphanous dependent actin assembly in the Drosophila syncytial embryo. Ezgi Kunttas-Tatli[1], Rebecca Webb[2], Orr Rozov[1], Kelly Shibuya[1], Brooke M. McCartney[1]. 1) Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Biology Department, University of Pittsburgh at Johnstown, Johnstown, PA.

Cell division, cell shape change and cell migration are a few of the many cellular processes that require precise cytoskeletal rearrangements mediated by an orchestra of actin and microtubule-associated proteins. The colon cancer tumor suppressor Adenomatous polyposis coli (APC), a well-known component of the Wnt signaling pathway, is a poorly understood regulator of the actin cytoskeleton. The Drosophila syncytial embryo is a powerful *in vivo* model system to study the role of APC in actin reorganization. The early embryo undergoes synchronous nuclear divisions without cytokinesis, and the actin cytoskeleton undergoes dynamic changes coordinated with the cell cycle. During interphase, actin is organized in caps above each nucleus, and in metaphase actin is reorganized into pseudocleavage furrows that act as physical barriers to ensure mitotic fidelity. A complex of APC2 and the formin Diaphanous (Dia) regulates the formation and extension of the furrows. Mapping of the binding domains revealed that APC2 interacts with Dia via the 20 amino acid repeats (20Rs) and deletion of these domains results in furrow extension defects. Because the 20Rs are highly phosphorylated, we predicted that phosphorylation of the 20Rs may play a role in the APC2-Dia interaction. Consistent with that hypothesis, targeted mutants that disrupt or mimic phosphorylation both disrupt furrow extension. While our original *in vivo* analysis suggested a collaborative interaction between APC2 and Dia, *in vitro* analysis revealed that the APC2-20Rs potently inhibit Dia's actin assembly activity. To reconcile these findings, we are investigating the relationship between APC2 and Dia by live analysis of actin dynamics and actin filament turnover throughout the actin cycle in the syncytial embryo.

697B

Exploring mechanisms of Troponin-T isoform switching and regulation of stoichiometry in the Troponin complex of *Drosophila* Indirect Flight Muscles. Aditi Madan, Divesh Thimmaiya, Prabodh Kumar, Upendra Nongthomba. MRDG, Indian Institute of Science, Bangalore, India.

The indirect flight muscles (IFM) of *Drosophila melanogaster* have been used for decades to study muscle development and function. This is attributed to the extreme ease of genetic manipulability in this system, and high degree of homology between mammalian and Dipteran cytoskeletal proteins. Muscle contraction in striated muscles is regulated by the troponin (Tn) complex (Ca^{2+}-binding troponin-C, inhibitory troponin-I & tropomyosin-binding troponin-T). These subunits are present in a 1:1:1 ratio on thin filaments. 2 isoforms of TnT are alternatively spliced in the *Drosophila* thorax - one containing alternative exon 10a (expressed in adult IFM & TDT); and one containing alternative exon 10b (expressed in pupae & newly eclosed flies). A splice mutant of TnT - up^1 - results in exclusion of the adult-specific TnT isoform (TnT-10a) and leads to defects in myofibrillogenesis. This study aims to understand functional differences between the 2 TnT isoforms, and elucidate probable

mechanisms underlying the isoform switch. Transgenic *UAS* constructs have been used to rescue up^1, using muscle-specific *Dmef2-GAL4* driver. Rescue has been analysed at 5 levels - physiological(flight tests), morphological(polarised light microscopy), myofibrillar(confocal microscopy), expression(RT-PCR) and protein(2D-GE). The homozygous rescued flies are flightless, but show rescue at structural and myofibrillar level. Rescue in the $up^1/+$ background shows recovery of flight ability as well as muscle structure. Control experiments were performed to test for the effect of over-expression of TnT in WT IFM. These experiments have shed light on the importance of stoichiometry amongst troponin subunits, as over-expression leads to flight defects. We have attempted to rescue up^1 using an alternative strategy - by knocking down a splicing factor reported to regulate the temporal switch from TnT-10b to TnT-10a in IFM. We have succeeded in partially rescuing flight ability and muscle architecture.

698C

Polarized contraction coupled to F-actin turnover is required for pulsed contractions. Adam C. Martin, Frank M. Mason, Mike Tworoger. Biology, Massachusetts Institute of Technology, Cambridge, MA.

Apical constriction of epithelial cells promotes tissue folding during developmental processes such as gastrulation. Apical constriction and mesoderm invagination of the Drosophila ventral furrow is facilitated by pulsed contractions of an actin filament (F-actin) and myosin-II network that spans the apical surface and is coupled to circumferential adherens junctions. The mechanisms that generate pulsed actin-myosin contractions at the middle of the apical surface (medial cortex) are not known. Here, we combine live imaging, quantitative image analysis, spatially controlled drug injections, and genetics to demonstrate that F-actin turnover (assembly and disassembly) is critical for pulsed contractions. Apical constriction requires F-actin disassembly/remodeling after contraction pulses to prevent medial F-actin accumulation in ventral furrow cells. F-actin assembly, at least partially mediated by the formin Diaphanous, maintains a continuous medial actin-myosin meshwork, which is critical for contraction pulses and cell-cell adhesion. We show that actin-myosin contraction is directed towards medial Rho Kinase (Rok) foci and requires Rok activity. We propose that Rok and possibly other signals establish a “radial” planar cell polarity in ventral furrow cells that result in cycles of medial-directed contraction followed by actin-myosin network turnover to generate contractile pulses.

699A

Drosophila septins bundle and curve actin filaments. Manos Mavrakis[1], Yannick Azou[1], Feng-Ching Tsai[2], José Alvarado[2], Aurélie Bertin[3], Francois Iv[1], Gijsje Koenderink[2], Thomas Lecuit[1]. 1) Institut de Biologie du Développement de Marseille Luminy, CNRS UMR7288, Aix-Marseille University, Marseille, France; 2) FOM Institute AMOLF, Amsterdam, The Netherlands; 3) Institut de Biochimie et de Biophysique Moléculaire et Cellulaire, CNRS UMR8619, Université Paris-Sud, Orsay, France.

During cell division, actin filaments and myosin motor proteins form a membrane-anchored contractile ring that constricts the cell midzone to give rise to two daughter cells. However, how actomyosin assembles in a cytokinesis-competent network remains an outstanding question. Here we focus on septins, one of the conserved core components of cytokinesis implicated in cancer and neurodegeneration. Although septins bind independently membranes, Anillin (an actin crosslinker) and nonmuscle Myosin-II in different model systems, the role of septins in the organization of actomyosin is not understood, and their precise biochemical function remains unknown. We explore the role of septins during Drosophila embryo cellularization, when actomyosin assembles into a cytokinesis-like ring at the tips of invaginating membranes. Septin depleted embryos fail to accumulate and to stabilize actomyosin, whereas actomyosin fails to assemble into ring-like structures and instead forms a loose coat beneath the cell membrane, suggesting that septins could be involved in compacting or/and curving actomyosin. To test this hypothesis we reconstituted actin filaments in the presence of purified septin complexes in vitro. Biochemical and microscopy assays showed that Drosophila septins constitute a bona fide actin crosslinker and bundler, and that septins further bend actin filament bundles into rings and highly curved geometries. Our data suggest that the functional organization of actomyosin during membrane constriction relies on the bundling and curving activity of septins on actin filaments.

700B

The role of Dynein Heavy Chain in Drosophila bristle growth. Anna Melkov, Uri Abdu. Department of Life Sciences, Ben-Gurion University, Beer-Sheva 84105, Israel.

The highly elongated Drosophila sensory bristles have proven to be a valuable model system for studying cellular morphogenesis. Recently work in our lab showed that microtubuli (MT's) in the bristle are composed of two different MT's sets. The first MT population comprises stable, minus end-distal unipolarized MTs. The second MT population is dynamic and shows mixed polarity. However, the mechanism by which bristle MT's organize is still unknown. We focused our analysis on Dynein Heavy Chain (DHC) gene, since it was shown that DHC have a crucial role in controlling the orientation of axonal MT’s in Drosophila. We further characterized the bristle defects in several viable trans-heterozygous dhc mutants using scanning electron microscopy. We noticed that dhc macrocheata but not microcheata are much shorter than their counterpart wild type ones. Moreover, the first two-third of the bristles length (both in micro-and macrocheta) was wider with abnormally organized surface grooves, with the ridges not being parallel to each other. Then, the bristle becomes much thinner and flattened towards the bristle tip and its surface was smooth. More than 50% of the bristles were split at the base, with a minute extension in opposite direction to the bristle growth. In the next future we will study the mechanism by which dhc affects bristle growth.

701C

Mechanisms of APC-Diaphanous mediated actin assembly. Olivia Molinar[1], Richa Jaiswal[2], Aneliya Rankova[2], Vince Stepanik[1], Bruce L. Goode[2], Brooke M. McCartney[1]. 1) Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Department of Biology, Brandeis University, Waltham, MA.

Adenomatous polyposis coli (APC) negatively regulates Wnt signaling and stabilizes microtubules, and most recently has been implicated in actin assembly. Both vertebrates and *Drosophila* have two APC proteins, vAPC and vAPC2, and dAPC1 and dAPC2 respectively. In *Drosophila*, dAPC1 contains the basic domain necessary for actin assembly function in vertebrates, while dAPC2 does not. vAPC can bundle and nucleate actin filaments through its basic domain, and collaborate with the formin Diaphanous (Dia) to efficiently nucleate actin *in vitro*. To understand the *in vivo* function of this activity, our lab uses the assembly of actin caps and pseudocleavage furrows in the syncytial embryo as a model. We have shown that dAPC2 and Dia are required for pseudocleavage furrow extension, but the role of dAPC1 is not well understood. Taken together, it is clear that APC proteins contribute to actin assembly, but significant gaps exist in our understanding of both mechanism and physiological function. To address these gaps, we are taking a coupled *in vitro-in vivo* approach. *in vitro*, we have shown that dAPC1 shares a common mechanism of actin assembly with vAPC by exerting its activity through the basic domain and collaborating with Dia via the C-terminal DAD domain. In contrast, dAPC2 does not promote actin assembly alone, but instead inhibits the actin assembly activity of Dia through multiple dAPC2 domains that interact directly with Dia. dAPC2 does not inhibit the activity of other formins, arguing against the possibility that dAPC2 inhibits through a non-specific mechanism. Currently, we are working to understand the mechanism of dAPC2 dependent inhibition *in vitro*. In parallel, we are assessing the phenotypic consequences of loss of dAPC1 alone and together with dAPC2 and Dia to determine the *in vivo* role of APC-Dia interactions in actin assembly in the early embryo.

702A

A Genetic Screen Identifies Myo-V as a Component of Abl Signaling Pathways. Sierra K. Mosticone-Wangensteen, Traci L. Stevens. Biology, Randolph-Macon College, Ashland, VA.

Abl is a nonreceptor tyrosine kinase that regulates the cytoskeleton and cell migration. Mutations in *abl* alter actin structure and cell migration, and unregulated cell migration is a hallmark of cancer. Chronic myelogenous leukemia, which is caused by the expression of an activated form of Abl (Bcr-Abl) that results from a chromosomal translocation, is linked to the inappropriate migration of the white blood cells. In *Drosophila*, expression of Bcr-Abl in the epithelium causes defects in embryonic processes that require regulated cell migration such as head involution and dorsal closure. Although we know that Abl regulates cell migration, we do not know all the elements of the pathway by which it regulates the cytoskeleton. Our laboratory is doing a deficiency screen to identify regions that contain genes that interact with the activated form of Abl, Bcr-Abl, and therefore are likely to encode proteins that act in the Abl pathway. Here, our goal was to narrow down a region defined by two large overlapping deficiencies of the second chromosome that suppress the effects of Bcr-Abl expression to identify the gene(s) within this region that genetically interacts with Bcr-Abl. As a first approach, we tested seven smaller deficiencies that overlap with the deficiencies that suppress. Most of these smaller deficiencies did not interact, but surprisingly, we found that two of these smaller deficiencies enhanced the phenotypes associated with Bcr-Abl expression, suggesting that more than one gene in this region may genetically interact with expression of Bcr-Abl. In addition, we tested mutant alleles of fifteen individual genes in this region of the genome, and we found that two different mutant alleles of *didum*, which encodes Myosin-V (Myo-V), an unconventional actin-based motor, suppressed the phenotypes associated with Bcr-Abl expression. These results provide *in vivo* evidence that Myo-V plays a role in Abl signaling pathways, and future experiments will be done to examine the function of Myo-V in the pathways that regulate cell migration.

703B

A novel role for the non-catalytic intracellular domain of Neprilysins in muscle physiology. Mareike Panz[1], Arne Jendretzki[2], Jürgen Heinisch[2], Achim Paululat[1], Heiko Harten[1]. 1) Zoology, University of Osnabruck, Osnabruck, Germany; 2) Genetics, University of Osnabruck, Osnabruck, Germany.

Neprilysins are membrane bound M13-endopeptidases responsible for the activation and/or inactivation of peptide signaling events on cell surfaces. By hydrolyzing their respective substrates, mammalian neprilysins are involved in the metabolism of numerous bioactive peptides especially in the nervous, immune, cardiovascular and inflammatory systems. Based on their involvement in essential physiological processes, proteins of the neprilysin family constitute putative therapeutic agents as well as targets in different diseases, including Alzheimer's disease. We here demonstrate that overexpression of Neprilysin 4 (Nep4) in Drosophila melanogaster leads to a severe muscle degeneration phenotype. This phenotype is observed for overexpression of full length Nep4 in somatic muscles and is accompanied by severely impaired movement of larvae and lethality in late larval development. On the other hand, downregulation of expression causes only the latter two effects. By expressing several mutated and truncated forms of Nep4 in transgenic animals, we show that the intracellular domain is responsible for the observed phenotypes while catalytic activity of the enzyme is apparently dispensable. A Yeast two-hybrid screen identified a yet uncharacterized carbohydrate kinase as a first interaction partner of the intracellular domain of Nep4. These data represent the first report of an intracellular neprilysin domain being involved in muscle integrity.

704C

***Rho1* functions through multiple effectors for proper epithelial wound repair.** Travis K Rahe, Jeffrey M Verboon, Susan M Parkhurst. Division of Basic Sciences, Fred Hutchinson Cancer Research Institute, Seattle, WA.

Epithelial wound repair in embryos is essential for the maintaining of an external environmental barrier, tissue integrity, and survival. One family of proteins, the Rho family of GTPases, have been found to play a vital role in the epithelial wound repair process. The Rho family of GTPases interact with numerous effectors in their GTP bound form to regulate actin and microtubule cytoskeletal reorganization. We find that *Rho1* is required at every step of the epithelial wound healing process. We generated transgenic lines containing a series of substitution and point mutations that disrupt the functional and known protein binding domains of *Rho1*. Embryos expressing these various *Rho1* mutations, as well as fluorescent markers, were laser wounded. We quantitatively assessed actin and microtubule machineries in cell orientation, migration, and adhesion during the repair process. We find that specific *Rho1* effectors and/or molecular pathways are required at different steps in the wound healing process. We are currently investigating mutations in the effectors downstream of the relevant point mutations to integrate the *Rho1* pathways required for the repair process. By establishing these effector pathways we are elucidating the role of *Rho1* in the wound repair process, as well as gaining further insight into roles *Rho1* may play in the developing organism.

705A

The role of the motor protein, kinesin heavy chain, in Drosophila bristle development. Yasmin Simkhoni, Uri Abdu. Department of Life Sciences, Ben-Gurion University, Beer-Sheva, Israel.

Drosophila bristles serve as a good model to understand the role of cytoskeleton fibers in polarized cells. Previously it was shown that mutation in microtubule (MT) plus end motor protein, kinesin heavy chain (khc), affected bristle morphology. Previous study revealed that null mutation of khc resulted in short bristles, as well as flattened, flared, or twisted tips compared to WT. Another phenotype that has been demonstrated was a thinner cuticle of the bristle. In this study we would like to understand the molecular mechanism by which khc affects bristle growth. We show that knocking down khc specifically in the bristle by RNAi leads to defects in bristle tip morphology accompanied with uneven distribution of MTs at the bristle tip. We found that in khc RNAi knockdown flies the function of MTs within the bristle was not affected. Tracking of MTs polymerization using GFP-EB1 (End Binding protein 1) revealed an area that lacked microtubule movement, which suggested that a physical barrier at the bristle tip might have blocked MT distribution. To identify the nature of this physical barrier at the bristle tip, we first characterized the organization of mitochondria, golgi and ER within the bristle cell. We demonstrated that mitochondria were localized at the bristle cell base as well as along the entire bristle shaft. Moreover, live imaging of mito-GFP revealed stationary, anterograde and retrograde movements within the bristle shaft. Whereas Golgi and ER were localized mostly to the bristle cell body, Golgi outposts were found at the lower part of the bristle shaft and ER was evenly distributed along the bristle. Our future plans are to study the effect of khc RNAi downregulation on the organization of the above organelles.

706B

Morphogenetic apoptosis : a force generation mechanism. Magali Suzanne, Melanie Gettings, Bruno Monier, Thomas Mangeat. Paul Sabatier University, LBCMCP, Toulouse, France.

Apoptosis is involved in many crucial morphogenetic processes. However the question remains as to how dying cells influence their neighbors in order to drive tissue remodeling. We are currently investigating the role of apoptosis in the formation of Drosophila leg-joint, structures that separate each leg segment. We are focusing on the first morphogenetic event taking place during joint formation, which correspond to the folding of an epithelial tissue at the origin of adult leg, the leg disc. The analysis of cell shape organization revealed that cells in the presumptive joint are significantly stretched and aligned during fold formation. This suggests two things: the potential existence of a cellular boundary on one hand, and a discrepancy in cellular actin-myosin based tension between cells at the presumptive joint and cell within a leg segment on the other hand. The existence of a restriction boundary was confirmed by clone analysis and the cellular tension has been characterized by laser ablation, revealing a higher tension in a ring of cells at the future joint. We further observed that the apoptotic domain coincide with this stretched domain. These data suggested that the ring domain of higher tension observed in the fold is formed by the combination of a restriction boundary at the presumptive joint and a localized cell death. Analysis of cell shape in the absence of apoptosis indicates that cells are aligned but no longer stretched, suggesting that apoptosis induces cell stretching. The restriction of cell death by a physical boundary would ensures cell stretching rather than cell rearrangement. Thus the loss of cells through apoptosis would be compensated by cell stretching in order to maintain epithelial integrity. Thus, we hypothesize that the presence of a boundary is required at the presumptive joint to convert the elimination of cells into a morphogenetic driving force.

707C

Identification of cytoskeletal genes that are essential for lifelong maintenance of muscle function. Guy Tanentzapf, Alexander D. Perkins, Michael Lee, Fayeza Islam. CPS Dept, Univ British Columbia, Vancouver, BC, Canada.

In the adult animal, muscles maintain their function while bearing substantial mechanical loads and undergoing numerous contraction/extension cycles. How muscles are able to maintain function for long periods of time is presently not well understood. Drosophila melanogaster provides an attractive system in which to study the genetics of muscle tissue maintenance. The basic unit of muscle is the sarcomere which is primarily composed of cytoskeletal proteins. We therefore

hypothesised that cytoskeletal proteins are required to maintain muscle function in the adult fly. A recently developed gene expression system, TARGET, has made it possible to limit gene expression to adults. We used the TARGET system to carry out a systematic RNAi-meditated knockdown screen of cytoskeletal genes in the adult fly. We used robust and sensitive behavioural assays to perform high-time resolution analysis of fly muscle function. This approach identified 48 genes that caused a significant decline in adult muscle function and of these, 40 had not been previously implicated in muscle maintenance. Detailed analysis of candidate genes using confocal and electron microscopy showed that while muscle architecture was largely maintained after gene knockdown, defects in sarcomere length were observed suggesting that that these genes are required to maintain tension in sarcomere. Consistent with this hypothesis we used pulse-chase experiments to show that some of the candidate hits we identified undergo turnover in sarcomeres at the Z-disc. Together, our results provide direct evidence of in vivo muscle protein turnover, identify new candidate genes required for muscle tissue maintenance, and identify specific functional defects associated with knockdown of key sarcomeric components in the adult animal.

708A

Calling the Shots: Prostaglandins Temporally Regulate Actin Remodeling During *Drosophila* Oogenesis. Tina L. Tootle, Andrew J. Spracklen, Xiang Chen. Anatomy and Cell Biology, University of Iowa, Carver College of Medicine, Iowa City, IA.

The actin cytoskeleton is spatially and temporally regulated during both development and adult homeostasis. Temporal misregulation of actin remodeling directly contributes to the development and progression of multiple cardiovascular diseases, including developmental defects, heart attack, stroke, and cardiomyopathies. In the case of heart attack and stroke, such misregulation results in aberrant platelet activation and aggregation, resulting in vessel occlusion. Platelet activity is regulated by the opposing actions of prostaglandins (PGs), lipid signals. Thus it is critical for human health to understand the mechanisms by which PGs temporally regulate actin remodeling. Towards this goal we have established *Drosophila* oogenesis as a model for studying PG-dependent actin remodeling. PGs are required to prevent premature actin remodeling, as the loss of PG synthesis results in early, aberrant actin filament and/or aggregate formation (stage 9). We are taking advantage of this misregulation of actin remodeling to identify actin binding proteins that tightly control the onset of actin remodeling downstream of PG signaling. Specifically, we are using immunofluorescence to identify factors that co-localize to these aberrant, early actin structures and genetic interaction studies to identify factors that suppress or enhance their formation. We have found that Enabled, an actin elongation and anti-capping factor, localizes to the aggregates. Current efforts are focused on determining the signaling cascade by which PGs regulate Enabled activity to prevent premature actin remodeling. By combining correlative microscopy with robust genetic analysis, we can begin to determine how PG signaling temporally regulates actin cytoskeletal remodeling. Understanding the molecular mechanisms by which PGs temporally regulate actin remodeling during *Drosophila* oogenesis will provide insight into the conserved mechanisms by which PG signaling temporally regulates the actin cytoskeleton during human development and homeostasis, including events contributing to cardiovascular disease.

709B

Characterization of the Bristle mutant in *Drosophila melanogaster*. Pooneh Vaziri, Eduardo Gonzalez-Niño, Jennifer Curtiss. New Mexico State University , Las Cruces, NM.

The regulation of cell adhesion plays a crucial role in the formation and maintenance of tissue during development of multicellular organisms. The Rap1 small GTPase regulates adhesion between cells and affects localization of adherens junctions. Small GTPases are molecular switches that are active when bound to GTP and inactive when bound to GDP. GEFs promote the active state of the GTPase and GAPs promote the inactive form. To further investigate the mechanisms by which Rap1 regulates cell adhesion, we performed a screen to identify mutants that modify a Rap1 mutant eye phenotype in*Drosophila melanogaster*. One of the mutants that we identified is the *Bristle (Bl)* mutant, which enhances the *Rap1* loss of function eye phenotype. In the *Bl* mutant the bristles that cover all parts of the fly are shorter and thicker than in normal flies. Since it was not previously known which gene is affected by the *Bl* mutation, we performed complementation tests with mutants that lie close to the *Bl* locus. Mutations in the *skywalker (sky)* gene fail to complement the *Bl* mutation, suggesting that *Bl* is an allele of *sky*. The *sky* gene encodes a GAP that is known to inactivate another class of small GTPases: the Rabs. Rab proteins are important for formation and localization of vesicles in specific trafficking pathways. Also it has been demonstrated that Rab35, which is a previously known target of Sky, is involved in actin bundling during the formation of bristle in *D. melanogaster*. We overexpressed the *sky* gene in bristle precursors, and found that the resulting adult bristles have defects similar to those in the *Bl* mutant, suggesting that the *Bl* mutation results in the overexpression of the *sky* gene. To confirm that *Bl* is an allele of *sky*, we are performing quantitative PCR to determine whether expression of the *sky* gene is altered in *Bl* flies compared to wild-type flies, and we are sequencing *sky* genomic DNA from *Bl* flies to determine the nature of the mutation. In addition, we are performing genetic experiments to elucidate the relationship between *Rap1* and *sky*.

710C

The Identification and Characterization of a New Protein Essential for Drosophila Myotendinous Junction Formation. Zongheng Wang. University of Missouri-Kansas City, Kansas City, MO.

The myotendinous junction (MTJ) provides the primary site to transmit the force generated during muscle contraction from the interior of the muscle cell, across its membrane and the extracellular matrix (ECM), to the epithelial tendon cell for

locomotion. It has been reported that any decrease in MTJ formation and/or stability leads to muscle detachment in diverse organisms, which underlies a series of congenital, progressive myopathies in humans. While it is know that integrins are required to form cohesive attachments with the ECM that lies between the muscle and tendons, the molecular mechanisms that govern formation and maintenance of MTJs in any model system are poorly understood. As an entry point to uncover proteins that contribute to MTJ development and maintenance, we utilized an in vivo proteomics approach. We have identified three new proteins that are required for the stable attachments of muscles to their target tendon cells. One of these evolutionarily conserved candidates, named Clueless (Clu), has been chosen for further study. A series of clu mutant alleles were generated using P-element excision techniques. Immunostaining using an antibody against Myosin heavy chain reveals that the muscles in clu mutant embryos migrate to their target tendon cells. Moreover, the expression and localization of molecular markers that function in muscle migration, such as dGit1 and activated focal attachment kinase, are not affected in the clu mutant embryos. However, upon muscle contraction, the muscles detach from their corresponding tendon cells and round up. The expression of integrin components at the MTJ is reduced in clu mutant embryos, suggesting a failure to form strong interactions between the muscle and the tendon cell. Other data will be presented to determine in what cell type Clu functions in muscle-tendon attachment; and how Clu functions at the molecular level to mediate proper MTJ formation and/or maintenance.

711A

A Screen to Identify Genes that Interact with Abl Tyrosine Kinase in *Drosophila*. Selena Washington, Traci L. Stevens. Biology, Randolph-Macon College, Ashland, VA.

Actin filaments are highly dynamic, thin polymers found throughout eukaryotic cells. Actin is highly concentrated in the cortex, where it directs cell movement and shape. The tyrosine kinase Abl regulates cell morphogenesis by directing the organization of the actin cytoskeleton. In cell culture, expression of an activated form of Abl, Bcr-Abl, alters actin structures and cell migration, and in *Drosophila*, expression of Bcr-Abl disrupts processes that require regulated cell migration, such as dorsal closure and head involution. In order to identify components of Abl signaling pathways that regulate cell migration, our laboratory is conducting a genetic screen that has identified mutant alleles of several genes that modify phenotypes associated with Bcr-Abl expression. Here, we tested these interacting mutations in an *abl* loss-of-function background in order to provide additional evidence of a relationship with wild-type *abl*. First, we used embryos that lack maternal Abl but receive a wild-type copy of *abl* paternally. These *abl* mutant embryos are viable; however, heterozygous mutations of *abi* or *garz*, two genes that modify phenotypes associated with Bcr-Abl expression, resulted in lethality, providing more general support for a role of the products of these genes in normal Abl signaling pathways. Mutant alleles of all other Bcr-Abl interacting genes, however, did not affect the phenotypes of abl mutant embryos; therefore, we increased the sensitivity of this assay by removing the paternal copy of *abl*. Embryos that lack both maternal and zygotic Abl die with defects in epithelial morphogenesis. Thus far, we have tested mutant alleles of two genes that modify Bcr-Abl dependent phenotypes, *Khc* and *garz*, and found that heterozygous mutations in either enhanced the defects of embryos lacking maternal and zygotic Abl. These results suggest that this may be a more sensitive background in which to detect interactions with loss-of-function *abl* and provide additional support for a relationship between wild-type Abl and the products of *Khc* and *garz* in cell migration.

712B

Twinstar is required for muscle development. Shannon F. Yu[1,2], Mary K. Baylies[2]. 1) Gerstner Sloan-Kettering Graduate School, New York, NY; 2) Sloan-Kettering Institute, New York, NY.

A conserved feature of skeletal muscle development is the formation of muscles of various shapes and sizes. These differences are intimately linked to their function; yet little is known about how a muscle achieves its distinct morphology. *Drosophila* provides an excellent model system to study this process: the muscle pattern, while relatively simple, consists of a repetitive arrangement of 30 discrete muscles, each defined by their shape and size. The requirement for the actin cytoskeleton and its modifiers, including Arp2/3, WASp and SCAR, has been demonstrated during embryonic, larval and adult muscle development. To date, however, only activators of actin polymerization have been shown to be necessary for proper muscle development in *Drosophila*. Twinstar (Cofilin) is a conserved member of a family of actin-binding proteins that disassemble actin filaments. We show that *twinstar* is expressed in the embryonic muscle and that loss of *twinstar* results in muscle loss as well as aberrant muscle-tendon attachment and sarcomere formation. Further, muscle-specific depletion of *twinstar* leads to embryonic lethality, due to aberrant sarcomere formation. Additionally, overexpression of wild-type and constitutively active *twinstar* in muscle phenocopies loss of twinstar in the embryo suggesting that Twinstar activity must be tightly regulated during muscle development. Assessment of muscle function when*twinstar* is overexpressed later in development indicates defects in flying, further implicating Twinstar in muscle function. Taken together, these data indicate *twinstar* plays multiple roles during myogenesis and suggest a critical role for actin disassembly during muscle morphogenesis.

713C

UBPY Controls the Stability of ESCRT-0 Complex in Development. Junzheng Zhang, Ying Su, Min Liu, Alan Jian Zhu. Dept. of Cellular & Molecular Medicine, Lerner Res Inst, Cleveland Clinic, Cleveland, OH.

Ubiquitinated developmental signaling proteins are often internalized and then sorted into the endocytic trafficking pathway for degradation. The deubiquiting enzyme UBPY is believed to prevent protein degradation by removing ubiquitin modifier

from internalized signaling protein cargos. This protective role of UBPY is consistent with recent *Drosophila* studies of endocytosed Frizzled2 and Smoothened. However, this canonical model is challenged by several studies on USP8, the vertebrate homolog of UBPY, in which USP8 promotes endocytosed cargo degradation. Here, we utilize both RNAi and loss of function allele of *Ubpy* to demonstrate that UBPY is not required for protecting cargo from degradation *in vivo*. Instead, in the absence of *Ubpy*, a panel of signaling proteins important for *Drosophila*development accumulates in enlarged Rab5-positive endosomes, a phenotype that is often associated with defects in the ESCRT (endosomal sorting complex required for transport) complexes. Indeed, we find that UBPY directly interacts with and deubiquitinates Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate), an essential component of the ESCRT-0 complex. Furthermore, the activity of the ESCRT-0 complex is compromised in *Ubpy*-null cells where ubiquitinated Hrs undergoes lysosome-mediated degradation. Consistently, we find that developmental signaling proteins are enriched in early endosomes when *hrs* activity is abolished in *Drosophila*. Finally, we provide evidence that USP8 stabilizes Hrs in vertebrate cells. Thus, our study uncovers a critical role of UBPY in protecting the ESCRT-0 complex from degradation, a control mechanism in degradative protein sorting that is also conserved in higher organisms.

714A

Cardiac integrity and function depends on the ADAMTSL protein Lonely heart. Maik Drechsler, Ariane Schmidt, Heiko Meyer, Achim Paululat. Department of Zoology/Developmental Biology, University of Osnabrueck, Osnabrueck, Germany.

Changes in ECM composition, turnover or homeostasis are crucial mediators of human cardiovascular disease leading to life threatening conditions. The formation and maintenance of a tissue specific ECM is therefore a vital task to maintain organ functionality. Using Drosophila as model of cardiac integrity we identified the ADAMTSL protein Loh as essential for the assembly and proper organization of a cardiac matrix composed of the collagen Prc. We found that mutations in *loh* lead to the disability of Prc to integrate correctly into the cardiac ECM during embryogenesis. Consequently, the adhesion of pericardial cells and alary muscles towards the heart tube get disrupted during post-embryonic stages, causing a loss of cardiac integrity and function. This results in the abolishment of hemolymph flow and a dramatic reduction of the fly's life span. On the mechanistic level we provide further evidence that Loh is able to recruit Prc to target tissues *in vivo* and therefore acts as a secreted receptor of this collagen. Our data, in combination with previous findings in mammals, demonstrate that the function of ADAMTS-like proteins in facilitating matrix formation and stability is evolutionary conserved and constitutes an important task to allow cardiac homeostasis.

715B

Molecular mechanisms underlying the intracellular distribution of ZP proteins for epidermal differentiation. Francois Payre[1,2], Helene Chanut-Delalande[1,2], Delphine Menoret[1,2], Serge Plaza[1,2]. 1) Centre for Developmental Biology, University of Toulouse, Toulouse, France; 2) CNRS, UMR5547, Toulouse, France.

During late embryogenesis, the morphological differentiation of epidermal cells leads to the production of finely shaped apical extensions, called trichomes. A scaffold of Zona Pellucida (ZP) proteins that locally modify the apical extracellular matrix is required to sculpt the shape of trichomes. We have recently shown that 8 ZP proteins are localized in, and define, distinct apical regions, along the growing trichome (Fernandes et al, 2010). ZP proteins thus reveal a sub-compartimentalization of the apical domain. To understand how ZP proteins are addressed to sub-apical regions of the plasma membrane, we have undertook a genetic screening aiming at identifying genes required for ZP protein distribution. We focused on candidate genes displaying a strong alteration of trichome morphogenesis. We will present recent data showing the role of different classes of regulator of ZP distribution in the morphologicla differentiation of trichomes.

716C

Disruption of Rab protein vesicle transport by loss of huntingtin in vivo. Shermali D. Gunawardena, Derek Power, Shruthi Srinivasan. Biological Sciences, SUNY at Buffalo, Buffalo, NY.

Previous work put forth a tantalizing proposal that disruption of axonal transport within long, narrow-caliber axons caused accumulations that could elicit cell death, ultimately resulting in neuronal dysfunction observed in Huntington's Disease. Although a role for the Huntington's disease protein huntingtin (HTT) has been reported in axonal transport, it is unclear if HTT affects the transport of all vesicles or if HTT affects a specific class of vesicles. In this context, here we tested the hypothesis that disruption of Rab protein vesicle transport within axons mediated by HTT can contribute to early neuropathology observed in HD. Using in vivo motility analysis we found that HTT influences Rab11, Rab32 and Rab4X vesicles, but not Rab5 vesicles. While reduction of HTT perturbed the transport of Rab 11 and Rab32 vesicles, reduction of HTT rescued RabX4-mediated transport defects. Although Rab11, Rab32 and RabX4 are all thought to be on recycling endosomes, while Rab 5 is on early endosomes, all these Rab proteins show bi-directional movement. Reductions in kinesin and dynein motors also perturbed Rab11 vesicle transport indicating that these motors are required for bi-directional transport of Rab11. These results suggest that HTT plays a key role in the movement of Rab11, Rab32 and Rab4 vesicles within axons. Thus disruption of Rab vesicle transport mediated by mutant HTT could contribute to early neuropathology observed in Huntington's diseases.

717A

The Role of *tbc-1* in Drosophila Salivary Gland Development. Dorothy M Johnson, Deborah Andrew. Cell Biology, Johns

Hopkins School of Medicine, Baltimore, MD.

Rabs are small GTPases involved in vesicle targeting, tethering, and fusion. Rabs' GTPase activity is accelerated by Rab-GAPs (GTPase activating proteins). Recently, a highly conserved Drosophila Rab-GAP, known as *tbc-1*, was discovered to be expressed in the embryonic salivary gland under control of the FoxA transcription factor Fork head (*Fkh*). An analysis of deficiencies and RNAi of *tbc-1*revealed irregular apical membranes in embryos in which *tbc-1* was knocked down in the salivary gland, suggesting that *tbc-1* has a role in salivary gland development. Based on these preliminary findings, *tbc-1* knockout lines were generated by homologous recombination and verified by PCR analysis. Future plans include a full characterization of the null salivary gland and border cell migration phenotypes, as well as studies to learn which Rab and which membrane fusion events are normally modulated by this GTPase.

718B

Atlastin regulates lipid-droplet in Drosophila melanogaster fat bodies. Han Lee[1], Diana Jin[2], Yi Guo[2]. 1) Dept of Neurobiology of Disease, Mayo Graduate School, Rochester, MN; 2) Dept of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN.

Lipid droplet (LD) is the cellular energy storage hub in most cells. Neutral lipid synthesis enzymes mainly reside in the endoplasmic reticulum (ER). The newly synthesized triglycerides and sterol esters are packed into cytosolic LDs and bud off the ER membrane leaflet as proposed in the "budding" model. How the ER morphology affects LD formation and expansion and in which of the specific ER domain LD forms are largely unknown. We have identified that ER tubular network regulating gene, atlastin, is important in controlling LD size in Drosophila fat bodies. Fat body specific atlastin knockdown dramatically altered ER morphology, reduced whole body fat storage. As a consequence, these flies are much more sensitive to starvation challenge. Taken together, these results show that, Drosophila atlastin has a pronounced effect on LD size and energy homeostasis of the whole organism.

719C

AP-1-dependent E-Cadherin trafficking in *Drosophila* oogenesis. Nicolas Loyer, Roland Le Borgne. CNRS UMR 6290-IGDR, Rennes, France.

In yeast and mammals, clathrin adaptor protein complexes AP-1 function in transport between the trans-Golgi Network (TGN) and the endosomes, and in basolateral targeting in polarized epithelial cells. These functions of AP-1 are likely to be evolutionarily conserved. Here, we have investigated the function of AP-1 during oogenesis in Drosophila. Continuously growing immature egg chambers are composed of a monolayered follicular epithelium surrounding a germline cyst of 16 cells, interconnected by cytoplasmic bridges called ring canals. As reported in adult monolayered epithelium, loss of AP-1 does not cause a loss of establishment or maintenance of follicular epithelial cell polarity. In contrast, we observed two phenotypes in the germline cyst mutant for AP-1. First, plasma membrane separating the cells progressively disappeared in late stages, giving rise to multinucleated cells. Live imaging of cultured egg chambers revealed that this phenotype was due to plasma membrane detachment from ring canals actin structures. Second, the localization of E-Cadherin, an adhesion molecule homogeneously distributed along the germline cyst cells plasma membrane and enriched around their ring canals, was affected in AP-1 mutant cysts. E-Cadherin was found enriched in enlarged Rab11-positive recycling endosomes and was specifically excluded from the plasma membrane surrounding ring canals. We hypothesize a connection between these two phenotypes and are currently testing a working model involving an AP-1 dependent E-Cadherin targeting to ring canals, where it contributes to plasma membrane anchorage to ring canals actin structures, most likely through its binding partners alpha- and beta-catenins.

720A

Roles of phosphatidylinositol 4-phosphate in Drosophila larval secretory granule biogenesis. Cheng-I J Ma[1,2], Jason Burgess[2,3], Lauren M Del Bel[2,3], Barbara Barylko[4], Gordon Polevoy[2], Janet Rollins[5], Joseph P Albanesi[4], Helmut Krämer[6], Julie A Brill[1,2,3]. 1) Institute of Medical Science, University of Toronto, Toronto, ON, Canada; 2) Program in Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada; 3) Department of Molecular Genetics, University of Toronto, ON, Canada; 4) Department of Pharmacology, UT Southwestern Medical Center, Dallas, TX, USA; 5) Division of Natural Sciences, The College of Mount Saint Vincent, Riverdale, NY, USA; 6) Department of Neuroscience, UT Southwestern Medical Center, Dallas, TX, USA.

Phosphatidylinositol 4-kinases (PI4Ks) are responsible for production of the lipid phosphatidylinositol 4-phosphate (PI4P), a crucial resident of Golgi membranes that regulates membrane trafficking events such as secretion. Interestingly, we have discovered that PI4KII but not fwd function is required for normal development of the Drosophila larval salivary gland. In PI4KII mutants, mucin-containing glue granules were considerably smaller than in wild type. Furthermore, PI4KII formed dynamic tubular networks along microtubules. Portions of these tubules colocalized with late endosomes and lysosomes marked with YFP-Rab7, suggesting these PI4KII positive tubules were dynamic endosomal structures. In PI4KII mutants, these tubular structures were absent and enlarged lysotracker positive endosomes were observed. These enlarged acidic endosomes contained mucin cargo proteins as well as the glue granule associated SNARE SNAP24. Our data thus far suggested that PI4KII function is required for the proper trafficking of granule proteins, where mistrafficked granule proteins accumulated in the late endosome. To further examine the role of PI4P in granule biogenesis, we are employing a reverse genetic screen to identify potential genetic interactions using publicly available transgenic RNAi lines. A selection of candidate genes including Rabs, SNAREs, and BAR domain containing proteins will be screened to identify players involved in the PI4KII

mediated membrane tubulation, fusion and fission. We will also examine which of the known PI4P binding proteins play a role in glue granule biogenesis.

721B

Rab8 is Required for the Regulation of Invagination of the Furrow Canal in Cellularization During *Drosophila* Embryogenesis. Lauren Mavor, J. Todd Blankenship. Biological Sciences, University of Denver, Denver, CO.

Epithelial sheets create a boundary between the exterior and interior environments of organisms. The maintenance of adhesion within these sheets is critical to the function of the tissue and loss of this maintenance drives the metastasis of many epithelial cancers. In *Drosophila*, the epithelium is created via a process known as cellularization. Cellularization requires the coordinated invagination of plasma membrane to create an epithelial sheet 30µm tall. In the presented work, we show that vesicular trafficking is necessary for this invagination process to occur. Using fixed and live-imaging *in vivo*, we have shown that Rab8, a late exocytic vesicle marker, localizes both to metaphase furrows during syncytial divisions prior to furrow ingression and to the Furrow Canal (FC) during cellularization. Prior to the onset of cellularization, Rab8 forms tubule-like projections, which mark future ingression furrows and precede F-actin, a cytoskeletal element known to play a vital role during this process. During cellularization, these same tubules follow the length of the FC and lead the basal-most portion of the FC. Knockdown of Rab8 via RNAi leads to a failure in formation of the FC and thus failure to initiate cellularization. Previous reports have shown that membrane trafficking via the recycling endosome and Golgi body are required to drive the cellularization process; however, in the presented work we show that Rab8 is both more dynamic and functions at an earlier stage of the ingression process than these two compartments. Thus we propose that polarized vesicular trafficking via late exocytic pathways is the primary pathway required for providing the membrane components necessary to drive the invagination of the FC and thus form the epithelial sheet of the Drosophila embryo.

722C

Ykt6, a conserved v-SNARE, is required in neuronal function and maintenance. Kai Li Tan[1], Shinya Yamamoto[2], Manish Jaiswal[2], Hector Sandoval[2], Gabriela David[1], Bo Xiong[1], Wu-Lin Charng[1], Ke Zhang[4], Vafa Bayat[3], Hugo J Bellen[1,2,4,5,6]. 1) Program in Developmental Biology; 2) Department of Molecular and Human Genetics; 3) MSTP; 4) SCBMB Program; 5) HHMI; 6) Department of Neuroscience; Neurological Research Institute at Baylor of College of Medicine, Houston, TX.

Membrane fusion is required for vesicular trafficking between various organelles and compartments. Many cell functions rely on membrane fusion, including intracellular transport, hormone or enzyme secretion and maturation of organelles. In particular, neurons utilize membrane fusion for activities that are important for neuronal function and maintenance such as neurotransmission, protein recycling and degradation. Some of the key players of membrane fusion are the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs). In a forward genetic screen on the *Drosophila* X-chromosome to identify mutations that cause neurodegenerative phenotypes we identified 2 mutations in *Ykt6*, a gene that encodes the *Drosophila* homolog of mammalian Ykt6. *Ykt6* is a highly conserved gene which encodes a v-SNARE. It is widely accepted that Ykt6 is involved in ER-Golgi anterograde transport based on yeast and mammalian studies. However, no mutants have been reported for Ykt6 in higher eukaryotes. Using the *Ykt6* mutants, we examined the role of Ykt6 in photoreceptor function and maintenance. ERGs (electroretinogram recordings) of *Ykt6* mutant clones show defects in neurotransmission, and a progressive decline of the amplitude of the ERGs as the flies age, a hallmark of neurodegeneration. The functional neurodegenerative phenotype was confirmed with TEM. We found that several photoreceptor-specific transmembrane proteins like Rhodopsin1 and Chaoptin are localized to the cell body rather than in the cell membrane. Biochemical studies suggest that these proteins are trapped in the ER. We are postulating that the accumulation of proteins in the ER in mutant photoreceptors leads to ER stress and neurodegeneration. This study will therefore be able to assess the role of Ykt6 in a multicellular organism in neuronal maintenance.

723A

The retromer complex is required for photoreceptor maintenance and Rhodopsin recycling. Shiuan Wang[1], Bo Xiong[1], Shinya Yamamoto[2], Kai Li Tan[1], Hector Sandoval[2], Manish Jaiswal[2], Vafa Bayat[3], Ke Zhang[4], Wu Lin Charng[1], Gabriela David[1], Hugo Bellen[1,2,4,5,6]. 1) Program in Developmental Biology; 2) Department of Molecular and Human Genetics; 3) MSTP; 4) SCBMB Program; 5) HHMI; 6) Department of Neuroscience; Neurological Research Institute at Baylor of College of Medicine, Houston, TX.

Rhodopsin 1 (Rh1) is internalized and degraded upon light exposure, but it remains unknown if Rh1 can be recycled and if Rh1 recycling is required to maintain photoreceptor (PR) function. In a forward genetic screen for mutations that cause neurodegeneration we isolated mutations in *Vps26*. *Vps26* mutant PRs exhibit progressively worsening electroretinograms (ERGs) and morphological defects when kept in the light/dark cycle (LD). However, darkness strongly suppresses both defects, suggesting that PR degeneration of *Vps26* mutants are light dependent. *Vps26* encodes a protein which together with Vps35 is part of the retromer complex. To address if retromer function is required to maintain PRs, we performed ERGs and morphological assays in *Vps35* mutants. Similar to *Vps26*, *Vps35* mutant PRs show degenerative phenotypes in LD, implicating the retromer in PR maintenance. The retromer recycles membrane proteins from endosomes and prevent their degradation in lysosomes. Indeed the number and size of lysosomes in mutant PRs is vastly expanded. Moreover, Western Blots revealed a decreased level of Rh1 in *Vps26* mutants, suggesting that more Rh1 is delivered and degraded in lysosomes and that Rh1

recycling is impaired in *Vps26* mutants. Increased Rh transport into lysosomes may increase the burden on the endolysosomal pathway. To reduce Rh1 endocytosis, we performed ERG in *Vps26 shibire*double mutants and observed a strong suppression of the degenerative phenotype. Therefore, we propose that the retromer complex recycles Rh1 and that impaired Rh1 recycling increases Rh1 transport to lysosomes. This in turn causes the demise of *Vps26* mutant PRs. Reducing internalized Rh1 alleviates the stress and suppresses PR degeneration.

724B

Klar modulates *oskar* RNP transport in the *Drosophila* oocyte. Michael A. Welte[1], Imre Gáspár[2], Yanxun V. Yu[1,3], Anne Ephrussi[2]. 1) Dept Biol, University of Rochester, Rochester, NY; 2) Developmental Biology Unit, EMBL, Heidelberg, Germany; 3) Dept Biology, Brandeis University, Waltham, MA.

In *Drosophila* oocytes, the plus-end motor kinesin-1 delivers *osk* RNP particles to the posterior pole, where they initiate germ-plasm assembly. Although many components important for *osk* delivery have been identified, it remains unclear how this process is temporally controlled. The Klarsicht protein regulates kinesin-1-driven lipid-droplet motion in early embryos and has been proposed to modulate transport efficiency. Here we find that the β isoform of Klar is expressed during oogenesis and accumulates in distinct puncta at the posterior pole of mid-late stage oocytes, mimicking *osk* RNP accumulation. Klar co-immunoprecipitates with kinesin-1, and its accumulation at the posterior depends on kinesin-1 and microtubules, but not on *osk* or germ-plasm assembly. We conclude that Klar travels to the posterior pole with some kinesin-dependent cargoes. Klar puncta do not correspond to the previously known Klar cargoes nuclei and lipid droplets. Rather, we believe that one of these cargoes is *osk* RNPs: live imaging and particle tracking reveal that lack of Klar alters specific parameters of *osk* RNP motility; in particular, RNP run lengths are significantly increased. Using FISH analysis, we find that - compared to similarly staged wild-type oocytes - the *osk* RNP population is shifted towards the posterior pole in *klar* mutants. Based on this enhanced transport, we conclude that, in wild-type oocytes, Klar restricts *osk* RNP motion. In *klar* mutants, posterior *osk* RNA and Osk protein are not as tightly cortical as in the wild type, but spread out over a larger area, suggesting that prematurely delivered *osk* RNPs are inefficiently anchored at the cortex. Accumulation of germ-plasm components in ectopic dots suggests that this delocalization impairs germ-plasm assembly. We conclude that Klar synchronizes *osk* RNP arrival with the establishment of new anchoring sites. Thus, Klar plays a role in temporally integrating multiple processes during oocyte maturation.

725C

Crag is a GEF for Rab11 and regulates Rhodopsin trafficking in adult photoreceptor cells. Bo Xiong[1*], Manish Jaiswal[2], Ke Zhang[3], Hector Sandoval[4], Wu-Lin Charng[1], Tongchao Li[1], Gabriela David[1], Shinya Yamamoto[1,4], Hugo Bellen[1,2,3,4,5,6]. 1) PROGRAM IN DVELOPMENTAL BIOLOGY, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX; 2) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 3) Program in Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX; 4) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 5) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 6) Neurological Research Institute, Baylor College of Medicine, Houston, TX.

Rhodopsins (Rh) are G-protein coupled light sensors, and Rh1 is the major Rh in *Drosophila* which is mainly localized to the rhabdomere membrane. Upon photoactivation, a fraction of Rh1 is internalized and degraded, but it remains unclear how the rhabdomeric Rh1 pool is replenished and what molecular players are involved. In this study, we show that Crag, a DENN domain containing protein, is required for the homeostasis of Rh1 upon light exposure. The absence of *Crag* causes a light induced accumulation of cytoplasmic Rh1 and vesicles, leading to a retinal degeneration in adult flies. When endocytosis of Rh1 is triggered by blue light, Rh1 internalization and degradation is not affected in *Crag* mutant cells. However, a persistent accumulation of Rh1 is observed in *Crag* mutant cells but not in wild type cells after a recovery period. The accumulated Rh1 is partially associated with the trans-Golgi compartment. We therefore conclude that Crag is required for post-Golgi trafficking of newly synthesized Rh1. Furthermore, we show that Crag is a guanine nucleotide exchange factor for Rab11. Knockdown of *Rab11* leads to a similar light dependent retinal degeneration phenotype and overexpression of a constitutive active form of Rab11 partially rescues the defects associated with loss of Crag. We propose that upon light stimulation, Crag is required for trafficking of Rhodopsin from the Trans-Golgi network to rhabdomere membranes via a Rab11 dependent vesicular transport.

726A

Basal cell protrusive activity is a primary determinant of follicle cell planar polarity. Maureen P. Cetera[1], Lindsay Lewellyn[1], Michael J. Fairchild[2], Guy Tanentzapf[2], Sally Horne-Badovinac[1]. 1) Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL; 2) Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC.

Coordinated cell migration is critical for organ formation. We are using the *Drosophila* egg chamber as a highly tractable model to investigate morphogenetic mechanisms during organogenesis. The initially spherical egg chamber elongates along its A-P axis to form an elliptical egg. This morphogenesis depends on an unusual form of planar polarity at the basal surface of the egg chamber's outer follicle cell (FC) layer. Here, linear actin filaments and fibril-like structures in the basement membrane (BM) both align perpendicular to the elongation axis, where they are thought to function as a molecular corselet that restricts egg chamber growth to the A-P axis. Recently, follicle cell planar polarity has been shown to correlate with a dramatic collective migration of the FCs, a phenomenon that causes the entire egg chamber to rotate within the external BM. However,

the relationship between the planar polarity and the FC migration is unknown. By temporally manipulating migration either before or after planar polarity establishment we have determined their dependence on one another. The Arp2/3 activator Scar/WAVE is planar polarized at the front of the migrating FCs where it is cell-autonomously required for formation of basal protrusions at the leading edge of each FC. Reducing Arp2/3 activity in the entire epithelium before planar polarity is established blocks FC migration and planar polarity is never established. Conversely, culturing egg chambers with an Arp2/3 inhibitor after polarity establishment also blocks migration but does not influence the tissue-level alignment of basal actin filaments. These data suggest that Arp2/3's ability to drive FC migration is a primary determinant in the establishment, but not the maintenance, of basal actin filament planar polarity.

727B

Differential phosphorylation of the myosin light chain by multiple kinase pathways is required for collective cell migration. Jocelyn A. McDonald[1,2], Pralay Majumder[1], George Aranjuez[1,2], Ashley Burtscher[1]. 1) Molecular Genetics, Cleveland Clinic, Cleveland, OH; 2) Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH.

Despite the importance of collective cell movement to processes such as organ morphogenesis and tumor invasion and metastasis, how groups of cells escape from epithelia in tissues and subsequently coordinate their motility is still poorly understood. We use the border cell model to uncover novel mechanisms that regulate collective cell migration. Border cells migrate as a cohesive group of 6-10 cells during *Drosophila* oogenesis, where they detach from the follicular epithelium and migrate 100 μm to reach the oocyte. During migration, border cells extend directed protrusions to sense guidance cues and provide traction to their substrate. We discovered that the serine-threonine kinase and cell polarity protein Par-1 is required for efficient detachment of border cells from the epithelium and for protrusion dynamics and directionality. Moreover, we found that a major role of Par-1 in border cells is to specifically regulate the dynamic subcellular localization and activation of myosin-II (myo-II). Par-1 phosphorylates and inactivates myosin phosphatase, thus increasing the levels of active myo-II through enhanced phosphorylation of the myosin regulatory light chain (MRLC/Sqh). Here, we provide evidence that Par-1 integrates with a second kinase, Rho-kinase (Rok), which directly activates myo-II and inactivates the myosin phosphatase. Loss of both *Rok* and *par-1* severely impairs the ability of border cells to detach and migrate. We establish that these two kinases (and additional myo-II activating kinases) differentially regulate mono- versus di-phosphorylation of MRLC, thus resulting in distinct pools of active myo-II. While Rok and myosin phosphatase are distributed uniformly in the border cell cluster, Par-1 localizes to the cluster rear; this gives rise to polarized active myo-II. We propose that Rok, localized Par-1 and other kinases jointly activate myo-II in a spatio-temporally defined manner during the detachment and protrusion extension of border cells.

728C

Emergence of embryonic pattern through contact inhibition of locomotion. Brian M Stramer, John Davis, Chieh-Yin Huang, Jennifer Zanet, Daniel Soong, Graham Dunn. Randall Division of Cell and Molecular Biophysics, King's College London, London, United Kingdom.

The pioneering cell biologist, Michael Abercrombie, first described the process of contact inhibition of locomotion more than half a century ago when migrating fibroblasts were observed to rapidly change direction and migrate away upon collision. Since this initial observation, we have gleaned little understanding of how contact inhibition is regulated and only lately observed its occurrence in vivo. We recently revealed that Drosophila hemocytes require contact inhibition for their uniform embryonic dispersal (Stramer et al., J. Cell Biol. 2010). To investigate the role that contact inhibition plays in the patterning of this migration, we have now mathematically analyzed and computationally simulated their contact repulsion dynamics. Taking into account only the kinematics (i.e. acceleration and velocity) of freely moving and singly colliding cells, our data reveal that the final hexagonally arrayed pattern of hemocyte distribution, and the details and timing of its formation, can be explained entirely by contact inhibition dynamics within the geometry of the Drosophila embryo. This also highlights that the contact inhibition process is regulated by precise rules, and analysis of actin and microtubule dynamics around collisions suggests that there is a complex mechanical coupling between colliding cells allowing for the exact coordination of the response in colliding partners. These results have broader implications for morphological development suggesting that Michael Abercrombie's "social behavior" of cells, in the absence of elaborate external cues, can be a significant driving force for embryonic pattern formation.

729A

The influence of the myosin converter domain on muscle function in *Drosophila*. Bernadette Glasheen, Seemanti Ramanath, Qian Wang, Debra Sheppard, Lauren Riley, Douglas Swank. Center for Biotechnology and Interdisciplinary Studies, Department of Biology, Rensselaer Polytechnic Institute, Troy, NY.

Muscles must generate different amounts of force and operate at various speeds to power a wide variety of movements. A major protein component that is critical to setting these properties is the myosin isoform present in the muscle. However, how myosin isoform functional variation is determined by myosin structure is unknown. We studied the influence of the converter, a major myosin structural region, using *Drosophila* as it generates all myosin isoforms by alternative mRNA splicing from a single myosin heavy chain gene. The converter domain has five different alternative versions (11a-e), the most of the six alternative regions in the myosin gene. We created transgenic fly lines expressing myosin constructs that each contained a single alternative version of the converter and crossed them into the *Mhc^{10}* myosin null background. This forces expression of

the four non-native converters (11b-e) in the indirect flight muscles (IFM). Currently, we have tested the 11b, 11d, and 11e lines and found that wing beat frequency for all three lines was 11-15% less than control lines, and flight ability was 34% lower in 11b and 11d lines and 42% lower in the 11e line. We measured the mechanical performance of isolated single muscle fibers and found that the 11b converter version decreased power production by 40% while 11d and 11e decreased power by 60%. Muscle speed (the frequency at which maximum power was generated) was also affected. 11e was the slowest, with a 44% decrease compared to control lines, followed by 36% for 11d, and 22% for 11b. Force production does not appear to be significantly altered by these converter regions. Thus, the converter is critical for setting optimal muscle power production and functions primarily by varying muscle speed.

730B

Characterization of septate junction biogenesis during embryogenesis in Drosophila. Sonia Hall, Jennifer Mendez, Sam Long, Robert Ward. Molecular Biosciences, University of Kansas, Lawrence, KS.

Polarized epithelial cells form tissues that provide a protective barrier from the outside environment and allow for the compartmentalization of internal organs. The ability of an epithelium to act as a barrier is a universal requirement for all multicellular organisms, and is mediated by the formation of cellular junctions along the lateral membranes between epithelial cells. Tight junctions serve this function in vertebrate organisms, whereas septate junctions (SJ) are used in invertebrate organisms. Although tight and septate junctions are ultrastructurally distinct, they share several molecular components including proteins of the claudin and MAGUK families. Genetic studies in Drosophila have identified more than 20 genes that function in the assembly or maintenance of SJ, and recent studies have begun to shed light on the process of biogenesis. Most SJ genes are zygotically expressed and initially trafficked to the basolateral membrane. Many of these proteins are subsequently endocytosed and retargeted to the apical lateral region. Mature SJ are composed of highly crosslinked protein complexes that show very little mobility in the plane of the membrane. Interestingly, SJ proteins exhibit an interdependence, in which the loss of one core protein results in the mislocalization and increased mobility of all other SJ proteins along the lateral membrane. We noticed that the degree of mislocalization of certain SJ proteins is dependent upon which core component is missing. We are therefore extending these studies by examining the localization of ~10 SJ proteins in more than 20 different mutant backgrounds. In addition, we are using immunohistochemistry to track the colocalization of many SJ proteins as they are trafficked to the SJ during its biogenesis in wild type cells. These analyses should allow us to make inferences about the substructure of SJs during their biogenesis and maintenance.

731C

Formation and remodeling of the muscle cell T-tubule membrane network. Amy Kiger, Jen Nguyen, Ines Ribeiro, Naonobu Fujita. Cell & Dev Biol, Univ California, San Diego, La Jolla, CA.

Muscle cells rely on the extensive membrane network of the excitation-contraction coupling system for synchronous contraction. Transverse (T)-tubule membranes extend continuously from the plasma membrane into the muscle interior to couple surface signals with sarcomere function. Whereas the physiological significance of T-tubules is established in fly and human mobility and myopathy, little is understood about T-tubule formation or remodeling with muscle use. We discovered that the T-tubule network undergoes a dramatic, regulated remodeling during wildtype metamorphosis, providing an ideal developmentally programmed system in flies to study mechanisms of T-tubule remodeling. In pupal abdominal myofibers, we show that T-tubules disassemble at the larval-pupal transition and then rapidly reform in pharates. The extent of T-tubule disassembly correlates with an accumulation of membrane inclusions marked with T-tubule proteins, suggesting a membrane reservoir that serves in T-tubule reassembly. Using this system, we found that *shibire* (*shi*) dynamin GTPase and *myotubularin* (*mtm*) phosphoinositide 3-phosphatase play opposite roles in T-tubule remodeling. Muscle-targeted depletion of *shi* blocked regulated T-tubule disassembly, while in contrast, *mtm* depletion blocked T-tubule reassembly. Moreover, GTPase-dependent *shi* overexpression was sufficient to drive persistent T-tubule disassembly and Shi localization at T-tubule remnants. Double mutant analyses indicated that *shi* and *mtm* genetically interact in a common pathway dependent on the Class II PI3-kinase, *Pi3K68D*. Altogether, our results indicate that dynamin acts directly in T-tubule membrane scission under phosphoinositide regulation. Importantly, these and our additional findings with disease mutations provide insight into dominant and recessive forms of human centronuclear myopathy associated independently with Dynamin-2 and MTM1, respectively. Our work here and from ongoing screens reveals the dynamic nature and specific mechanisms of T-tubule membrane formation.

732A

Rab-mediated secretion of lipoproteins in *Drosophila melanogaster*. Sebastian Dunst, Marko Brankatschk, Anja Zeigerer, Marino Zerial, Suzanne Eaton. MPI-CBG, Dresden, Germany.

The mammalian liver secretes very low density lipoproteins (VLDL) to export synthesized lipids to extrahepatic tissues. ApolipoproteinB-100 (apoB-100) is a principal scaffold protein that forms VLDL particles. It has an important role for the assembly of VLDL particles and their secretion through the secretory pathway that is essentially coordinated by Rab GTPases and their effectors. Although the disruption of Rab-mediated transport has been implicated in several inherited human disorders, an *in vivo* model system for studying vesicular transport in tissue development and function is still lacking.

We used homologous recombination to generate a comprehensive Rab library in *Drosophila melanogaster* that includes *rab* genes fused to a fluorophore, modified by a proteolytic cleavage site, as well as precise loss-of-function alleles. We

utilized this novel resource to screen for Rabs that are involved in the secretion of lipoproteins from the larval fat body, an organ analogous to the mammalian liver and white adipose tissue. The fat body produces and secretes two apoB-100 functional homologues of systemic lipid carriers, Lipid transfer particle (LTP) and Lipophorin (LPP), which are required to shuttle dietary lipids from the gut to peripheral organs (Palm *et al*., 2012).

Our Rab screen identified two Rabs, whose localization suggested a role in trafficking of LPP and LTP. The fat body-specific RNAi-mediated knock down of these Rabs causes late larval lethality due to a specific depletion of LTP. The resulting mis-lipidation of LPP and lipid uptake defect from the midgut resembles the *ltp* mutant phenotype. Our data further suggests that the two Rabs cause LTP loss-of-function through different mechanisms as indicated by co-localization studies, qPCR and lipoprotein density gradient fractionation. Since *Drosophila* Rab proteins are more than 80 percent similar to their mammalian homologues, we further assess the role of Rab-mediated apoB-100 VLDL secretion and lipidation in primary mouse hepatocytes.

733B

Apical targeting of Diaphanous mediates polarized secretion in tubular organs. Eyal D. Schejter, Tal Rousso, Rada Massarwa, Erez Geron, Ben-Zion Shilo. Dept Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

The epithelial cells comprising tubular organs are highly polarized, a feature that enables efficient execution of specialized functions, such as vectorial secretion and nutrient absorption. We have previously shown that secretion from these epithelial cells into the organ lumen utilizes Myosin V-mediated transport of secretory vesicles over an apical layer of microfilaments, generated by the formin Diaphanous (Dia). This secretory mechanism, which is common to all Drosophila embryonic tubular organs examined, appears to be evolutionarily-conserved, as we have recently demonstrated that it underlies apical secretion in the mouse exocrine pancreas and salivary glands. Apical restriction of Dia activity is the central feature ensuring formation of the critical microfilament array that promotes apical secretion in Drosophila tubular organs. In the current study we have examined the mechanistic basis for this polarization of Dia activity. We find that Dia apical targeting requires the coincident detection of both PIP2 and the GTPase Rho1 on the apical surface of the tubular organ cells. We show that PIP2 levels regulate Dia localization. An N-terminal basic domain of Dia is critical for this regulation, indicative of direct interaction between Dia and membranal PIP2. Our data indicate that apical enrichment of PIP2 is a common feature of tubular organs in the fly embryo and larva, attributed in part to apical restriction of the PIP5 kinase (PIP5K) Skittles. We further show that Rho1 facilitates Dia apical targeting, both by inducing the open, active conformation of Dia, thereby exposing N-terminal domains that are critical for localization, as well as by physically anchoring Dia to the apical surface. This anchoring appears to be essential for the Dia-PIP2 interaction, leading to synergistic relations between the two apical cues. The mechanism we describe relies on the utilization of inherent and global features of tubular organs, in order to achieve a distinct localization pattern of Dia, thereby enabling proper execution of oriented secretion in these organs.

734C

Novel interactions between the NF-κB and BMP signaling pathways in the *Drosophila melanogaster* embryo. Sophia Carrell, Gregory Reeves. Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.

Many diseases, most notably cancer, occur when cells misinterpret or ignore signals regarding proliferation, migration, and/or apoptosis, implying these signaling pathways must be tightly regulated. Since these same signals are highly conserved between species, we study the regulation of signaling pathways in the context of patterning the dorsal-ventral (DV) axis in the early *Drosophila melanogaster* embryo.

The transcription factor Dorsal, homologous to NF-κB, is responsible for expression of the genes that generate the DV pattern in the Drosophila embryo. Dorsal is present in the nuclei in a gradient, with the highest concentration at the ventral midline and a steady decay to about 40% of the embryo's circumference. This gradient is established on the ventral side of the embryo by signaling through the Toll receptor, which phosphorylates the inhibitor protein Cactus (homologous to I-κB), marking it for degradation. In the absence of Cactus, Dorsal is free to enter the nuclei and direct expression of target genes in a concentration-dependent manner. On the dorsal side of the embryo, the bone morphogenic protein (BMP) ligand Dpp is present in a graded fashion, establishing DV gene patterns beyond the spatial range of the Dorsal nuclear gradient.

Because the Dorsal gradient is essential to correct patterning of the early embryo, there likely exist multiple sets of regulatory loops to ensure proper development in the face of perturbed conditions. Preliminary evidence indicates the existence of such feedback loops through the BMP signaling network. We have found that there is interplay between the gradient of Dorsal, expressed highly on the ventral side of the embryo, and that of BMP signaling, expressed in an opposing gradient with its peak at the dorsal side of the embryo. We believe that the interactions between these two gradients are essential for proper gene positioning along the DV axis of the developing *Drosophila* embryo.

735A

Lack of Functional Conservation in Early Axial Patterning of the Drosophila Embryo. Jackie F. Gavin-Smyth[1], Daniel R. Matute[2], Martin Krietman[1], John Reinitz[1]. 1) Ecology and Evolution, University of Chicago, Chicago, IL; 2) Dept. of Human Genetics, University of Chicago, Chicago, IL.

A long held tenet of developmental biology is that the early, most critical patterning genes and networks are also the most functional constrained and conserved throughout evolution. Recent work (Matute et al. 2010), however, has shown that within a relatively short evolutionary time frame, a large number of genes are sufficiently diverged as to disallow viability when

hemizygous in a hybrid embryo between two species. This hemizygous inviability in the hybrid context implies a lack of functional compensation between the gene networks of the different species' genomes. Specifically, many of the early embryonic patterning genes are not able to functionally compensate in hybrid embryos derived from *Drosophila melanogaster* and *Drosophila santomea*, a species whose last common ancestor with *D. melanogaster*was less than 13 million years ago. Here we present evidence that the early, critical axial patterning networks of the *Drosophila* embryo, including the gap gene *giant* (*gt*), have diverged rapidly on both a sequence and functional level. This divergence leads to novel, antimorphic patterning defects when juxtaposed in the hybrid context. Quantitative imaging analysis and genetic experiments confirm the loss of *gt*expression and subsequent phenotypic defects which result in the failure to develop a viable hybrid.

736B

Essential roles for stat92E in patterning the proximodistal axis of the Drosophila wing imaginal disc. Victor Hatini, Ela Kula, David Nusinow, Steven DelSignore. Dept Anatomy & Cellular Biol, Tufts Univ, Boston, MA.

The Drosophila wing imaginal disc is subdivided along the proximodistal axis into the distal pouch, the hinge, the surrounding pleura, and the notum. The mechanisms that subdivide the wing proximodistal axis into smaller domains and regulate their scope and limits are incompletely understood. Here we investigated the role of the stat92E signal transducer and activator of transcription in wing proximodistal patterning. We mapped Stat92E activity from early stages and employed genetic loss- and gain-of-function analysis to investigate the role of stat92E in wing proximodistal axis patterning. We find that Stat92E is active ubiquitously in early wing imaginal discs where it acts to inhibit the induction of ectopic wing fields. As development proceeds, Stat92E activity is downregulated in the notum and distal pouch. This downregulation coincides with and contributes to the expansion and patterning of these structures. At late stages, Stat92E activity is progressively restricted to the lateral border of the notum, the pleura and hinge. During this period, stat92E specifies dorsal pleura identity and inhibits notum identity. Additionally, stat92E contributes to the expansion of gene expression domains along the hinge proximodistal axis. Overall, we find important roles for stat92E in wing proximodistal patterning and in controlling the scope and limits of gene expression domains along the wing proximodistal axis.

737C

Reduced cell number in the hindgut epithelium disrupts hindgut left-right asymmetry in a mutant of *pebble*, encoding a RhoGEF, in *Drosophila* embryos. Mitsutoshi Nakamura[1,2], Kenjiroo Matsumoto[1,2], Yuta Iwamoto[1,2], Ryo Hatori[1,2], Kenji Matsuno[1]. 1) Dept, Biol Sci, Osaka univ, Toyonaka, Japan; 2) Dept, Bio/Tech, Tokyo Univ of Sci, Noda, Japan.

Animals often show left-right (LR) asymmetry in their body structures. In some vertebrates, the mechanisms underlying LR symmetry breaking and the subsequent signals responsible for LR asymmetric development are well understood. However, in invertebrates, the molecular bases of these processes are largely unknown. Therefore, we have been studying the genetic pathway of LR asymmetric development in Drosophila. The embryonic gut is the first organ that shows directional LR asymmetry during Drosophila development. We performed a genetic screen to identify mutations affecting LR asymmetric development of the embryonic gut. From this screen, we isolated pebble (pbl), which encodes a homolog of a mammalian RhoGEF, Ect2. The laterality of the hindgut was randomized in embryos homozygous for a null mutant of pbl. Pbl is a multi-functional protein required for cytokinesis and the epithelial-to-mesenchymal transition in Drosophila. Consistent with Pbl's role in cytokinesis, we found reduced numbers of cells in the hindgut epithelium in pbl homozygous embryos. The specific expression of pbl in the hindgut epithelium, but not in other tissues, rescued the LR defects and reduced cell number in embryonic pbl homozygotes. Embryos homozygous for string (stg), a mutant that reduces cell number through a different mechanism, also showed LR defects of the hindgut. However, the reduction in cell number in the pbl mutants was not accompanied by defects in the specification of hindgut epithelial tissues or their integrity. Based on these results, we speculate that the reduction in cell number may be one reason for the LR asymmetry defect of the pbl hindgut, although we cannot exclude contributions from other functions of Pbl, including regulation of the actin cytoskeleton through its RhoGEF activity.

738A

Dorso-ventral patterning of the embryonic epidermis. Francois Payre[1,2], Ahmad Alsawadi[1,2], Robin Vuilleumier[3], Philippe Valenti[1,2], Jennifer Zanet[1,2], Giorgos Pyrowolakis[3], Serge Plaza[1,2]. 1) Developmental Biology, University of Toulouse, Toulouse, France; 2) CNRS, UMR5547, Toulouse, France; 3) Institute for Biology I, University of Freiburg, Freiburg, Germany.

BMP signalling is broadly conserved across bilateral animals in setting up the Dorso-Ventral (DV) axis and patterning of the neuroectoderm. Although many works have deciphered the network of factors underpinning BMP signalling, how these DV cues influence programs of terminal differentiation remains not fully understood. The fly embryonic epidermis ultimately produces cuticle extensions called trichomes, which display various organization and morphologies along the body plan therefore providing an exquisite readout of terminal differentiation. Combining large-scale genetic and molecular screenings, we identified transcription factors and cis-regulatory elements that implement the DV patterning of epidermal trichomes in response to BMP (Dpp) signalling. We found that DV cues modify transcriptional outputs at different levels of the developmental program of trichome differentiation, from upstream regulatory factors to terminal effectors. These results further reveal an unexpected prevalence of seemingly redundant regulatory interactions, showing that both enhancers and transcription factors of similar activities are used during terminal differentiation to achieve a robust phenotype.

739B

The maternal-effect phenotype of the *delorean* mutation in *Drosophila melanogaster*. Georgette Sass, Sarah VanOeveren. Biology, Grand Valley State University, Allendale, MI.

We have previously described the *delorean* mutation in *Drosophila melanogaster*; a recessive, gain-of-function allele of the *protein kinase N* (*pkn*) gene (Sass, VanOeveren, Burke, and Ostrow 2012 A. Dros. Res. Conference 53; 572B). The PKN protein is required for the process of dorsal closure during embryogenesis and has been identified as an effector of Rho1, a member of the Ras GTPase superfamily (Betson and Settleman 2007 Genetics). To further investigate the role of PKN in embryogenesis, we have characterized embryonic phenotypes associated with the *delorean* mutation. Females that are homozygous for the pkn^{dln} allele were found to exhibit a maternal-effect phenotype. Embryos derived from these mothers show moderate to severe head defects as well as alterations in denticle patterning. To expand upon these results, we have generated germ-line clones of the pkn^{dln} allele and find that the embryos produced have similar embryonic defects. Furthermore, the head and segmental defects that we see are analogous to those seen in other systems such as in germline clones of a loss-of-function allele of *nemo* (Mirkovic et al, 2002 Mechanisms of Development). The cause of such defects is postulated to be due to disruption of programmed cell death and we are specifically interested in how apoptosis via the JNK pathway may be perturbed in *delorean* mutants. For this reason, we will present our analysis of genetic interactions between the pkn^{dln} allele and mutants of the JNK signaling pathway.

740C

Taranis regulates posterior identity during imaginal disc regeneration. Keaton J Schuster, Andrea Skinner, Rachel K Smith-Bolton. Cell & Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL.

During regeneration, proper patterning must be re-established in order to generate a functional organ or appendage. How a regenerating structure repatterns is still an open question. We address this problem using a genetic ablation system in wing imaginal discs of *Drosophila melanogaster*. Our initial characterization of patterning during regeneration showed dramatic changes in gene expression, signaling and patterning. Furthermore, we observed pattering intermediates not seen during normal development. Therefore, we hypothesized that regeneration-specific mechanisms help pattern the regrown tissue after damage. To discover novel factors that regulate cell fate and patterning during regeneration, we are performing a dominant modifier screen using isogenic deficiencies. We identified a deficiency that, when heterozygous, resulted in regenerated wings with posterior to anterior fate transformations, but did not affect normal development. In these mutant regenerating wing discs, expression of the posterior selector gene *engrailed* was initially elevated before decreasing, allowing cells to adopt anterior fates. Alleles of *taranis* (*tara*), which encodes a putative Trithorax Group protein and a member of the TRIP-Br family, recapitulated this regeneration-specific transformation phenotype. We will present evidence that Tara regulates the expression of *engrailed* during regeneration to ensure maintenance of posterior cell fate. Thus we have both demonstrated that regeneration-specific mechanisms are key for regulating cell fate during regrowth, and identified and characterized one such mechanism.

741A

***Drosophila microRNA-9a* modulates the process of muscle attachment assembly via downregulation of Dystroglycan.** Andriy S. Yatsenko, Halyna R. Shcherbata. MPRG Gene Expression and Signaling, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.

Coordinated transcription factor networks are prominent regulators of cell fate during embryonic development and adult life. Now it is becoming evident that in conjunction with transcription factors at least three epigenetic elements (chromatin structure, DNA methylation, and microRNAs) help to form a reciprocal regulatory circuit to maintain cell identity and differentiation. miRNAs, based on their paradoxical properties, e.g., being highly evolutionarily conserved but not essential, have been proposed to play a role in generating biological robustness as canalization factors to buffer gene expression against perturbation or variability. We identified new roles for the ECM receptor Dystroglycan and miR-9a in establishment of muscle-tendon connections. Dystroglycan is specifically enriched at the termini of the growing muscles facing the tendon matrix and absent from tendons. This differential localization is crucial for proper muscle-tendon attachments and is adjusted by miR-9a. Interestingly, various critical genes required for muscle development are putative miR-9a targets and miR-9a is expressed in non-mesodermal cells. Since exogenous expression of miR-9a in mesoderm completely abolishes muscle formation, we hypothesize that miR-9a acts as a guardian to prevent noisy muscle gene expression in the epidermal tendon precursor cells that can be challenged upon stress or transcriptional noise.

742B

Homeodomain interacting protein kinase promotes normal and ectopic eye development through the repression of *pax6* paralogs *twin of eyeless* and *eyeless*. Jessica A Blaquiere[1], Wendy Lee[1,2], Esther M Verheyen[1]. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) Dermatology and Cell Biology, NYU Langone Medical Center and School of Medicine, New York University, New York, NY, USA.

The retinal determination gene network (RDGN) encompasses a group of nuclear factors responsible for mediating eye development. Pax6 paralogs Twin of eyeless (Toy) and Eyeless (Ey) sit atop this network. A striking feature common to most RD factors is their ability to coax non-retinal tissue to adopt retinal fate upon mis-expression. We further investigate the mechanism of ectopic eye development, specifically in the leg by using *dpp-GAL4, UAS-toy*. We utilized *toy* in our assay due to our interest in examining the Ey domain within the presumptive ectopic eye field; although in an exogenous tissue, Ey is under

control of its endogenous promoter. Interestingly, we found much like the normal eye, Ey must be restricted away from developing photoreceptors (PRs) for them to be specified. This observation led us to identify a morphogenetic furrow within the ectopic eye field and to the conclusion that ectopic eye development is progressive in nature exactly like the normal eye. In addition, we provide further evidence that Ey is a repressor of PR differentiation and highlight the importance of Ey repression for normal and ectopic eye development to occur. Using genetic analyses we have identified Homeodomain interacting protein kinase (Hipk), a serine-threonine kinase, as a transcriptional repressor of both *toy* and *ey*. We have previously shown that Hipk promotes Notch (N) mediated growth of the eye disc by repressing the global co-repressor Groucho and here, we provide evidence that Hipk's involvement with *toy*/*ey* is separate from its role in the N pathway. Both loss-of-function and over-expression experiments reveal that Hipk represses *toy* and *ey* in the eye-antennal disc and this relationship is in fact conserved in the ectopic eye. Collectively, our data suggests Hipk promotes normal and ectopic eye development by repressing*toy*/*ey*.

743C

Blimp-1 Participates in Patterning during Pupal Eye Development. Carrie L Jenkins[1], Gerald B Call[2]. 1) Biomedical Sciences, Midwestern University, Glendale, AZ; 2) Dept. of Pharmacology, Midwestern University, Glendale, AZ.

B lymphocyte-induced maturation protein (Blimp-1) was identified in *Drosophila melanogaster* nearly 7 years ago, but its function is still largely unknown. Mosaic eye tissue (generated from the *ey-Flp/FRT* system) reveals a unique raised glossy lens surface in mutant tissue in adults and significant interommatidial bristle patterning defects in both adults and pupae. Staining larval eyes with various developmental markers indicates normal development up until this stage. However, data from staining pupal eye discs reveals that the *Blimp-1* mutation leads to nonautonomous ommatidial patterning defects including loss and mispatterning of bristles (64% vs. 2% in control eye discs) and secondary pigment cells (17% vs. 0%), but not in tertiary pigment cells. Cone cells are still being analyzed. Current analysis of cell adhesion molecules essential to ommatidial patterning in *Blimp-1* mosaic eye tissue is underway to determine mechanisms behind this mispatterning. To further investigate the adult raised glossy eye phenotype the interaction between Blimp-1 and Crystallin, the main lens protein, is being studied through the use of *Cry-lacZ* reporters. When raised at 25°C, normal *Cry-lacZ* expression begins in primary pigment cells (PPC) sporadically distributed throughout the eye disc at about 35-40 hours after puparium formation (APF), and slowly ramps up into full production in all PPCs and cone cells (CC) by 45-50 APF, and then ceases by 65 APF. In striking contrast, by 35 APF at 25°C, all photoreceptors (R) strongly express *Cry-lac*Z, and continue to do so through 50 APF in mosaic *Blimp*-1 eye discs in a non-autonomous manner. At 35APF, all PPCs and some CCs in *Blimp-1* mosaic eye discs show premature *Cry-lacZ* expression in a non-autonomous pattern. However, at 50-55 APF, PPCs appear to maintain a higher expression level of *Cry-lacZ* in the *Blimp-1* mutant cells. This autonomous misexpression pattern of *Cry-lacZ* might explain the raised lens phenotype observed in the adult mosaic eyes. These findings suggest that Blimp-1 regulates multiple pathways involved in late eye development.

744A

Midline Functions within the Notch-Delta Signaling Pathway Regulating Interommatidial Bristle Complex Formation within the Developing Eye of *Drosophila* . Sandra M. Leal, Sudeshna Das. Dept Biological Sci, Univ Southern Mississippi, Hattiesburg, MS.

The *Drosophila midline* (*mid*) gene encodes a highly conserved invertebrate ortholog of the mammalian *Tbx20* transcription factor gene and is essential for proper development of the embryonic central nervous system (CNS). At present, the exact mechanisms by which *mid* regulates CNS development are not completely understood. Thus, to resolve *mid* function, we have been using the *Drosophila* eye as a practical model system to efficiently undertake a genetic modifier screen and RNAi methodology to identify *mid*-interacting genes for subsequent translational studies in CNS tissues. One *mid*-interacting gene we identified from the screen, *extramacrochaetae* (*emc*), functions within the Notch-Delta signaling pathway specifying the fates of sensory organ precursor cells (SOPs). We carried out genetic epistasis studies to confirm that *mid* functions within the Notch-Delta signaling pathway critical for specifying SOPs. SOPs give rise to mechanosensory bristle complexes that are each composed of a shaft, socket, neuron and sheath cell. Based upon the results of these studies, we propose a model suggesting that *mid* regulates the specification of pI neuroblasts that differentiate into SOPs. Since *emc* and *mid*are widely expressed in the embryonic CNS, we are now examining whether they interact within unique subsets of neurons to specify their fates.

745B

***defective proventriculus (dve)*, a new member of DV patterning in the eye.** Oorvashi Roy G. Puli[1], Takeshi Yorimitsu[3], Hideki Nakagoshi[3], Amit Singh[1,2,4]. 1) Department of Biology, University of Dayton, 300 College Park Drive, Dayton, OH; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton, OH; 3) School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan; 4) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

Axial patterning is crucial to eye development. During eye development, Dorso-ventral (DV) axis determination is the first lineage restriction event. The early eye primordium begins with the default ventral fate on which the dorsal eye fate is established by expression of a GATA-1 transcription factor, *pannier (pnr)*. There is a need to identify new components to understand the mechanism of DV patterning. We have identified *defective proventriculus (dve)* as a new dorsal eye gene. Loss-of-function (LOF) of *dve* in the eye results in dorsal eye enlargements, ectopic eyes, antennal duplications and loss of ocelli. Gain-of-function (GOF) of *dve* suppresses the eye fate by regulating the RD genes. *dve* misexpression does not affect Ey but

downregulates the downstream targets *eyesabsent(eya)*, *sine oculis (so)* and*dachshund (dac)*, suggesting that *dve* acts downstream of *ey* and is involved in blocking retinal differentiation to promote the dorsal head fate. Using genetic epistasis we found that dve acts downstream of*pnr* and upstream of *wingless (wg)*. We found that *dve* is involved in regulating the Wg morphogen gradient in the eye. Here we present *dve* as a novel dorsal gene required in the dorsal eye during development.

746C

Segregation of Eye and Antenna Fates: Initiation and Maintenance. Y. Henry Sun[1,2], Cheng-Wei Wang[1,2], Hui-Yu Ku[1,2]. 1) Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan, Republic of China; 2) Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, Republic of China.

A general problem in development is how do adjacent primordia adopt different developmental fates and stably maintain their distinct fates. In Drosophila, the adult eye and antenna originate from the embryonic eye-antenna primordium. We used the G-TRACE lineage tracing method to show that these cells proliferate to form the larval eye-antenna disc. Classical mitotic clonal analysis and disc transplantation experiment suggested that the eye and antenna fates segregated before late second instar (L2). However we found, by twin-spot MARCM, that a significant proportion of clones induced at late L2 can still cross the eye and antenna disc boundary. In L1 eye-antenna disc, the nuclear factors eyeless (ey), twin of eyeless (toy), sine oculis (so), eye gone (eyg), homothorax (hth), and teashirt (tsh) are expressed uniformly. The expression became segregated at mid-L2 with the expression of Cut (Ct) in the antenna disc and the restriction of Ey in the eye disc. We have recently demonstrated that once segregated, the eye and antenna fates are stably maintained by the mutual repression between the eye pathway factors (Ey and So) and antenna pathway factors (Ct and Hth) (Wang and Sun, 2012, Development 139:3413-21). However, these cell-autonomous repressions can only create a salt-and-pepper pattern. A coordinated segregation of eye and antenna cells would require additional patterning mechanism. We found that the initial bias of the two fields comes from induction by an external signal, the Egfr ligand Spitz (Spi). Spi, acting through the Egfr signaling pathway, induced Ct expression in the presumptive antennal field. As Ct and Spi spreads progressively through the antennal field, Ey expression was driven out by Ct. This initial bias thus directed the progressive segregation of the two fields. Our results revealed the molecular mechanisms for the initiation and maintenance of eye and antenna fate segregation.

747A

Domain specific function of Cullin-4 to promote cell survival in the ventral eye compartment in *Drosophila* . Meghana Tare[1], Madhuri Kango-Singh[1,2,3], Amit Singh[1,2,3]. 1) Department of Biology, University of Dayton, 300 College Park Drive,Dayton, OH 45469; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton OH 45469; 3) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, 300 College Park Drive, Dayton OH 45469.

Axial patterning is required for transition of a monolayer organ primordium to a three-dimensional organ. In the *Drosophila* eye, the first lineage restriction event of generation of dorsal and ventral compartments is an outcome of domain specific expression and/or function of proteins during early larval development. We identified an ubiquitin ligase *cullin-4* (*cul-4*) as a new member of ventral eye gene hierarchy. Loss-of-function of *cul-4* results in the preferential loss of ventral eye cells due to Wg mediated induction of cell death. Wg, acts as a negative regulator of eye development, and is involved in induction of cell death through activation of Caspases as well as JNK signaling pathway. Blocking Wg signaling does not completely rescue *cul-4* mutant phenotype. We looked for other targets of Cul-4 in the eye. We found that a transcription factor dE2F1 (a regulator of G1-S transition during cell cycle), a reported target of *cul-4*, may also be responsible for loss of ventral eye phenotype of cul-4 mutant. It is known that optimum levels of E2F1 are required for growth. Increase/decrease of E2F1 from its optimum levels results in induction of cell death in the developing tissues. Here we present the mechanism by which Wg and dE2F1 are involved in cell survival function of *cul-4* in the ventral eye. Our studies will help to discern a novel mechanism by which cell cycle genetic circuitry participate in axial patterning in order to promote cell survival during organogenesis.

748B

Abams is a member of the neprilysin family of metallopeptidases that affects signaling pathways during *Drosophila melanogaster* eye development. Christine Woods, Landry Nfonsom, Jennifer Curtiss. New Mexico State University, Las Cruces, NM.

To better understand *Drosophila melanogaster* eye development, we used mRNAseq and microarray analyses to identify targets co-regulated by Eyeless (Ey) and Hedgehog (Hh), Decapentaplegic (Dpp) or Notch (N) signaling. From this work, we identified a novel gene: *abnormally blistered and misshapen eyes* (*abams*). The predicted protein encoded by *abams* is a member of the neprilysin family of metallopeptidases, although it lacks certain highly conserved catalytic residues, suggesting that it is not capable of peptidase activity. RNA interference of *abams* (using an $abams^{IR}$ construct) resulted in smaller and misshapen adult eyes. Expression of the eye proneural factor Atonal (Ato) initiates ahead of the furrow in $abams^{IR}$ eye discs. However, proneural enhancement of Ato in intermediate groups does not occur and very few R8 cells expressing Ato emerge from the furrow. Surprisingly, $abams^{IR}$ eye tissue lacking R8s is still able to develop photoreceptors. Peptidases of the neprilysin family are involved in cleaving and thereby regulating activity of signaling peptides. During *Drosophila* eye development, Hh and Dpp signaling are responsible for initial activation of *ato* ahead of the furrow, while N signaling is required for *ato* upregulation in the furrow. In addition, ectopic Egfr activity has been shown to lead to photoreceptor development in the absence of R8 cells. Accordingly, *abams* RNAi resulted in premature*hh* and *dpp* expression as well as

down-regulation of N signaling and up-regulation of Egfr signaling. Based on this data and the fact that Egfr is known to activate *hh* and *dpp* expression, we hypothesize that the Abams protein physically binds to signaling proteins in the N and/or Egfr pathway, thereby regulating *Drosophila* eye development. Current efforts involve using genetic, molecular and biochemical techniques to determine which specific component(s) of the N and/or Egfr pathway are being targeted by Abams.

749C

Retrograde trafficking of apical extracellular matrix protein regulates epithelial tube geometry. Bo Dong[1], Ken Kakihara[1,2], Tetsuhisa Otani[1], Housei Wada[1], Shigeo Hayashi[1,2]. 1) Riken CDB, Kobe, Japan; 2) Department of Biology, Kobe University Graduate School of Science.

Apical extracellular matrix filling the lumen controls the morphology and geometry of epithelial tubes, yet the regulation of luminal protein composition and its role in tube morphogenesis are not well understood. Here, we show that an endosomal-retrieval machinery consisting of Rab9, retromer, and actin nucleator WASH regulates selective recycling of the luminal protein Serpentine in the Drosophila trachea. Secreted Serpentine is endocytosed and sorted into late endosome. Vps35, WASH, and actin filaments differentially localize at the Rab9-enriched subdomains of the endosomal membrane, where Serpentine containing vesicles bud off. In Rab9, Vps35 and WASH mutants, Serpentine was secreted normally into the tracheal lumen, but the luminal quantities were depleted at later stages, resulting in excessively elongated tubes. In contrast, secretion of many luminal-proteins was unaffected, suggesting that retrograde trafficking of a specific class of luminal proteins is a pivotal rate-limiting mechanism for continuous tube-length regulation.

750A

Screening for regulatory sequences that pattern the Drosophila eggshell. Nicole Pope, Maira Farhat, Robert A. Marmion, Nir Yakoby. Biology, Rutgers University- Camden, Camden, NJ.

Organogenesis relies on extensive tissue patterning by regulating the expression of genes in a spatiotemporal manner. During oogenesis, the follicle cells, a monolayer of epithelial cells surrounding the developing oocyte, are patterned to drive the formation of the Drosophila eggshell; an organ that shelters the developing embryo. While follicle cells' patterning has been vastly documented, the regulatory domains that govern tissue patterning are mostly unknown. To find regulatory domains, we cross-listed the 81 genes known to be expressed during oogenesis with the large collection of the Rubin's Lab GMR lines, and we found 19 common genes. These genes are represented by 230 GMR lines. Of great advantage, all GMR lines are driving the expression of a GAL4 thus providing an opportunity to screen these lines by crossing them to a UAS-GFP. Approximately 25% of the tested GMR lines express GFP during oogenesis, and 25% of those lines recapitulate the partial or full endogenous patterns of their corresponding genes. We found that regulatory information is enriched in certain positions of the genes' locus. We also demonstrated the use of the GMR lines to disrupt eggshell morphologies. Our comprehensive screen identified multiple regulatory DNA fragments that governs eggshell patterning.

751B

Function of PCP effector proteins, In, Fy and Frtz, in regulating planar cell polarity. Ying Wang[1], Jie Yan[1], Paul Adler[1,2]. 1) Biology, University of Virginia, CHARLOTTESVILLE, VA; 2) Cell Biology,University of Virginia, CHARLOTTESVILLE, VA.

The *frizzled/PCP* pathway plays a fundamental role in *Drosophila melanogaster* to establish and regulate planar cell polarity. Inturned (In), Fuzzy (Fy) and Fritz (Frtz) are PCP effector proteins that accumulate on the proximal side of wing cells. They function downstream of the core protein, such as Frizzled (Fz), and Disheveled (Dsh), but upstream of Multiple-wing-hairs (Mwh). Our research focuses on how effector proteins interact with other pathway proteins, including upstream core proteins and the downstream protein Mwh, and how this network functions.

Our hypothesis is that In, Fy and Frtz work as a group by binding to each other, through protein-protein binding domains, to transfer the signals from the core proteins to Mwh, which regulates the cytoskeleton to insure hair initiation is restricted to the distal most part of the cell. We found that Fy and Frtz both bind to In, but not to each other. Our data argues that In, Fy and Frtz form a protein complex and function together in the *frizzled/PCP* pathway. We also found that Mwh interacts with In, but not with the upstream core protein Vang and Prickle. This suggests that Mwh is recruited to the proximal side of wing cells by the In protein. We have also found that under certain circumstances the over expression of a PPE protein can alter hair polarity and the asymmetric accumulation of upstream proteins such as Starry Night (Stan). The mechanisms by which the "downstream" components influence the localization of "upstream" proteins is under investigation.

752C

Systematic Identification of Ftz and Ftz-F1 Responsive Target Genes and Their Enhancers. Amanda Field[1], Ray Anderson[1], Jie Xang[1], Leslie Pick[1,2]. 1) Program in Molecular & Cell Biology, University of Maryland, College Park, MD; 2) Department of Entomology, University of Maryland, College Park, MD.

Fushi tarazu (Ftz) and its obligate cofactor Ftz-F1 cooperatively bind to DNA and co-regulate the transcription of genes responsible for segmentation in the early embryo. Ftz is a homeodomain protein which binds DNA promiscuously, whereas Ftz-F1 is an orphan nuclear receptor with a well characterized DNA binding sequence. To identify the range of targets regulated by Ftz/Ftz-F1, a microarray analysis was performed comparing blastoderm/gastrulation stage wild type and ftz-f1 mutant embryos. This resulted in a short list of candidate targets that were downregulated in the absence of Ftz-F1 protein.

The microarray data was combined with the blastoderm staged ChIP-chip data sets, generated by the BDTNP, showing where Ftz protein is bound in the genome. This combination produced a testable list of novel candidate Ftz/Ftz-F1 target enhancers near the genes of interest from the microarray. To test whether these regions correspond to Ftz/Ftz-F1-dependent enhancers, reporter genes were constructed in which these genomic regions are fused upstream of a basal promoter and E. coli lacZ. Reporter gene expression was analyzed in wild type, ftz and ftz-f1 mutant transgenic Drosophila. Once Ftz-responsive enhancer regions are well defined, this will be used to computationally extract the code for Ftz/Ftz-F1 DNA binding.

753A

Gap-gap cross-regulation in mid-embryo pattern formation: deterministic and stochastic modelling of *hunchback-Krüppel* interactions. David M. Holloway[1], Alexander V. Spirov[2,3]. 1) Dept Mathematics, British Col Inst Tech, Burnaby, BC, Canada; 2) Computer Science and CEWIT, Stony Brook University, NY, USA; 3) Sechenov Institute for Evolutionary Physiology and Biochemistry, St. Petersburg, Russia.

hunchback (*hb*) is an evolutionarily conserved gap gene involved in early specification of anterior-posterior (AP) position in the embryo. In previous work, we used a stochastic model of *hb* cis-regulation to characterize the relative contributions of maternal Bicoid (Bcd) activation and *hb* self-regulation to *hb* expression noise, suggesting selective influences for multiple cooperative Bcd binding and *hb*autofeedback. Here, we explore the role of gap-gap interactions in constraining expression noise at the mid-embryo, at the sharp Hb transition from high anterior expression. *Krüppel* (*Kr*) is a gap gene expressed just posterior to this boundary, which has long been known to interact with *hb*. We build on a recently published model incorporating experimental evidence for dual action of Hb on *Kr* expression - activating at low concentration, inhibiting at high concentration. We find that inhibitory feedback of Kr on *hb* can play a role in decreasing expression noise (e.g. between- and within-nucleus mRNA variability). We find that the observed loss of the mid-embryo (parasegment 4) *hb* stripe in *Kr*- embryos can be accounted for by a reciprocal dual action of Kr on *hb*. The model gives quantitative predictions for both the averaged expression patterns and the stochastic variability of these patterns.

754B

Describing the balance between cooperative binding and self-activation during pattern formation in *Drosophila melanogaster*. Francisco J P Lopes[1,2], Alexander V Spirov[3,4], Paulo M Bisch[1]. 1) Instituto de Biofisica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; 2) Polo de Xerem, Universidade Federal do Rio de Janeiro, Duque de Caxias, Brazil; 3) Laboratory of Evolutionary Modelling, the Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, Saint-Petersburg, Russia; 4) Computer Science Department and Center of Excellence in Wireless & Information Technology, State University of New York at Stony Brook, Stony Brook, USA.

In *Drosophila* embryonic development, the gap gene *hunchback* (*hb*) is regulated in response to maternal signals like the antero-posterior gradient of Bicoid protein. This developmental gene has a strong anterior expression and a sharp on-off boundary at mid-embryo. Two major factors determine *hb* expression pattern: the cooperative binding of Bcd to the *hb* regulatory region and *hb* self-activation. A debate about the role of these mechanisms in the patterning of the *hb* sharp border has been established. Some experimental data indicate that Bcd cooperative binding would be sufficient for *hb* sharp border although some data indicate that *hb* self-activation could perform a critical role. In order to contribute to this discussion, we determined the Hill coefficient (n_H) required for Bcd to generate the sharp border of Hb at different stages into cycle 14A [1]. We found that the n_H ranges from 4 to 6 during the first half of cycle 14A and from 6 to 9 during the second half of this cycle. This result indicates that Bcd cooperative binding cannot account for *hb* sharpness at late embryos because this high n_H is likely unachievable for Bcd binding to the *hb* promoter. To verify our results we estimated the n_H required to pattern the Hb profile of 15 embryos expressing an hb^{14F} allele that is defective in self-activation and found n_H to be 3.0. Our results indicate that there are two different stages during *hb* pattern formation: a Bcd-dependent stage at early stages of cycle 14A and an Hb self-dependent stage at late stages of this cycle. [1] Lopes FJP, et al (2012). Developmental Biology 370(2): 165-172.

755C

Signals from the pouch and notum restrict JAK/STAT signaling to the hinge to insure proper wing development. Erika Bach, Aidee Ayala-Camargo, Aloma Rodrigues, Marc Amoyel, Maria Sol Flaherty. Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY.

JAK/STAT pathway activity localizes to the hinge domain of the wing disc, but its function there has not been reported. Here we show that STAT activity is necessary and sufficient for hinge development through autonomous induction of hinge-specific factors. We find that mutual negative interactions between Iroquois-complex factors and Stat92E repress JAK/STAT pathway activity from the notum. The pouch factor Nubbin represses Unpaired, the JAK/STAT ligand, and STAT activity out of the pouch. These data suggest that JAK/STAT signaling in the pouch is deleterious to wing development. Indeed, mis-expression of Unpaired within the presumptive pouch causes small, stunted adult wings. Within the hinge, JAK/STAT pathway activity becomes restricted to the gap domain in cells that lack Nubbin and Teashirt. We report the autonomous lack of growth of gap domain cells lacking STAT function. Finally, JAK/STAT signaling does not perturb the Wingless inner or outer ring, indicating that JAK/STAT and Wingless pathways control hinge growth independently. We conclude that JAK/STAT signaling is critical for hinge fate specification and growth of the gap domain.

756A

BMP signaling requires an inwardly rectifying K+ channel to pattern Drosophila wing. Giri Raj Dahal, Brandon Gassaway, Ben Kwok, Emily Bates. Chemistry and Biochemistry, Birgham Young University, Provo, UT.

Mutations that disrupt the Inwardly rectifying K+ (Irk) channel Kir2.1 cause periodic paralysis, heart arrhythmias and dysmorphic features in humans. Morphological defects were also observed in mice and flies when a homologous channel is inhibited. Using Drosophila genetics we found the molecular mechanism that underlies the developmental phenotypes. We eliminated or reduced functional Irk2 channel in Drosophila wing and characterized the phenotypes, which are similar to Bone Morphogenetic Protein (BMP) signaling defects. We found that antagonizing Irk channels reduces BMP signaling and leads to wing defects. We found that Irk2 is necessary downstream of BMP transcription and upstream of phosphorylation of the BMP type 1 receptor in the signaling cascade. Our data demonstrate that developmental signaling cascades can sense membrane potential.

757B

Inverse Regulation of Target Genes at the Brink of the Dpp Morphogen Activity Gradient. Offer Gerlitz[1], Oren Ziv[1], Rutie Finkelstein[1], Yaron Suissa[1], Tama Dinur[1], Girish Deshpande[2]. 1) Developmental Biology and Cancer Research, IMRIC, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; 2) Department of Molecular Biology, Princeton University, Princeton, NJ 08540.

Dpp-dependent patterning in the wing imaginal disc of Drosophila melanogaster serves as a paradigm to understand how morphogen gradients specify various cell fates in a concentration-dependent manner. According to the current model, profile of the transcriptional response to the graded activity of Dpp, relies upon two opposing gradients of pMad and Brinker (Brk). However, this patterning model is inadequate to explain the expression of target genes, like vestigial and spalt, in lateral regions of the wing disc, where Dpp signal decline and Brk levels peak. We show that in contrast to the reciprocal repressor gradient mechanism, where Brk represses Dpp targets in medial regions, in lateral regions target expression is downregulated by Dpp signaling and activated by Brk. How is this inverse regulation achieved? By studying spalt expression in the different regions of the wing disc, we uncovered a novel circuitry where Brk induces expression of spalt at the periphery of the wing disc indirectly through repression of a negative regulator (NRS). On one hand, NRS represses spalt expression by binding to a cis-regulatory element that does not contain Brk binding sites. On the other, NRS is itself negatively regulated by Brk. Our findings constitute an important first step towards unraveling the workings of a morphogen gradient at the edges.

758C

The TSC1/2 complex controls Drosophila pigmentation through TORC1-dependent regulation of catecholamine biosynthesis. Fabrice Roegiers, Diana Zitserman. Inst Cancer Res, Fox Chase Cancer Ctr, Philadelphia, PA.

In Drosophila, the pattern of adult pigmentation is initiated during late pupal stages by the production of catecholamines DOPA and dopamine, which are converted to melanin. The pattern and degree of melanin deposition is controlled by the expression of genes such as ebony and yellow as well as by the enzymes involved in catecholamine biosynthesis. In this study, we show that the conserved TSC/TORC1 cell growth pathway controls catecholamine biosynthesis in Drosophila during pigmentation. We find that high levels of Rheb, an activator of the TORC1 complex, promote premature pigmentation in the mechanosensory bristles during pupal stages, and alter pigmentation in the cuticle of the adult fly. Disrupting either melanin synthesis by RNAi knockdown of melanogenic enzymes such as tyrosine hydroxylase (TH), or downregulating TORC1 activity by Raptor knockdown, suppresses the Rheb-dependent pigmentation phenotype in vivo. Increased Rheb activity drives pigmentation by increasing levels of TH in epidermal cells. Our findings indicate that control of pigmentation is linked to the cellular nutrient-sensing pathway by regulating levels of a critical enzyme in melanogenesis, providing further evidence that inappropriate activation of TORC1, a hallmark of the human tuberous sclerosis complex tumor syndrome disorder, can alter metabolic and differentiation pathways in unexpected ways.

759A

The role of dietary restriction in age-related muscle atrophy. Guiping Du, Jennika Krisa, Patrick Li, Subhash Katewa, Aric Rogers, Matthew Laye, Pankaj Kapahi. Buck Institute for Research on Aging, Novato, CA.

Age-related loss of muscle mass (sarcopenia), is one of the major problems compromising the health condition of the aged population. Therefore, uncovering the molecular mechanisms of age-related muscle atrophy and understanding the correlation between muscle activity and aging progression are of great importance. Dietary restriction (DR), which extends lifespan and slows down the progression of various age-associated diseases, enhances spontaneous activity in flies. After 60 days of different diets treatment, flies under DR show higher locomotor ability comparing to ad libitum (AL) diet treatment, indicating DR preserves muscle function. We detected dramatic transcriptional upregulation of myofibril genes upon DR in both young and old flies. To assess whether these mRNAs were also translated, we performed translation state array analysis (TSAA), and found increased binding of myosin, troponin, tropomyosin and paramyosin mRNAs to polysomes upon DR. Our data support the idea that DR increases myofibril protein expression which eventually increases muscle mass and slows down age-related sarcopenia progression. Then, we examined the effect of Insulin/IGF signaling, which has been shown to regulate muscle mass, on myofibril protein expression. Muscle-specific expression of dominate negative insulin receptor (InR) decreases DR-dependent myofibril protein expression, while expression of constitutive active InR increase myofibril protein level on DR. Muscle-specific knockdown of myosin or troponin compromised DR-dependent lifespan extension. Furthermore, expression of dominate negative InR in the muscle also shortens lifespan under DR, while dominate negative InR in the muscle

shows extended lifespan in AL flies. In summary, DR triggers Insulin/IGF signaling in the muscle to prevent the decline of muscle mass, which is required for DR-dependent lifespan extension.

760B

A metabolomics approach identifies β-Sitosterol to increases Longevity in adult *Drosophila melanogaster*. Matthew J. Laye, Kisha Barrett, Pankaj Kapahi. Buck Institute, Novato, CA.

The restriction of specific nutrients without causing malnutrition, dietary restriction (DR), is a robust nutritional intervention capable of increasing lifespan in organisms ranging from yeast to mammals. However, DR also leads to changes in specific metabolites that might be critical for the increased lifespan and associated phenotypes that accompany DR. Thus, we sought to determine whether feeding metabolites that increase when *Drosophila melanogaster* are fed a DR diet of 0.5% Yeast diet while fed a 5% Yeast nutrient rich diet (referred to as AL from here) was sufficient to increase lifespan. As expected DR decreased the concentration of amino acids and increased the concentration of AMP and nicotinic acid, demonstrating efficacy of the DR. Interestingly, β-sitosterol, a sterol metabolite with documented beneficial metabolic effects, increased in the DR condition relative to the AL. β-sitosterol increased lifespan of flies fed an AL diet, but not a DR. Furthermore, feeding β-sitosterol increased metabolism indicated by increased VO2 and VCO2, which was consistent with a decreased resistance to starvation on AL, but not DR diet. However, the increased general metabolism was not associated with increased levels of activity, AMPK phosphorylation, or mRNA of several genes important for fatty acid metabolism. In conclusion, the sterol β-sitosterol has beneficial effects on lifespan in flies fed a nutrient rich diet through an unknown mechanism.

761C

Is oxygen limitation a cue for the initiation of molting in Drosophila? Viviane Callier[1], Colin Brent[2], Jinkyu Kim[1], Shampa M. Ghosh[3], Alexander W. Shingleton[3], Jon F. Harrison[1]. 1) School of Life Sciences, Arizona State University, Tempe, AZ; 2) USDA-ARS Arid-Land Agricultural Research Center, Maricopa, AZ; 3) Department of Zoology, Michigan State University, East Lansing, MI.

Body size profoundly affects many aspects of animal biology, yet it remains one of the fundamental unsolved problems of developmental biology. Attainment of a critical weight causes Drosophila larvae to terminate growth and initiate metamorphosis, processes regulated by a hormonal cascade. Although the endocrinology of molting is well understood, the sensory mechanisms used by a larva to determine the timing of the hormonal cascade is largely unknown. The result is a conspicuous gap in our understanding of the mechanisms that regulate body size. A putative developmental pacer is oxygen availability. As larvae grow through an instar, oxygen supply structures are largely fixed, but metabolizing tissues increase in mass. As the ratio of demand to supply grows, internal hypoxia may occur, serving as a physiological cue to initiate a molt. To test for late-instar oxygen limitation, we measured respiration rates of Drosophila larvae across the third instar. Our data indicate that respiration rates level off at the critical weight, consistent with the hypothesis of oxygen-limitation. To test whether larvae experience internal hypoxia late in the instar, we used a GFP reporter to assess the activity of Hypoxia-Inducible Factor (HIF). We found that HIF activity is increased in late-instar larvae relative to early-instar larvae. To further test the role of internal hypoxia in pacing development, early instar larvae were reared in either hypoxic (10%) or normoxic conditions. Hypoxia induced earlier metamorphosis and a reduced critical weight. Oxygen deprivation also caused an elevation in ecdysteroid levels, the hormones driving the molting process. Collectively, these data support oxygen limitation as a cue used by Drosophila to regulate developmental pacing. The research was supported by NSF IOS 1122157 to JFH, and IOS 0845847 to AWS.

762A

Trans-interactions at Men in Drosophila melanogaster demonstrate environmental plasticity. Xinyang Bing[1], Teresa Rzezniczak[2], Thomas Merritt[1]. 1) Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario, Canada; 2) Institute of Biochemistry, Carleton University, Ottawa, ON, K1S 5B6, Canada.

We found that trans-regulation, a little understood but potentially wide-spread form of transcriptional regulation, is sensitive to changes in both large-scale genetic background and environmental conditions. Interestingly, these trans-interactions may be sensitive to background and environment even when the better understood cis-interactions are not. Further, these trans-interactions appear to be mediated through changes in binding transcription factors, possibly in a tissue specific manner. Together, our results support a dynamic view of the Drosophila genome, including complex chromosomal interactions on a genome-wide basis, in response to changes in the environment. Specifically, we changed the post-eclosion environment of a series of adult flies that exhibit varying levels of trans-interactions at Malic enzyme (Men) in different genetic backgrounds, and assayed MEN activity. We found that shifting the post-eclosion environment to higher or lower temperatures significantly reduced MEN activity overall. Post-eclosion temperature also significantly reduced the amount of trans-interactions observed, and the amount of interaction between each knockout allele and genetic background. However, cis-interactions were not significantly affected by post-eclosion temperature. Next, using qPCR, we found significant differences in Men expression across post-eclosion temperature, and significant correlations between Men expression and MEN activity with the expression of Abd-B and mirr. Lastly, we demonstrate that knockdown of Abd-B and mirr significantly affected both Men expression and MEN activity.

763B

The effect of altered mitochondrial function on larval development and adult lifespan. Rachel T. Cox, Aditya Sen. Dept Biochemistry and Molecular Bio, Uniformed Services University, Bethesda, MD.

Mitochondria are highly dynamic organelles that are responsible for making ATP. The structural and functional integrity of mitochondria are vital for proper cellular function. During development, different tissues at different stages vary in their need for mitochondrial output. In order to understand how mitochondrial requirements and dynamics change during development, we are characterizing the gene *clueless*(*clu*). Homologs for *clu* are found in all eukaryotes for which there is genomic sequence available. *clu* mutant adults are small, highly uncoordinated and have greatly reduced lifespans. Part of the coordination deficits are caused by defects in muscle mitochondria, however we have found Clu is highly expressed in larval neuroblasts and other regions of the dividing larval brain. Mitochondria in *clu* mutant neuroblasts are mislocalized during the cell cycle, but overall brain morphology appears to be normal. Mutations in two other genes important for mitochondrial function, *technical knockout* and *stress sensitive B*, do not mislocalize neuroblast mitochondria, suggesting this defect in mitochondrial dynamics is *clu* specific. Even though there are high levels of Clu expression in the brain, and very few *clu* mutant adults in any given culture, Clu does not appear to be required for overall larval development. In uncrowded conditions, larval development and pupation are only slightly delayed. However, only 40% of the flies are able to eclose, and *clu* mutant adults die after only three days. In addition, ATP levels are normal in *clu* mutant larvae and they do not suffer mitochondrial oxidative damage. In contrast, when the flies eclose, ATP levels plummet and mitochondria accumulate oxidative damage. These results support that Clu functions upstream of electron transport and oxidative phosphorylation and helps to suppress mitochondrial oxidative damage in the cell. In addition, these results support previous work indicating larvae do not use their mitochondria for oxidative phosphorylation, but rather rely on aerobic glycolysis for ATP (Tennessen et al, 2011).

764C

Drosophila melanogaster harbor the machinery to mediate an insulin-responsive sugar uptake response. Georgeta Crivat[1*], Vladimir Lizunov[2], Caroline Li[1], Karin Stenkula[3], Joshua Ziammerberg[2], Samuel Cushman[2], Leslie Pick[1]. 1) Entomology, University of Maryland, College Park, MD; 2) Laboratory of Cellular and Molecular Biophysics, Program on Physical Biology, Eunice Kennedy Schriver National Institute of Child Health and Human Development, and the Experimental Diabetes, Metabolism, and Nutrition Section, Diabetes, Endocrinology, and; 3) Department of Experimental Medical Science, Lund University BMC F10, SE-221 84 Lund, Sweden.

Although insulin-signaling pathways are shared between mammals and insects, the extent to which this reflects conserved physiology is unclear. The Drosophila insulin receptor is structurally similar to the mammalian insulin receptor and was shown to auto-phosphorylate in response to mammalian insulin in cell culture. Here, we asked whether flies harbor the machinery to mediate an insulin-responsive sugar uptake response similar to mammals, by generating transgenic Drosophila expressing the human insulin-responsive sugar transporter GLUT4. We generated transgenic flies that carry UAS-HA-GLUT4-GFP: an HA tag inserted in the first exofacial loop of GLUT4 to monitor translocation of GLUT4 at the cell surface, and GFP at the C-terminus to monitor expression and trafficking. The GAL4/UAS system was used to express HA-GLUT4-GFP in the fat body. We used TIRF microscopy to examine the subcellular localization and trafficking of HA-GLUT4-GFP in living Drosophila fat body cells. We found that fat from transgenic animals expressing human GLUT4 responded to mammalian insulin with an increased rate of GLUT4 trafficking and translocation to the plasma membrane. This is, to our knowledge, the first study utilizing TIRFM to study insulin-mediated responses in the living cells of an insect and the first to show that insects harbor the machinery to mount a hormone-mediated sugar uptake response. Future studies will identify the endogenous Drosophila sugar transporter(s) that mediate this response.

765A

The TGF-β/Activin ligand *daw* regulates sugar and pH homeostasis in *Drosophila melanogaster*. Arpan Ghosh, Michael O'Connor. Gen Cell & Development, Univ Minnesota Twin Cities, Minneapolis, MN.

TGF-β/Activin ligands regulate a plethora of biological processes in all multicellular organisms and consequently have emerged as potential therapeutic solutions for multiple conditions. Our study aims at understanding the role of TGF-β signaling in regulating metabolism by studying the metabolic consequences of manipulating TGF-β signaling in *Drosophila melanogaster*. We show that *Drosophila* TGF-β/Activin ligand *dawdle* (*daw*) dose-dependently regulates sugar and pH homeostasis in the fly larvae. *daw* nulls show significantly higher circulating sugar concentration, whereas over-expression of *daw*leads to a significant drop in hemolymph sugar. This diabetic phenotype of *daw* mutants is caused by a block in the release of Insulin from the Insulin producing cells since *dIlp* mRNA expression remains unchanged, but DIlp peptides accumulate in the IPCs. These mutants also show higher total triacylglycerol, glycogen and glucose, all hallmarks of insulin resistance in *Drosophila* larvae. Additionally, high-throughput GC/MS metabolomic analysis showed significant increase in multiple sugar metabolism intermediates in *daw* mutants, indicating a potential involvement of *daw* in regulating peripheral sugar metabolism. *daw* null mutants also show a significant drop in hemolymph pH that can be rescued by ectopic expression of *daw*. Challenging the *daw* mutants with high sugar and/or low pH food conditions lead to a severe drop in larval viability highlighting potential physiological significance of altered sugar and pH homeostasis in these mutants. While acidification (pH ~4.3) of food by any acid increased *daw*lethality, Propionic acid (PA) in particular most severely affected larval viability. The mechanism by which PA causes this acidity-independent effect may involve the role of PA as a metabolite or signaling molecule. Collectively, our study shows widespread involvement of TGF-β signaling in metabolism and homeostasis

in *Drosophila* and opens up the possibility of using this model organism to develop mechanistic paradigms for how TGF-β signaling might regulate metabolism.

766B

The Control of Lipid Metabolism by mRNA Splicing in *Drosophila*. Robert Gingras[1], Bijal Kakrecha[3], Nicole Chichearo[3], Spencer Ng[2], Justin DiAngelo[1], Alexis Nagengast[2]. 1) Dept Biol, Hofstra U, NY; 2) Dept Biochem, Widener U, PA; 3) Dept Biol, Widener U, PA.

The *Drosophila* fat body responds to different nutrient conditions and controls overall energy metabolism by regulating long-term storage of triglycerides in structures called lipid droplets (LDs), thereby serving a function similar to mammalian liver and adipose tissue. Recent genome-wide RNAi screens in *Drosophila* cells identified mRNA splicing factors as playing a role in LD formation; their decreased expression results in fewer LDs. Using RNAi under GAL4-UAS control in the fat body of larvae, we have identified several splicing factors that control lipid storage *in vivo*. Larvae raised on both high and low nutrient food demonstrate a visibly lean phenotype with decreased fat body expression of U1-70K, U2AF-50 or U2AF-38 and prp19 and this lean phenotype corresponds to a decrease in triglyceride levels as measured by quantitative assays. Interestingly, knockdown of the SR protein 9G8 in the larval fat body is male lethal and leads to increased triglycerides on low nutrient food, and decreased triglycerides on high nutrient food. To further probe the role of 9G8 in controlling lipid storage, LD morphology was assessed by staining 9G8 RNAi fat bodies with the lipid stain BODIPY. Fat body-specific knockdown of 9G8 results in more LDs of a medium size compared to control fat bodies that contain a wide range of LD sizes. Previous *in vitro* studies have implicated 9G8 in the control of doublesex (DSX) splicing by binding to transformer (TRA) and transformer2 (TRA2) to regulate sex determination. To determine whether DSX, TRA or TRA2 play a role in lipid accumulation, LD staining was performed on fat bodies lacking these genes. While knockdown of TRA or TRA2 had little effect on LD morphology, decreasing fat body DSX leads to an increase in LD size. Together, these results suggest a link between mRNA splicing, sex determination and lipid metabolism and may provide insight into the mechanisms underlying tissue-specific splicing and nutrient storage in the fat body.

767C

Lost in Translation: mitochondrial and nuclear incompatibility results in reduced longevity and increased oxidative stress resistance in *Drosophila*. Marissa A. Holmbeck, David M. Rand. Bio-Med, Brown University, Providence, RI.

Communication between the mitochondrial and nuclear genomes is vital for cellular function and influences aging. Coordinated expression of both genomes is required for the function of jointly encoded respiratory enzymes that produce the majority of energy for the cell. We have developed a model in which mitochondrial and nuclear genomes can be jointly manipulated in *Drosophila*. mtDNA from different strains of *Drosophila simulans (Dsim)* and *D. melanogaster (Dmel)* have been introduced into controlled *Dmel* nuclear backgrounds. We previously found that a specific *Dsim* mtDNA, $simw^{501}$, shows a strong epistatic interaction with the *OreR* nuclear background resulting in a suite of compromised phenotypes including developmental delay and reduced mitochondrial function. Genetic mapping studies have identified a mutation in the nuclear encoded mitochondrial tyrosyl-tRNA synthetase, and a mutation in the mtDNA encoded tyrosine-tRNA as the source of this epistatic interaction (C.D. Meiklejohn et al. manuscript in review). Our working model is that this mito-nuclear interaction compromises translation within mitochondria. We have examined the effects of this epistasis on mortality: the $simw^{501}$*; OreR*(mito; nuclear) genotype reduces lifespan, but does not have elevated reactive oxygen species (ROS) levels. Interestingly, $simw^{501}$*; OreR* displays increased resistance to paraquat treatment. These phenotypes are not seen when the $simw^{501}$ mtDNA is placed on an *Aut* nuclear background. To confirm the source of this epistasis, a transgenic approach was used to generate rescue strains with alternative*OreR* and *Aut* nuclear alleles of the identified tyrosyl-tRNA synthetase inserted into the genome at the same location. The strain containing the transgenic *OreR* allele also displays reduced lifespan when paired with the $simw^{501}$ mtDNA in a matched genetic background, supporting the model of epistasis. This study provides insight into the joint genetic architecture that regulates mitochondrial function and aging and suggests that mitochondrial defects can reduce longevity without increasing ROS.

768A

Regulation of fatty acid metabolism by the nuclear receptor DHR78. Stefanie M. Marxreiter, Carl S. Thummel. Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

DHR78 encodes the single fly ortholog of the mammalian orphan nuclear receptors TR2 and TR4. Previous studies of *DHR78* mutants showed a severe developmental defect where larvae fail to molt their trachea correctly and display roving behavior, reduced growth, and eventually die as third instars. A recent paper described a similar phenotype in animals lacking acetyl-CoA-carboxylase (ACC), which acts as the rate-limiting step in fatty acid synthesis (Parvy et al. (2012) *PLoS Genetics* 8: e1002925). Consistent with this observation, Northern blot analysis revealed that *ACC* expression is significantly reduced in *DHR78* mutants. Moreover, recent studies of *TR4* mutant mice indicate that it regulates a metabolic transcriptional program, including many genes involved in lipid metabolism. These animals are also protected against obesity when fed a high-fat diet. Antibody staining revealed that DHR78 is not only expressed in the trachea, but is also expressed in key metabolic tissues such as the midgut and fat body. Triacylglycerol (TAG) and glycogen assays combined with Oil Red O staining revealed significant reductions in stored energy in *DHR78* mutant larvae. Current efforts are focused on using tissue-specific genetic rescue experiments, metabolomics, and RNA-seq to define the role of DHR78 in metabolism and growth.

769B

A recessive X-linked mutation causing a 3-fold reduction in total body zinc content is widespread within *Drosophila melanogaster* laboratory strains. Fanis Missirlis[1], Negar Afshar[2], Bilge Argunhan[2], Lucia Bettedi[2], Joanna Szular[2]. 1) Departamento de Fisiología, Biofísica y Neurociencias, CENTRO DE INVESTIGACIÓN Y DE ESTUDIOS AVANZADOS (CINVESTAV), Mexico City, D.F., Mexico; 2) School of Biological and Chemical Sciences Queen Mary, University of London Mile End Road, London, United Kingdom E1 4NS.

A 3-fold increase in zinc accumulation was previously reported in *fumble[1]* heterozygous mutants and a deficiency strain uncovering the *fumble* locus (Gutiérrez et al., FEBS Letters, 584:2942). However, dietary interventions that changed systemic zinc content and genetic elimination of *Metal Transcription Factor 1* had minimal impact on the *fumble* phenotype. Here we show that the *fumble* gene does not contribute to the substantial change in total zinc content between the aforementioned genotypes and multiple fly strains used as controls. Indeed, outcrossed *fumble[1]* heterozygous mutant flies with low zinc content were recovered. We excluded a purely maternal transmission of this trait and confirmed that the condition of low zinc is recessive and segregates with the X-chromosome. As several other *Drosophila*species tested have total body zinc concentrations in the range of 200 mg per g dry weight (Sadraie and Missirlis, Biometals, 24:679) we have concluded that the trait of low (approximately 70 mg per g dry weight) concentration of zinc is widely present in laboratory strains. We postulate this trait can be attributed to a single recessive mutation on the X-chromosome.

770C

Transgenerational Inheritance of Metabolic State in *Drosophila*. Rebecca A. Somer, Carl S. Thummel. Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

Poor nutrition has been implicated as a key causal factor in the development of metabolic syndrome. Recent data, however, has suggested that poor nutrition and the resulting altered metabolic state in the parental generation can also have a dramatic impact on the health of subsequent generations. Several human studies have shown that nutrient deprivation, gestational diabetes, and obesity have an effect on the metabolic state of children at both adolescence and adulthood. In addition, studies in rodents have shown that the adult progeny of mothers subjected to nutrient depletion display hallmarks of obesity and diabetes. Similar results are seen in the progeny of male mice fed a low protein diet, along with changes in the expression of genes involved in lipid metabolism (Carone et al 2010 Cell). These results suggest that the inheritance of a metabolic program is more than a gestational effect. At best, however, these studies are correlative, and the mechanism of the inheritance of metabolic state is still unknown. To determine if parental metabolism influences progeny metabolism in *Drosophila*, we have used a combination of dietary and genetic methods to alter the parental metabolic state while maintaining a constant environment for subsequent generations. Our preliminary studies reveal changes in the metabolite levels of adult progeny when the parental generation is subjected to a dietary or genetic metabolic insult as compared to the progeny of control parents. We propose that these changes indicate that the progeny are inheriting an altered metabolic program in order to adapt to a new nutritional environment. This data provide a foundation to characterize the genetic and molecular mechanisms underlying the transgenerational inheritance of metabolic state in an easily manipulable genetic system.

771A

The role of FoxO in integrating insulin and ecdysone signaling during body size regulation. Takashi Koyama, Christen Mirth. Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Body size regulation, a conspicuous and fundamental process in any organism, is best understood in insects. In the fruit fly Drosophila melanogaster, larvae regulate their final body size by controlling both growth rate, through insulin/insulin-like growth factor signaling (IIS) and target of rapamycin signaling, and regulating the duration of the growth period, via the steroid hormone ecdysone. Recent studies have shown that these two processes interact; IIS stimulates ecdysone synthesis from the prothoracic gland by up-regulating ecdysone biosynthesis genes. How this occurs is still unclear. In mammalian and nematode cells, the IIS modulates steroid hormone signaling through the interaction between a downstream component of the IIS, forkhead box type O (FoxO), and nuclear hormone receptor superfamily members. Given this, we have found that a heterodimeric partner of Ecdysone Receptor, Ultraspiracle (Usp), binds to FoxO. Furthermore, we found that overexpression of FoxO RNAi in the prothoracic gland (PG) induces premature patterning of the imaginal discs in early third instar larvae fed on sucrose alone, suggesting ecdysone synthesis occurs prematurely. This FoxO/Usp association occurs independently of 20-hydroxyecdysone, suggesting that the complex may potentially regulate ecdysone signaling in a nutrient-dependent, but ecdysone-independent manner. To explore the function of FoxO/Usp complex, we have created two different types of transgenes that alter the state of FoxO/Usp complexes without disrupting independent transcriptional activity of FoxO. We will discuss about the function of the FoxO/Usp complex on growth period determination.

772B

***Mio* acts in the brain to control feeding and metabolism in *Drosophila*.** Joseph Manno, Jacqueline McDermott, Justin DiAngelo. Department of Biology, Hofstra Univ, Hempstead, NY.

After a meal, multiple organs recognize the availability of nutrients and increase the intake of these nutrients and their storage as glycogen and fat. For example, populations of neurons in the brain sense changes in nutrient levels leading to alterations in an animal's feeding behavior, amount of food consumed and energy expenditure. However, the molecular

mechanisms underlying nutrient sensing and subsequent changes in behavior and metabolism are not fully understood. Our lab has previously shown that *Mio*, the *Drosophila* homolog of carbohydrate response element binding protein (ChREBP), functions in the fat body of the fly to control triglyceride storage as well as feeding, suggesting that Mio may act as a nutrient sensor to coordinate food consumption and metabolism. In this study, we characterized the role of *Mio* in the brain to control feeding and nutrient storage in larvae and adult flies. Lowering *Mio* levels using RNAi specifically in the central nervous system leads to a decrease in triglycerides in 3rd instar larvae. However, decreasing neural *Mio* expression in adult flies has little effect on triglycerides and glycogen. Interestingly, depleting *Mio* in neurons results in increased food consumption in adult flies. These data indicate a role for *Mio* in neurons to control feeding and metabolism and suggests that Mio may act as a nutrient sensor in the brain to coordinate behavior with nutrient availability.

773C

Gustatory-mediated Neuronal Circuits Regulate *Drosophila* Physiology and Longevity. Michael J. Waterson[1], Tammy P. Chan[2], Zachary M. Harvanek[3], Ivan Ostojic[4], Joy Alcedo[4,5], Scott D. Pletcher[1,3]. 1) Cellular and Molecular Biology Graduate Program, University of Michigan; 2) Department of Developmental Biology, Baylor College of Medicine, Houston, TX; 3) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 4) Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland; 5) Department of Biological Sciences, Wayne State University, Detroit, MI.

The integration of environmental information within the central nervous system is crucial to the regulation of aging, yet the circuitry of this regulation remains poorly understood. *D. melanogaster* offers a simple, yet conserved neuronal architecture and the genetic reagents needed to tackle the difficulty of mapping neural regulatory networks. Previous work has provided strong evidence for chemosensory inputs in modulating longevity. We have thus focused on one such set of these signals - gustatory inputs - as a model to understand this regulation.

To determine which specific gustatory inputs regulate certain aging phenotypes, we utilized multiple genetic techniques to systematically manipulate the expression of single gustatory inputs and measured their effects on lifespan. Our work has identified three gustatory genes, representing three distinct taste modalities, as potent regulators of fly longevity.

Our initial work in elucidating this gustatory-mediated neuronal circuitry has focused on one of these inputs, an ion channel critical for the activation of one subtype of gustatory receptor neurons. Importantly, we have found that this input can regulate nutrient homeostasis, and that lifespan extension mediated by this circuit requires two neuroendocrine signaling pathways. These data suggest a model in which gustatory signals, transduced to the CNS, modulate signaling responsible for the systemic control of metabolic homeostasis and the regulation of an organism's physiological state. The cumulative effects of these corresponding physiological changes, in turn, contribute to the rate at which the animal ages.

774A

Dietary Composition Regulates *Drosophila* Mobility and Cardiac Physiology. Sara Ginzberg, Brian Bazzell, Lindsey Healy, Robert Wessells. The University of Michigan, Ann Arbor, MI.

The impact of dietary composition on exercise capacity is a subject of intense study in both humans and model organisms. Interactions between diet and genetics are a critical component in optimized dietary design. However, the genetic factors governing exercise response are still not well understood. The recent development of invertebrate models for endurance exercise is likely to facilitate study designs examining the conserved interactions between diet, exercise, and genetics. As a first step, we use the *Drosophila* model to describe here the effects of varying dietary composition on several physiological indices, including fatigue tolerance and climbing speed. We find that flies of two divergent genetic backgrounds optimize endurance and cardiac performance on relatively balanced low calorie diets. When flies are provided with unbalanced diets, diets higher in sugar than in yeast facilitate greater endurance at the expense of cardiac performance. Lastly, we use dissected cardiac muscle as a model muscle to analyze the effects of diet on intramuscular lipid storage, autophagy levels, and structural preservation of muscle fibers.

775B

Food pH and microbial growth modulate *Drosophila* longevity. Sany Hoxha, Ryuichi Yamada, Christine Mak, Brooke Hunter, William Ja. Department of Metabolism & Aging, The Scripps Research Institute, Jupiter, FL.

Dietary pH potentially influences a number of sensitive biological parameters such as microbial growth, gut homeostasis, and feeding behavior. In most *Drosophila* studies where dietary or drug interventions are employed, the effects of these manipulations on food pH and microorganism growth are ignored. To investigate the effect of food pH on survival, we measured fly adult lifespan on media buffered to different pH. We show that flies maintained on acidic buffered medium are long-lived compared to flies kept on buffered food of neutral or basic pH. To determine whether microorganism growth and food pH interact to affect longevity, we compared the lifespan of flies under conventional and axenic (germ-free) conditions. Under conventional conditions, microbial growth rapidly acidifies the food surface. Conversely, medium pH remains stable when flies are developed axenically and no microorganisms are present. In both conventional and axenic conditions, maximal fly lifespan is achieved on acidic medium. Moreover, flies maintained under acidic conditions consume more food, suggesting that longevity is not a result of caloric restriction. Our results show that dietary pH and microbial growth can mutually influence fly lifespan. Hence, effects that are currently attributed solely to nutritional or pharmaceutical interventions may rather be due, at least in part, to changes in medium pH, microorganism growth, and food buffering capacity.

776C

The Insulin and Tor signaling pathways directly regulate cuticle melanization in *Drosophila*. Jennifer A. Kennell, Iryna Shakhmantsir. Biology, Vassar College, Poughkeepsie, NY.

In addition to its mechanical properties, the pigmented cuticle of insects can play a role in thermoregulation, desiccation tolerance, mimicry and sexual selection. Cuticle pigmentation is a phenotypically plastic trait, with environmental factors such as temperature and nutrition influencing the extent of melanization in *Drosophila*. We present evidence suggesting a novel role for the nutrient sensing Insulin and Target of Rapamycin (Tor) pathways in directly regulating pigmentation of adult cuticle in *Drosophila melanogaster*. We found that activation of Insulin/PI3K signaling along the dorsal midline using the *pnr-Gal4* driver results in a cell autonomous increase in melanization of the adult cuticle. Conversely, inhibition of the pathway causes decreased pigmentation of both the abdomen and thorax. Insulin/PI3K signaling likely promotes pigmentation in part through inhibition of dFOXO. Given that the Insulin/PI3K pathway interacts with the Tor pathway, we also tested the ability of Tor signaling to regulate pigmentation. We found that activation of the Tor pathway causes increased pigmentation, as does activation of S6K, an effector of the Tor pathway. Interestingly, modulation of these pathways using the *pnr-Gal4* driver could change pigmentation without altering other aspects of cuticle development such as segment and bristle formation, suggesting this effect on pigmentation is specific and not due to the indirect effects of altered cell migration, division or differentiation. Previous studies in *Drosophila* and other insects have found a positive association between nutrient status and melanization. Some have proposed that this connection between nutrition and pigmentation may be due to changes in availability of substrates for melanin production, such as tyrosine. Our data suggests that nutritional control of cuticular melanization in *Drosophila melanogaster* may be mediated by the Insulin and Tor signaling pathways.

777A

Towards complete ecdysteroidome of *Drosophila melanogaster*. Oksana Lavrynenko, Suzanne Eaton, Andrej Shevchenko. MPI CBG, Dresden, Germany.

Ecdysone and 20-hydroxyecdysone control molting metamorphosis during *Drosophila* development [1]. Fruit flies are auxotrophs and produce ecdysteroids solely from dietary sterols. However, the exact relationship between hormone profiles and the sterol composition of food remains unclear [2]. Enzyme immunoassay (EIA) may estimate the total content of ecdysteroids, however it lacks specificity to distinguish and quantify individual hormones. We developed a LC-MS/MS strategy for ecdysteroidome (i.e. the full complement of ecdysteroid species) mapping. It relies on the targeted accurate mass screening, while species candidates are confirmed by MS/MS. Chemical modification of the total extract with Girard reagent followed by specific detection and fragmentation of candidate precursors enhanced the analysis specificity. In the series of proof-of -principle experiments we were able to identify polar conjugates (glycosides, phosphates and sulfates), catabolytes or any structurally modified hormones at the picograme level. We demonstrated that 20-hydroxyecdysone and makisterone A are synthesized independently from different precursors and could not be converted into each other. We also identified a new 20-hydroxyecdysone -related hormone that is highly enriched at the embryonic stage: previously, high ecdysteroid content was detected by EIA, but was not associated with any known hormone. Our approach provided further insight into the hormones catabolism pathways: we detected and quantified the carboxyl acids produced by C-26 specific oxidation of both 20-hydroxyecdysone and makisterone A acids. The new hormones and their metabolites could be quantified with acceptable accuracy with no recourse to synthetic standards. 1.Schwedes, C. C.; Carney, G. E., Ecdysone signaling in adult Drosophila melanogaster. J Insect Physiol 2012, 58 (3), 293-302. 2.Carvalho, M.; Schwudke, D.; Sampaio, J. L.; Palm, W.; Riezman, I.; Dey, G.; Gupta, G. D.; Mayor, S.; Riezman, H.; Shevchenko, A.; Kurzchalia, T. V.; Eaton, S., Survival strategies of a sterol auxotroph. Development 2010, 137 (21), 3675-3685.

778B

Brain Vacuolization and Muscle Protein Aggregation as Potential Biomarkers of Aging in Drosophila. Atanu Duttaroy[1], Kristopher Bcekwith[2], Peter Kibanyi Kibanyi[1], Eva Polston Polston[2]. 1) Dept Biol, Howard Univ, Washington, DC; 2) Department of Physiology and Biophysics, Howard University, Washington, DC.

Vacuoles appear in the form of clear spaces in Drosophila brain as it ages. This progressive vacuolization event is presumably related to the loss of CNS neurons, so the appearance of vacuoles in the brain is used as a typical hallmark of neurodegeneration in Drosophila. In order to compare the extent of brain vacuolization and its relationship with the amount of neuronal loss during biological aging we selected three fly strains CantonS (wild type strain), Methuselah (long lived strain) and White1118, the latter of which shares identical genetic background with methuellah. H&E stained sections of the fly brains from different ages were obtained and quantitative morphometric measurement was performed to analyze vacuole numbers and volumes, as well as neuronal numbers. Our observation revealed that the number and volume of vacuoles formed in the brain is directly correlated with the biological age of the fly; as the age of the animal increased, the number and sizes of vacuoles also increased. Next, we asked if the amount of vacuolization is related to the loss of brain cells. Number of nuclei counted from the same brain sections showed a surprising result, in that the numbers of neuronal nuclei remained same across all ages in all three genotypes. Our results therefore indicate that progressive brain vacuolization in Drosophila is not related to neurodegeneration. Aging muscle in Drosophila shows progressive accumulation of protein aggregates characterized by increased ubiquitination causing impaired muscle function at older age. Same three Drosophila genotypes were chosen: CantonS (wild type), w1118 and methuselah, and polyubiquitination of muscle proteins were monitored in immuno-histological sections as a function of age with an anti-ubiquitin antibody as well as by Western analysis. Our data

shows that muscle ubiquitination is simply an age associated event.

779C

Loss of the mitochondrial matrix protein Shaken not Stirring causes bang-sensitivity and early adult lethality. Daniel K. Bricker[1,3], Jon Van Vranken[2,3], Kelly J. Beumer[2,3], Dana Carroll[2,3], Jared Rutter[2,3], Carl S. Thummel[1,3]. 1) Department of Human Genetics; 2) Department of Biochemistry; 3) University of Utah School of Medicine, Salt Lake City, UT.

Mitochondria are complex organelles that have important roles in energy production, intermediary metabolism, signal transduction and apoptosis. Consistent with these activities, mitochondrial dysfunction is associated with a wide range of human diseases, including myopathies, cancers and neurodegenerative disorders. Given these critical cellular functions and links to disease, major efforts have been made to determine the identity of all mitochondrial proteins. The most comprehensive study to date identified ≈1200 proteins in the mouse mitochondrial proteome. Remarkably, about one fifth of these proteins have unknown functions. Moreover, many are represented by one or a few genes in most species, ranging from yeast to humans, suggesting they have a critical function maintained through evolution. We are characterizing a subset of these evolutionarily-conserved genes through a collaborative effort in both flies and yeast. Our current studies are focused on a protein that we have named Shaken not Stirring (SST) in *Drosophila melanogaster*. The yeast ortholog of *sst* encodes a mitochondrial matrix protein that is required for growth on media containing acetate. This phenotype can be complemented by expression of either the fly or human orthologs of the gene, indicating an evolutionarily-conserved function. Mutations in *Drosophila sst* have been generated using transcriptional activator like effector nucleases (TALENs) and by imprecise excision of a P-element. These null mutants are viable, but display a severely reduced lifespan under normal laboratory conditions. Interestingly, *sst* mutants are sensitive to paralytic seizures caused by mechanical stress, a classic phenotype termed "bang sensitivity". This phenotype is closely associated with neuronal dysfunction and neurodegeneration. We are currently determining how SST maintains neuronal function through a combination of metabolomic analysis and a detailed examination of mitochondrial function in *sst* mutants.

780A

Morphological and molecular characterization of adult midgut compartmentalization in *Drosophila* . Nicolas Buchon[1,2], Dani Osman[2], Fabrice David[2], Jean-Philippe Boquete[2], Bart Deplancke[2], Bruno Lemaitre[2]. 1) Cornell University, Ithaca, NY; 2) EPFL, Lausanne, Switzerland.

The gut is a central organ of Eumetazoans and has critical roles both in health and disease. However our understanding of gut morphological and molecular features remains rudimentary, calling for integrative studies to understand its structure and function. In our study, we generated a comprehensive atlas of the *Drosophila* adult midgut based on analyses of microanatomy and of spatial transgene expression mapping along the gut. We uncovered a fine-resolution regional organization consisting of 14 sub-regions with distinct morphometric, histological and genetic characteristics. Further genomic analysis of the main gut regions revealed that immune, physiological and homeostatic properties also vary in those regions, suggesting functional compartmentalization. In addition, we showed that *Drosophila* intestinal regionalization is defined after adult eclosion, remains stable throughout life, and re-establishes following acute tissue damage, but is lost upon aging. Finally, we started to unravel the molecular determinants that control midgut compartmentalization, showing that regions are maintained by the interplay between pan-gut and regionalized transcription factors, in concert with the spatial activity of morphogens. Interestingly, disruption of midgut compartmentalization leads to intestinal disorders characterized by an increase in stem cell proliferation and aberrant immune responses. Together, our study analyzes*Drosophila* midgut compartmentalization in an integrative manner and provides new insights into the conserved mechanisms underlying intestinal regionalization in metazoans.

781B

***SERF1* contribution to protein homeostasis in *Drosophila melanogaster*.** Swagata Ghosh, Adna Karic, Susan Harrison, Douglas Harrison, Brian Rymond. Biology, University of Kentucky, Lexington, KY.

SERF1 is a gene well conserved in species ranging from baker's yeast to human but its natural biological function is not known in any organism. *SERF1* is a candidate modifier of the autosomal recessive form of Spinal Muscular Atrophy (SMA) (Scharf et.al, 1998), the leading genetic cause of human infant mortality. Moreover, *SERF1* in C. elegans has been shown to modify proteotoxicity and the accumulation of amyloid aggregates associated with disease related human amyloid proteins, thus indicating its potential role in cellular protein homeostasis (Van Ham et.al, 2010). Here we are investigating the role of *SERF1* in cellular protein homeostasis, known to play critical role in tissue aging (Demontis & Perrimon, 2010) using *Drosophila melanogaster* system. We have created a number of *SERF1* mutant backgrounds by imprecise P-element excision, *SERF1* mis-expression as well as RNAi mediated down regulation, in order to analyze its impact on protein homeostasis in adult fly muscle. Preliminary observations suggest possible *SERF1* contribution to age dependent aggregation of natural ubiquitinated proteins in the adult thoracic muscle tissue. In addition, we are also investigating *SERF1* contribution to the Parkinson's disease model of *Drosophila* in which mutant forms of human alpha synuclein protein are expressed using conventional UAS-Gal4 binary expression system (Feany et.al, 2000). The impact of*SERF1* expression on the accumulation of 'Lewy-body' like aggregates of alpha-synuclein in the fly brain will be scored by immune detection. With this study we aim to shed light on *SERF1's* natural biological function and develop tools helpful in the investigation of age related neurodegenerative disease.

782C

Effects of rearing oxygen level on the structure of the adult tracheal system in *Drosophila melanogaster*. Jon F Harrison, James Waters, Stephanie Heinrich, Taylor Biddulph, Sandra Kovacevic. School of Life Sciences, Arizona State University, Tempe, AZ.

Insect tracheal systems are known to respond in a compensatory manner to rearing oxygen level, but the functional extent of compensation remains unclear, as does whether compensation extends from the larval to the adult stage. In this study, we investigated the structure, and phenotypic plasticity of the tracheal system of *Drosophila melanogaster* . Flies were reared from egg through adulthood in 10, 21 or 40% oxygen atmospheres, and their tracheal system assessed on the fourth day of adulthood. The tracheal system of the whole body was assessed with a 3D tomographic technique using synchrotron x-rays at Argonne National Laboratory. In addition, tracheoles in the flight muscle were imaged using confocal microscopy. We did not detect changes in the branching structure or number of branches in the major, large-diameter tracheae of the thorax. However, there was strong compensatory variation in the number and density of tracheoles in the flight muscle. In addition, we were surprised to find that the diameter of the terminal tracheoles decreased for flies reared in hypoxia. These results contrast to observations in the tracheal trunks of larvae, which can show increases in diameter in hypoxic-reared animals. Perhaps the decreased diameters of tracheoles benefit oxygen diffusion into muscle by increasing surface/volume ratios of these tubes. This research was supported by NSF 0938047to JFH.

783A

The role of the adiponectin receptor homolog in Drosophila melanogaster oogenesis. Kaitlin Laws, Leesa Sampson, Daniela Drummond-Barbosa. Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

The ability of a stem cell to sense and respond appropriately to systemic cues is integral for the coordination of its behavior with whole organism physiology. The Drosophila melanogaster ovary is a stem cell-based system that rapidly responds to the diet of the organism through hormonal and local nutrient-sensing pathways. Previous work in our lab demonstrated that Drosophila ovarian germline stem cells (GSCs) require several nutrient-sensing pathways, including insulin signaling directly for their proliferation and indirectly for their maintenance. It is unclear, however, whether adipose tissue secreted proteins play a role in the modulation of stem cell activity. In mammals, the adipose tissue secretes adipokines that regulate organismal metabolism and homeostasis. The adipokine adiponectin is of particular interest because of its well-described role as an insulin-sensitizing agent. Although there is no obvious Drosophila homolog of adiponectin based on primary sequence, a homolog of the adiponectin receptor, CG5315, has been identified. We have generated a null CG5315 mutant allele, obtained CG5315 hairpin lines for RNAi, and are in the process of generating CG5315 rescue lines and analyzing the ovary autonomous and non-autonomous roles of CG5315 during Drosophila oogenesis. These studies will provide us with a more complete view of how stem cells respond to various diet-dependent cues to coordinate tissue behavior with the physiology of the organism.

784B

The regulation of muscle function by *Mio* in *Drosophila*. Grzegorz Polak, Justin DiAngelo. Department of Biology, Hofstra Univ, Hempstead, NY.

All cells require energy to perform their specialized functions. Muscle is particularly sensitive to the availability of nutrients due to the high energy requirement for muscle contraction. Therefore the ability of muscle cells to obtain and store nutrients for energy is essential for the function of these cells. Our lab has recently identified *Mio*, the *Drosophila* homolog of carbohydrate response element binding protein (ChREBP), as a nutrient responsive transcription factor important for triglyceride storage in the fat body. However, the function of *Mio* in muscle is unknown. In this study, we characterized the role of *Mio*, in controlling muscle function in adult flies. Lowering *Mio* levels using RNAi specifically in muscle leads to a flight defect. This phenotype does not result from a lack of nutrient stores or the inability to utilize those stores as there is little effect on glycogen and triglyceride levels when *Mio* expression was decreased in muscle. These data raise the possibility that the flight defect observed in muscle-specific *Mio* knockdown flies may be due to effects on muscle structure and electron microscopy experiments are being performed to test this hypothesis. Together, these data indicate a novel role for *Mio* in muscle to control flight and may provide a molecular link between nutrient availability and muscle function.

785C

Expression of *drop-dead* (*drd*) in the tracheae is sufficient to prevent neurodegeneration, but not early lethality. Christine L. Sansone, Edward M. Blumenthal. Biological Sci, Marquette Univ, Milwaukee, WI.

Mutation of the adult lethal gene *drop-dead* (*drd*) causes a number of phenotypes in addition to short lifespan, including neurodegeneration, fragile tracheae, reduced movement of food through the gut and subsequent starvation, small body size, and female sterility. The *drd* gene product is an integral membrane protein with limited homology to prokaryotic acyltransferases, but its biochemical function remains unknown. In this study, we have used tissue-specific knockdown and rescue of *drd* expression in order to determine the cause of the neurodegeneration phenotype. Knockdown of *drd* in glia (*repo-Gal4* or*17A-Gal4*), neurons (*elav-Gal4*) or both cell types did not cause adult lethality. In contrast, knockdown of *drd* in the tracheae (*btl-Gal4*) caused early adult lethality and neurodegeneration. The gut phenotypes of starvation and reduced food movement were not affected by knockdown of *drd* expression in the tracheae. Surprisingly, rescue of *drd* expression in the

tracheae rescued neurodegeneration, but not adult lethality or gut dysfunction. From these data, we conclude that adult lethality in *drd* mutant flies has two independent causes: a tracheal defect that results in neurodegeneration and a gut defect that results in starvation. Either of these appears sufficient to cause early lethality. The fragile tracheae phenotype observed in *drd* mutants is predicted to cause hypoxia-induced neurodegeneration. To test this model directly, we crossed the hypoxia-sensitive *LDH-Gal4* and *LDH-LacZ* reporters onto a *drd* mutant background. Expression of these transgenes was not elevated in *drd* mutants, suggesting that these flies are not hypoxic. Therefore, our data suggest that the absence of *drd* expression in the tracheae causes neurodegeneration by a mechanism other than hypoxia. Supported by Marquette University, a U.S. Department of Education GAANN fellowship to C.L.S. and NIH 1R15 GM080682-01 to E.M.B.

786A

Aging affects circadian control of glutathione biosynthesis in *Drosophila melanogaster*. Eileen Chow[1], Vladimir Klichko[2], Joanna Kotwica-Rolinska[1,3], Dani Long[1], William Orr[2], Svetlana Radyuk[2], Jadwiga Giebultowicz[1]. 1) Department of Zoology, Oregon State University, Corvallis, OR, USA; 2) Department of Biological Sciences, Southern Methodist University, Dallas, TX, USA; 3) Department of Animal Physiology, University of Warsaw, Warsaw, Poland.

Circadian clocks control many biological processes that are vital to maintain health, including daily sleep/activity patterns and oscillations in neuronal, physiological, and metabolic functions. The focus of our work is to understand how clock-controlled processes are altered during aging in *Drosophila melanogaster*. We recently determined that levels of the major redox regulator glutathione (GSH) fluctuate in fly heads in a circadian manner. Significant rhythms were observed in the expression of genes encoding the catalytic (*Gclc*) and modulatory (*Gclm*) subunits of glutamate cysteine ligase (GCL), the rate-limiting enzyme in GSH biosynthesis, in the activity of the GCL, and expression of *GstD1* (which utilizes GSH in cellular detoxification). The circadian system consists of ~150 central pacemaker neurons which control rest/activity rhythms, and so-called peripheral oscillators in many tissues such as retinal photoreceptors, glia, and fat body. We are mapping which circadian loci contribute to rhythmic expression of*Gclc* by disrupting clocks in specific cells such as *Pdf* positive neurons, glia, and photoreceptors. In addition, we investigate age-related changes in GSH biosynthesis, as we and others determined that aging is associated with dampened expression of clock genes and proteins. Our data suggests that rhythmic expression of *Gclc* and *Gclm* is impaired in old flies. We are testing whether loss of circadian regulation leads to a decline in GSH levels and compromised redox homeostasis by evaluating reduced and oxidized glutathione, and analyzing the profiles of protein mixed disulfides across lifespan. Our results should provide insights into relationships between the clock system and GSH, a compound which acts as a major antioxidant, regulates activity of detoxification enzymes, and mediates redox-sensitive signaling.

787B

Effects of radiofrequency identifiers in embryos and pupae of *Drosophila melanogaster*. David A. Lavan[1,2,5], Luis Moreno[2], Rubén E. Acosta[1], Miguel Diaz[5], Marcos Moroto[3], Ricardo Yauri[1], Roxana Moran[1], Olga Bracamonte[2], Julio Valdivia-Silva[4], Daniel Diaz[1]. 1) National Institute Research of Telecommunications Training - INICTEL-UNI, Av. San Luis 1771, Lima 41, Peru; 2) Cytogenetics Laboratory, Universidad Nacional Mayor de San Marcos, Av. University s/n, Lima 1, Peru; 3) Department of Pharmacology and Therapeutics, School of Medicine, Universidad Autonoma de Madrid. Av. Morcillo 4 Madrid 28029, Spain; 4) Biotechnology and Space Medicine - NASA Ames Research Center, Build. N245 M/S:245-3, Moffett Field, CA 94035, USA; 5) Central Therapy Magnetic Field, International Institute of the Cancer and Pain; Av. Montero Rosas nº 1141 Lima 1, Peru.

In the last decade the wireless communications into a broad range of intelligent electronic devices are becoming to be an important key in the progress of different industrial and social areas, including biomedical applications, where ubiquitous sensor networks and RFID identifiers tags have a faster development than the past. In Peru, the technological advances in wireless communication systems have so far been developing the biomedical field due to the fear that exists in the effects that radiofrequency could lead in cells and tissues. Indeed, few studies have shown some non thermal effects of radiofrequencies on the integrity of the protein structure, levels of gene expression, and development In our laboratory at INICTEL has been developed a new type of RFID device that allow a rapid access and storage of information in a database which could be very useful to several biomedical applications. In this matter, the study of RFID effects on biological systems should be considered. In this work, we conducted two studies using *Drosophila melanogaster* flies as a biological system exposed to RFID devices. The first one compared the growth curve of flies exposed 22 hours at a frequency of 13.5 MHz versus non-exposed organisms since their embryonic stage. The other study analyzed mRNA expression profiles of the genes: G1 to G6, which are highly expressed during embryonic, larval, and puparia stages, under the same RFID exposition.

788C

A genetic approach reveals selective elimination of damaged mitochondria in healthy cells and tissue. Yun Qi[1*], Jahda Hill[1], Guofeng Zhang[2], Hong Xu[1]. 1) GDBC, NHLBI, bethesda, MD; 2) NIBIB, bethesda, MD.

Mitochondrial turnover has been postulated as a mechanism for mitochondrial quality control. However, it remains a question whether cells are indeed able to eliminate defective mitochondria selectively. Quantitative and live imaging assays are required to measure selective mitochondrial degradation and visualize this process in real time, while a genetic approach is essential to probe mitochondrial turnover in a physiological context. We expressed a toxic bacterial protein, PorB, to damage a subpopulation of total cellular mitochondria in cultured Drosophila cells and tissues. Damaged mitochondria concentrated with PorB were segregated from the mitochondrial network through a fission/fusion process and selectively

removed by lysosomes probably through the autophagy pathway in otherwise healthy cells. We also demonstrated in our model that the Parkin-dependent degradation of damaged mitochondria in an animal tissue, the Drosophila flight muscle. Our work proves in principle that defective mitochondria are selectively removed in healthy cells, and also provides a novel genetic approach to monitor mitochondrial turnover and dissect the underlying mechanisms.

789A

A Novel Role for the Ribosomal Protein RpL22 in Poly(ADP-ribose)polymerase 1-Dependent Transcriptional Regulation. Ernest Boamah, Alexei Tulin. Epigenetics and Progenitor Cells Program, Fox Chase Cancer Center, Philadelphia, PA.

Poly (ADP-ribose) polymerase 1 (PARP1), a nuclear protein, induces transcriptional activation or repression primarily through its ability to synthesize ADP-ribose (ADP) polymers. PARP1 generates ADP polymers using nicotinamide adenine dinucleotide (NAD+) as a substrate. The mechanisms linking PARP1 and ADP modification to chromatin regulation is currently being investigated with promising discoveries. Importantly, PARP1 has been shown to modify multiple cellular targets; however PARP1 only interacts with a select group of proteins. How these interactions impact PARP1 transcriptional regulation remains less understood. We recently discovered that RpL22, a ribosomal protein, interacts with PARP1. In this study, we evaluate the interaction of RpL22 with PARP1 and examine how this interaction affects PARP1 transcriptional regulation specifically at the hsp70 locus. Using the Drosophila model system, we observe that disruption of RpL22, using a P-element insertion, dramatically reduces PARP1-dependent hsp70 activation when larvae are exposed to heatshock. We therefore generated transgenic flies expressing CFP-tagged RpL22 to further examine this interaction and the impact on PARP1 transcription. We observed, using immunofluorescence analysis, that RpL22 predominantly localizes within active chromatin. Additionally, chromatin immunoprecipitation analysis also shows an even distribution of RpL22 within the hsp70 locus prior to heatshock. Together, our preliminary analyses suggest that RpL22 localization on chromatin may influence PARP1 transcriptional regulation. Our overall objective is to investigate PARP1-interacting proteins and their impact on PARP1 transcriptional activity.

790B

A transcriptional code for muscle fiber identity in Drosophila. Anton L. Bryantsev[1], Sandy Duong[1], Tonya M. Brunetti[1], Maria B. Chechenova[1], TyAnna L. Lovato[1], Cloyce Nelson[1], Elizabeth Shaw[1], Juli D. Uhl[2], Brian Gebelein[2], Richard M. Cripps[1]. 1) Department of Biology, University of New Mexico, Albuquerque, NM; 2) Division of Dev. Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

In this study we define a molecular switch that specifies muscle fiber fate in adult *Drosophila* flies, via activity of the homeodomain proteins Extradenticle (Exd) and Homothorax (Hth). The genes *exd* and*hth* are expressed in the fibrillar indirect flight muscles, but not in tubular jump muscles. When expression of *exd* or *hth* is knocked down, the flight muscles are transformed into a jump muscle fate. Conversely, if *exd* and *hth* are forcefully expressed in the jump muscles, these fibers now become flight muscles. In the flight muscles, *exd* and *hth* are genetically upstream of another muscle identity gene,*salm*. We further demonstrate that *exd* and *hth* are direct transcriptional regulators of the signature flight muscle structural gene, *Actin88F*, indicating that these factors represent a molecular switch to regulate muscle fiber fate. We also demonstrate that Exd and Hth impact muscle identity in other somatic muscles of the body, by cooperating with Hox factors. Since mammalian orthologs of exd and hth also contribute to muscle gene regulation, our study suggests that an evolutionarily-conserved genetic pathway determines muscle fiber differentiation.

791C

Multiple screening approaches identify novel transcription factor binding partners for Eyes absent. Trevor L. Davis[1,2], Ilaria Rebay[1,2]. 1) Committee on Development, Regeneration, and Stem Cell Biology, University of Chicago, Chicago, IL; 2) Ben May Department for Cancer Research, University of Chicago, Chicago, IL.

A long-standing question in developmental biology is how transcription factors direct different outcomes of gene expression depending on context. One way these proteins' activities change is through binding to different co-factors to create multi-protein complexes that uniquely affect transcription. In Drosophila, four transcription factors known as the retinal determination (RD) network control eye development, but little is known about how these proteins cooperate with others during development. To investigate transcriptional complex behavior in vivo, we study the RD transactivator Eyes absent (Eya). Loss of Eya or its co-activator, Sine oculis (So), abolishes retinal development, while their misexpression creates ectopic eyes. However, both proteins bind co-activators and So also functions in repressive complexes with Groucho, raising the possibility that both activation and repression by Eya-So are essential for eye development. Because additional proteins likely regulate these behaviors, we performed a yeast two-hybrid screen for novel Eya interactors. This approach identified putative Eya-binding transcription factors that we find are required for eye development. To assess which interactions are relevant in vivo, we asked whether knockdown of candidates modified the eya^{RNAi} reduced-eye phenotype. We hypothesized that genes whose knockdown suppressed this phenotype inhibit transcriptional activation by Eya, while those that enhanced it promote Eya-mediated gene expression. To test these ideas, we asked if Eya's ability to induce ectopic transcription was increased by expressing RNAi transgenes that suppressedeya^{RNAi} and vice versa. Based on these data, we selected putative co-repressors and co-activators that may regulate Eya's transcriptional function. Future work will test whether these complexes contain So and characterize the developmental processes and target loci that are controlled by Eya-candidate interactions.

792A

Building an interactome: Identifying novel Akirin-interacting factors. William Dawkins, Meghan Troutt, Aayushi Bhagwanji, Kate Majeski, Shelby Rogers, Scott J. Nowak. Department of Biology and Physics, Kennesaw State University, Kennesaw, GA 30144.

The specification and differentiation of muscle precursor cells, or myoblasts, by the action of the Twist mesodermal and muscle transcription regulator is a key event in the formation of the *Drosophila* larval musculature. However, despite the primary importance of myoblast specification and differentiation for building and patterning the musculature, the identities of many molecular players in this process remain unknown. We have recently determined that Akirin, a highly conserved nuclear protein, appears to play a critical role in the regulation of Twist-dependent gene expression during mesodermal specification and muscle development. We performed a genetic interaction screen to identify Akirin interacting-proteins that have essential roles during the process of muscle specification and patterning. Using our screening method, we have identified a number of loci that genetically interact with Akirin during muscle patterning. Our list of positive Akirin-interacting partners includes factors involved in general transcription initiation, as well as components of chromatin remodeling complexes. Identification of the cast of molecular players that work with Akirin will provide crucial insight into Akirin's mechanism of molecular action during myoblast specification and muscle patterning.

793B

Functions of the co-activator CBP in transcription and in control of early Drosophila embryo development. Mattias Mannervik. Wenner-Gren Institute, Stockholm University, Stockholm, Sweden.

The p300 and CBP co-activators are histone acetylases and central regulators of transcription in metazoans. The genomic occupancy of p300/CBP detected by ChIP-seq experiments can be used to identify transcriptional enhancers. Whether p300/CBP is preferentially involved in some gene regulatory networks is not known. We therefore compared the genome occupancy of Drosophila CBP (nejire) with that of 40 different transcription factors in early embryos. We found a striking overlap of CBP ChIP-seq peaks with regions bound by Dorsal. In mutant embryos where Dorsal fails to enter the nucleus, CBP peaks were less associated with Dorsal-binding regions, and instead best correlated with Dpp-signaling and Smad binding. Thus, two key processes in dorsal-ventral patterning, the Dorsal gene regulatory network and Dpp-signaling, overlap the genomic distribution of CBP most significantly, whereas anterior-posterior activators such as Bicoid and Caudal show little overlap. Perhaps CBP serves to coordinate the Dorsal and Dpp pathways in dorsal-ventral patterning. Surprisingly, although CBP occupancy in general correlates with gene activation, it can also be found at silent regions. At silent sites, CBP occupancy does not cause histone acetylation. One mechanism for preventing histone acetylation at these sites is methylation of H3K27 by the Polycomb complex PRC2. Interestingly, H3K27me3-repressed chromatin does not preclude CBP binding, resulting in a bivalent situation. The antagonism between H3K27ac and H3K27me3 indicates that CBP may be involved in switching between repressed and active chromatin states. We performed ChIP-seq of CBP in S2 cells, which showed that CBP occupancy depends of GAGA factor (GAF), and is preferentially found at promoters with a paused RNA polymerase. Using a CBP inhibitor, we show that CBP is required for pol II occupancy at GAF-bound paused promoters. Our studies show that there is a preference for some transcription factors over others in directing CBP to the genome, and that CBP is required for transcription from promoters with a paused polymerase.

794C

Pan-leg developmental regulators control pro-thoracic leg specific Scr expression. Christopher L McCallough, Ece Eksi, Emily R Wyskiel, Teresa V Orenic. Biology, University of Illinois at Chicago, Chicago, IL.

The Drosophila adult has one pair of legs on each of its three thoracic segments (T1-T3). Although these structures exhibit serial homology, the legs from different segments have distinct morphological features. One such feature is the patterning of the peripheral nervous system in the form of small mechanosensory bristles (mCs). In the T2 leg these mCs are organized into a series of longitudinal rows (L-rows) along the circumference of the tibia and tarsal segments. However, at specific positions along the circumference and proximal/distal axis of the T1 leg, the L-rows are replaced by a group of mCs organized into transverse rows (T-rows) [1,2]. Studies have indicated that the position of T-row bristles on the tibia and basitarsus of T1 legs is established as a result of Hox gene modification of the L-row patterning pathway [3,4]. In T1 prepupal legs, Sex combs reduced (Scr) is expressed at elevated levels within the T-row primordia. We have found that Scr modifies the mC pattern on T1 legs via repression of Delta, a key regulator of leg mC patterning [4]. Our model for T-row patterning suggests that a central step in this process is establishment of spatially defined Scr expression within defined domains of the leg primordium in response to the global regulators of leg development. The mechanisms that generate morphological diversity among the legs will therefore require an understanding of the regulation of Scr in the T-row primordium. Here we will present our genetic studies on the regulation of Scr by genes known to pattern the leg along its circumference and P/D axis.

795A

Post-translational modification of Vestigial modulates transcriptional response in developing wing cells. Virginia Pimmett[1], Hua Deng[2], Andrew Simmonds[1]. 1) Cell Biology, University of Alberta, Edmonton, Alberta, Canada; 2) Molecular Biology & Genetics, Johns Hopkins University, Baltimore, Maryland, USA.

The transcriptional co-activator Vestigial (VG) is a key selector in determining cell fate in the developing wing disc. Together

with its partner transcription factor Scalloped (SD), VG coordinates activation of the expression of many key wing-specification genes through activation of target enhancers in cells within the wing pouch. It has been shown previously by Takanaka and Courey (Mech. Dev, 122, pp 1030-7, 2005) that VG is SUMOylated and that this might be important for VG function. We have found that specific post-translational modifications of VG through phosphorylation by mitogen-activated protein kinase (MAPK) and conjugation of a small ubiquitin-like modifier (SUMO) to a specific lysine residue are important in determining the ability of VG and SD to activate target enhancers *in vitro* and *in vivo*. We have identified the target lysine residue for SUMO conjugation as well as a key residue targeted by p38b MAPK that is important for proper transcriptional activation in wing discs. Mutation of these sites, as well as blocking the modification pathways, alters the ability of a SD/VG complex to activate the Vestigial quadrant (VgQ) enhancer *in vitro*. Furthermore, we show that blocking these modifications has transcriptional and phenotypic consequences *in vivo*. Post-translational modification of VG has been experimentally shown to be dependent on a scaffolding function of SD. Together this indicates a possible mechanism for restriction of target gene expression to specific cells within the developing wing primordia.

796B

Zelda sites activate expression and promote transcription factor binding in a strength-dependent manner. Zeba Wunderlich[1], Rahul Satija[2], Meghan D. Bragdon[1], Robert K. Bradley[3], Angela H. DePace[1]. 1) Systems Biology, Harvard Medical School, Boston, MA; 2) Broad Institute of MIT and Harvard, Cambridge, MA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA.

Genome-wide transcription factor (TF) binding cannot be predicted using TF binding motifs alone. Computational and experimental work has shown that the binding of Zelda, a key TF in the maternal-to-zygotic transition in Drosophila, strongly correlates with the binding of many TFs critical for patterning the embryo. The mechanism by which Zelda affects the binding of other TFs and how binding affects the expression of target genes is currently unknown. We investigate the link between Zelda binding, TF binding and the expression of 4 target genes using ChIP-PCR experiments and quantitative imaging-based measurements of gene expression in blastoderm-stage Drosophila embryos. We created 12 transgenic reporter constructs where lacZ expression is driven by *cis*-regulatory elements that include Zelda binding sites. For each reporter, we compare the wild-type sequence to variants with the weak, strong, or all Zelda sites deleted. We find that deleting Zelda sites lead to a reduction in expression that depends on the strength of the Zelda sites; deleting only weak sites leads to a slight decrease in reporter expression, while deleting strong sites leads to a larger decrease. ChIP-PCR experiments measuring the change in TF binding and histone modification state are also underway. Together, these experiments will help us understand how Zelda binding affects the binding of other TFs and will help us interpret how changes in Zelda binding sites between individuals and species may lead to changes in gene expression.

797C

pΔTubHA4C, a new versatile vector for constitutive expression in Drosophila. Stephanie M. Arcia[1,2], Yan Zhang[1], Pedro Fernandez-Funez[1,3,4], Diego E. Rincon Limas[1,4]. 1) Department of Neurology; 2) HHMI Science for Life Undergraduate Program; 3) Department of Neurosciences; 4) Genetics Institute and Center for Translational Research on Neurodegenerative Diseases; McKnight Brain Institute, University of Florida, Gainesville, FL.

Several vectors for gene expression are available in *Drosophila*, a hub for genetics and genomics innovation. However, the vectors for ubiquitous expression have a complex structure, including coding exons, that makes in-frame cloning of cDNAs very complicated. We describe a new *Drosophila* expression vector (pΔTubHA4C) for ubiquitous expression of coding sequences under the control of a minimal, 0.9 kb promoter of *α1tubulin* (*α1t*). This plasmid was designed to include optimized multiple cloning sites (polylinker) to provide flexibility in cloning strategies. We also added the option of double labeling the expressed proteins with two C-terminal tags, the viral epitope hemagglutinin (HA) and a synthetic tetracysteine (4C) tag that binds small fluorescent compounds. This dual tag allows both in situ and biochemical detection of the desired protein. In particular, the new 4C tag technology combines easy fluorescent labeling with small arsenical compounds in live or fixed cells and tissues, while producing minimal alterations to the tagged protein due to its small size. To demonstrate the potent and ubiquitous expression under the control of the ΔTub promoter, bacterial *lacZ* was expressed and monitored in cell culture and transgenic flies. We found that the modified 0.9 kb ΔTub promoter induced similar expression levels to the intact 2.6 kb *α1t* promoter, supporting the inclusion of all critical regulatory elements in the new and flexible ΔTubHA4C vector.

798A

Allele-specific expression analysis in a large panel of intraspecific Drosophila melanogaster crosses. Daniel Campo[1], Justin Fear[2], Rita Graze[2], Peter Poon[1], Matt Salomon[1], John Tower[1], Lauren McIntyre[2], Sergey Nuzhdin[1]. 1) University of Southern California, Los Angeles, CA; 2) University of Florida, Gainesville, FL.

Gene expression variation is an important source of phenotypic change and therefore it has important implications for small-scale evolutionary processes, like local adaptation, population differentiation, and speciation. Thus, understanding how gene expression is regulated at the genome level can shed light on how these processes shape phenotypic diversity. Here we investigate the contribution of cis- and trans- regulatory changes to allele-specific expression (ASE) differences in intraspecific hybrids of Drosophila melanogaster. We have re-sequenced the entire transcriptome of a panel of 115 F1 heterozygous genotypes, derived from a set of crosses between 115 isogenic lines (N Raleigh Drosophila reference panel lines plus N Winters lines) and the isogenized standard strain w[1118]. Whole genome sequences for all the parental isogenic lines are

available. For each F1 genotype, we estimated ASE using a mixed-effects model that accounts for differences between technical replicates. In each case, the p-value was adjusted for a False Discovery Rate (FDR) that takes into account all genotypes analyzed. We found significant ASE for the majority of genes. This amount of cis- regulatory divergence appears surprisingly high at first glance. However, previous studies focused on one or a few genotypes, and cis-regulation needs to be polymorphic to be revealed. In our large collection of genotypes, we are able to more effectively uncover cis-variation for the gene. The vast amount of cis-variation suggests that much of the adaptive evolution at the population level might be due to variation in gene expression rather than changes at the protein level.Additionally, because we used the common parental fly line w[1118] in all the crosses, we will be also able to identify the relative contribution of trans- regulatory changes. These differences in allelic expression will be further associated with specific polymorphisms at the nucleotide level.

799B

Identification of a tissue-specific transcription factor required for ecdysone production in the prothoracic gland of Drosophila. Erik Thomas Danielsen[1], Morten E. Møller[1], Rachel Harder[2], Michael B. O'Connor[2], Kim F. Rewtiz[1]. 1) Department of Biology, Copenhagen University, Faculty of Science, Copenhagen, Denmark; 2) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, USA.

In the Drosophila larva, molting and metamorphosis depend on the steroid hormone producing prothoracic gland (PG). The PG regulates transcription of genes required for the biosynthesis of the molting hormone ecdysone in response to both developmental and environmental cues. Ecdysone is produced specifically in this endocrine tissue, and thus a PG-specific expression profile of genes encoding enzymes involved in ecdysone synthesis is required. However, the underlying transcriptional mechanism which dictates the cell specific program still remains to be elucidated. We have identified a transcription factor (TF) that is predominantly expressed in the PG from the embryonic formation and throughout larval development and, have investigated the consequence of reducing its expression specific in the PG. Interestingly, the larva arrests in the first instar and fails to undergo metamorphosis. The PG is intact in these larvae suggesting that the TF regulates aspects of steroid synthesis and not cell fate. We observed a reduced expression of the Halloween gene, phantom, and a reduced level of an ecdysone-responsive target gene suggesting that the TF is implicated in transcriptional regulation of the steroidogenic pathway. We further demonstrate binding-sites for the TF in the regulatory promoter-region of phantom in vitro and in vivo. In conclusion we believe this ís a key factor implicated in Drosophila steroid synthesis by regulating the expression of a PG-specific gene encoding an enzyme in the biosynthetic pathway of molting hormone, ecdysone.

800C

The molecular basis of enhancer-promoter choice. Jia Ling, Theresa Apoznanski, Stephen Small. Department of Biology, New York University, New York, NY.

In Drosophila, AP patterning is mainly controlled by the maternally deposited TF, Bicoid (Bcd) and its target genes. Upon binding to an enhancer, Bcd is thought to interact with a target gene's core promoter, forming a complex by recruiting basal transcription factors. It is intriguing that when an enhancer is located close to two genes or promoters, it usually makes a choice to activate one, not both. For instance, one of the gap genes, hunchback (hb) has a Bcd-dependent enhancer P2 activating zygotic transcription from its proximal promoter, and another enhancer activating maternal transcription from a distal promoter. Our lab identified a new Bcd-dependent enhancer 4 kb upstream of the proximal promoter and next to the distal promoter. A series of reporter genes suggests that the second Bcd-dependent enhancer actually skips over its nearest basal promoter to activate the more distally located one. This leads to the hypothesis that enhancer-promoter interactions are specific, and that only certain enhancer-promoter pairs can activate transcription. Consistent with this, data have shown that an occupied enhancer does not necessarily correlate with gene activation. We are interested in the molecular basis of enhancer-promoter choice. We are focusing on the enhancer-promoter pairs of hb, performing a series of tests, such as mutating or adding motifs, and eliminating cooperative TF bindings. The chromosome conformation capture (3C) technique will be used to identify the DNA sequences involved in enhancer-promoter interaction. We also compared expression patterns of 32 Bcd-dependent enhancer lines with nearby genes' endogenous patterns and created a dataset of positive and negative promoters for motif searching. Taken together, hopefully we will find factors contributing to enhancer-promoter choice.

801A

KDM5 interacts with heat shock factor (Hsf) to regulate cellular response to oxidative stress. Xingyin Liu, Christina Greer, Juile Secombe. Genetics, Albert Einstein Med College, Bronx, NY.

Drosophila KDM5 (also known as Lid) and its four mammalian homologs, KDM5A, KDM5B, KDM5C and KDM5D, are multi-domain transcriptional regulators. In humans, KDM5A or KDM5B overexpression causes breast, gastric and prostate cancers, and loss of KDM5C results in intellectual disability. However, a confounding factor to the analysis of the four mammalian KDM5 paralogs is their functional redundancy. In contrast, Drosophila has a single, essential KDM5 protein, providing an ideal system to answer fundamental questions regarding the mechanisms by which KDM5 regulates gene expression, and to cast light on how KDM5 function goes awry in human disease. While KDM5 proteins are most famous for their JmjC domain-encoded histone demethylase activity, we have shown that this activity is not required for viability. To investigate demethylase-independent functions of KDM5, we use microarrays to identify genes differentially expressed in response to KDM5 overexpression. Gene ontology analysis revealed a significant enrichment of genes involved in the response to oxidative stress. Consistent with this, we find that modulating KDM5 levels confers resistance or sensitivity to the oxidative stress agent

paraquat when overexpressed or reduced, respectively. Because activation Hsp22 is essential for cells to survive conditions of oxidative stress, we are focusing on the mechanism by which KDM5 transcriptionally regulates this gene. Based on our preliminary data, we propose that KDM5 interacts with the renown stress response factor, Heat shock transcription factor (Hsf) to directly activate Hsp22 and that this occurs via KDM5-mediated inhibition of the histone deacetylase HDAC1. Our results provide a mechanistic basis for KDM5's role in the regulation of oxidative stress. Importantly, because increased levels of reactive oxygen species (ROS) is a feature of many neurological disorders, our data suggest that oxidative stress-induced cellular damage may be a major contributor to intellectual disability caused by mutations in human KDM5C.

802B

Modeling Dorsal Feedback Interactions in the Developing Embryo. Michael D. O'Connell, Gregory T. Reeves. Department of Chemical & Biomolecular Engineering, NC State University, Raleigh, NC.

Dorsoventral axis polarity in the developing embryo is largely determined by the transcription factor Dorsal prior to gastrulation. Dorsal acts as a morphogen by promoting gene expression along the ventral half of the embryo causing cells to adopt certain fates, leading to patterning of the endoderm, mesoderm, and neurogenic ectoderm. The classical morphogen hypothesis suggests that Dorsal targets are regulated in a concentration-dependent fashion, and that changing the concentration of Dorsal by altering its dosage will disturb the spatial pattern of target gene expression. However, recent work has shown (1) that the Dorsal concentration gradient is not simply proportional to the number of Dorsal alleles and (2) that gene expression is robust with respect to changes in the Dorsal gradient. These results are not explainable under the current model. In light of the fact that feedback loops provide many gene regulatory networks with robustness, we are building a more comprehensive model that includes possible regulatory interactions between Dorsal and its target genes. Our first step was to illuminate the effects of Cactus, a suspected Dorsal target that is also a Dorsal inhibitor, in regulating the shape of the Dorsal gradient. This and other potential feedback mechanisms, such as a Dorsal-Dpp interaction, may further explain experimental results. Developing a more accurate model will allow us to predict the outcomes of future studies and lead us to a deeper understanding of this complex system.

803C

Transcriptional Twister: characterizing the plasticity of a bipartite TCF binding motif. Hilary Cara Archbold[1], Ken M. Cadigan[1,2]. 1) Cellular and Molecular Biology Program, University of Michigan, Ann Arbor, MI; 2) Department of Molecular, Cellular and Developmental Biology , University of Michigan, Ann Arbor, MI.

In *Drosophila*, TCF/pangolin acts as a major transcriptional regulator of Wingless (Wg, a fly Wnt) signaling in multiple events that shape the developing organism, and maintain stem cell populations necessary for adult tissue homeostasis. The ability of TCF to bind the correct regulatory elements is critical for proper spatio-temporal expression of target genes. How TCF mediates such a broad range of outcomes is poorly understood. The HMG domain of TCF binds the sequence SSTTTGWW, and previous research in our lab identified a second sequence GCCGCCR (the "Helper site") which is required for activation of multiple Wg targets. Our data support a model where TCF binds HMG and Helper site pairs, via two closely spaced domains, the HMG and the C-Clamp. Surprisingly, spacing and orientation of these two motifs varies both within and between Wingless Response Elements (WREs). My research is focused on understanding how motif architecture regulates TCF binding and/or activity, and using this information to identify new WREs and target genes. Using a luciferase reporter gene system in *Drosophila* Kc cell culture, we have shown that in both synthetic and known target gene WREs, the optimal spacing of the Helper site is orientation specific, is relative to the HMG domain imposed bend of DNA, and can be located either up or downstream of the HMG site. Our bioinformatic analysis has also shown an enrichment of optimal pairs in regions identified as bound by TCF in fly embryos (using a ChIPseq data set from Junion et al. Cell 148: 473). We are currently investigating the correlation between activation levels and binding affinity, and are using site-specific integration to characterize reporter gene expression level and patterns driven by optimal and suboptimal motifs in transgenic *Drosophila*. In addition, with our awareness of optimal motif configuration, we have identified several novel candidate WREs which will be tested for Wg dependent activity in *Drosophila*.

804A

Mapping the cis-regulatory landscape of early embryonic development in Drosophila with hundreds of TFs. C Blatti[1], M Kazemian[1], S Celniker[2], M Brodsky[3], S Sinha[1]. 1) U of Illinois, Urbana, IL; 2) LBL, Berkeley, CA; 3) U Mass Med School, Worcester, MA.

While ModENCODE data enables the genome-wide annotation of potential regulatory elements in Drosophila, it does not generally provide their specific spatial-temporal activity pattern nor identify which transcription factors (TFs) and DNA binding sites drive those patterns. We developed a strategy to produce this type of comprehensive description of the cis-regulatory landscape by modeling TF occupancy from the binding specifies (motifs) for > 300 TFs and by examining sets of genes expressed in ~200 distinct early embryonic expression domains annotated in the BDGP in situ image database. First, we predicted each TF's genome-wide binding profile using a HMM-based motif-scanning method and stage-specific DNA accessibility data. Comparison of these profiles to data from 60 ChIP experiments revealed a high degree of agreement (avg corr coeff >0.6). Next, for each gene set from the ~200 expression domains, we searched for enrichments of predicted TF binding within the regulatory regions. This procedure generated a compendium of > 5000 significant associations between TFs and expression terms with 21% supported by the TF having the associated or a related expression pattern. For this

analysis, we identified TFs and expression terms with systematic biases for regulatory regions that are gene-proximal or distal. Finally, we annotated candidate enhancers, defined as stage-specific open chromatin regions, for the likely expression pattern they drive. To predict a specific pattern from regulatory sequence, we fit a regression model incorporating information from TF binding profiles, TF expression, and our functional associations. Our model accurately recovered REDfly enhancers for 18 separate expression domains. By leveraging available comprehensive sets of TF binding specificities and gene expression patterns, we are able to systematically describe embryonic development in terms of TFs and their target regulatory sequences.

805B

Thermodynamic models predict quantitative expression levels driven by synthetic *cis*-regulatory modules in the *Drosophila* embryo. Daniel K. Bork[1,2], Adam S. Brown[2], Lily Li[2], Robert A. Drewell[2], Jacqueline M. Dresch[1]. 1) Mathematics Department, Harvey Mudd College, Claremont, CA; 2) Biology Department, Harvey Mudd College, Claremont, CA.

Quantitative models of gene expression offer valuable insight into the molecular basis for activation and short-range repression in eukaryotic organisms. High-throughput sequencing and transcriptomics elucidate the sequence-level nature of specific transcription factor binding sites in the *Drosophila* embryo. Thermodynamic models can be used to predict quantitative levels of gene expression given the DNA sequence and concentration gradients of the TFs involved in regulation.

Thermodynamic models can also be fit to quantitative expression data obtained from *in situ* hybridization of synthetic reporter genes under the control of *cis*-regulatory modules, and begin to uncover the nature of TF-induced regulation of gene expression. The use of synthetic constructs with a small number of binding sites decreases the complexity of TF interactions, thus increasing parameter identifiability and model reproducibility. For this reason, we have applied thermodynamic models of gene regulation to synthetic *cis*-regulatory modules designed to investigate the binding strength of specific TFs and the role of certain TF interactions, such as quenching and competition, on short-range repression during early *Drosophila* development.

806C

A synthetic biology approach to investigate conserved regulatory motifs in *Drosophila melanogaster*. Adam S. Brown[1], Daniel K. Bork[1,2], Lily Li[1], Jacqueline M. Dresch[2], Robert A. Drewell[1]. 1) Biology Department, Harvey Mudd College, Claremont, CA; 2) Mathematics Department, Harvey Mudd College, Claremont, CA.

In multicellular organisms, development is a period in which precise gene expression is required to regulate cellular differentiation, leading to faithful production of the adult body plan. Differentiation is mediated by a complex network of genes that are controlled in large part by *cis*-regulatory modules (CRMs). CRMs are segments of non-coding DNA that bind transcription factors to up- or down-regulate expression of their target genes. Recent studies in *Drosophila* have identified minimal conserved motifs, consisting of multiple transcription factor binding sites, within CRMs that are capable of reproducing the function of the module. These functional motifs may represent the underlying molecular mechanism by which *cis*-regulation drives specific gene expression patterns. We are investigating how complex combinatorial activities, such as individual protein-protein interactions, as well as the concerted effort of multiple motifs, control the functional output of a single gene.

To understand the activity of these motifs and how they contribute to overall CRM function we are utilizing a synthetic biology approach, combining bioinformatic predictions, mathematical modeling and *in vivo* reporter gene assays. The goal is to functionally decode the CRM network that controls gene expression along the anterio-posterior axis in the early embryo.

807A

Temporal coordination of two enhancers relies on the modulation of a common inductive signal. Lily S. Cheung[1], Alisa Fuchs[2], David S. A. Simakov[3], Len M. Pismen[3], Giorgos Pyrowolakis[2], Stanislav Y. Shvartsman[1]. 1) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 2) Institute for Biology I, Albert-Ludwigs University of Freiburg, Germany; 3) Department of Chemical Engineering, Technion-Israel Institute of Technology, Israel.

Development is directed by temporal and spatial changes in gene expression. Although it is recognized that changes in gene expression patterns can result from stage-specific use of different enhancers, the mechanisms of coordination between enhancers remain less understood. We explore this question in *Drosophila* oogenesis, at stages when the expression of the transcription factor *broad* (*br*) evolves from an spatially uniform to a more complex, two-domain pattern that determines the formation of the dorsal respiratory structures in the eggshell. We identified two enhancers of *br* activated sequentially during mid-oogenesis that together recapitulate the dynamics of the gene. In addition to their temporal coordination, these enhancers also exhibit complementary spatial patterns due to differential regulation by the EGFR pathway. Here, we use RNAi-mediated downregulation of *br* to show that the activity of the early enhancer is required for the activation of the late one. Using a shorter version of the late enhancer, we also show that this effect does not depend on a direct positive autoregulation. Instead, we propose that modulation of the EGFR pathway by the protein produced by the early enhancer acts as a permissive signal for expression of the late one. We have formulated a computational model combining transient inductive signals with multiple enhancers that supports the feasibility of our mechanism. We use this model to guide our experimental effort, and to investigate the more general question of how a single signaling pathway is used recurrently during development to establish complex patterns of gene expression.

808B

Highly parallel assays of tissue-specific enhancers in whole *Drosophila* embryos. Stephen S. Gisselbrecht[1], Luis Barrera[1,2], Martin Porsch[1,3], Preston W. Estep[4], Anastasia Vedenko[1], Anton Aboukhalil[1,5], Alexandre Palagi[1,6], Yongsok Kim[7], Xianmin Zhu[7], Brian Busser[7], Alan M. Michelson[7], Martha L. Bulyk[1,2,8,9]. 1) Division of Genetics, Brigham & Women's Hospital, Boston, MA 02115; 2) Committee on Higher Degrees in Biophysics, Harvard University, Cambridge, MA 02138; 3) Institute of Computer Science, Martin Luther University of Halle-Wittenberg, 06099 Halle, Germany; 4) TeloMe, Inc., Waltham, MA 02451; 5) Department of Aeronautics and Astronautics, Massachusetts Institute of Technology, Cambridge, MA 02139; 6) Bioengineering Department, Polytech Nice Sophia, University of Nice Sophia Antipolis, 06903, France; 7) Laboratory of Developmental Systems Biology, Genetics and Developmental Biology Center, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892; 8) Dept. of Pathology, Brigham & Women's Hospital, Boston, MA 02115; 9) Harvard-MIT Division of Health Sciences and Technology (HST); Harvard Medical School, Boston, MA 02115.

Understanding transcriptional regulatory networks requires the identification and characterization of cis-regulatory modules (CRMs), DNA sequences which can direct expression of associated genes to a specific cell type and/or developmental stage. Reporter assays for the capacity of a candidate CRM to activate a heterologous promoter have been productive but suffer limited throughput or fail to convey information on cell type specificity. We have developed an assay in which reporter constructs containing a pool of candidate CRMs are introduced in parallel to *Drosophila* embryos along with a common cell-type-specific second marker; candidate CRMs isolated by PCR from FACS-purified double-positive cells can by quantitated by high-throughput sequencing, and their relative abundance compared to those in the input cell population to detect activity in the cell type (or types) of interest.

809C

***REDfly:* The Regulatory Element Database for *Drosophila*.** Marc S. Halfon[1,2,3,4], Jeffrey T. Palmer[2,5], Michael Simich[1,2], Benjamin Des Soye[1,2], Steven M. Gallo[2,5]. 1) Department of Biochemistry, SUNY at Buffalo, Buffalo, NY; 2) NYS Center of Excellence in Bioinformatics & Life Sciences, Buffalo, NY; 3) Department of Biological Sciences, SUNY at Buffalo, Buffalo, NY; 4) Molecular and Cellular Biology Department, Roswell Park Cancer Institute, Buffalo, NY; 5) Center for Computational Research, SUNY at Buffalo, Buffalo, NY.

The REDfly database is a highly-curated portal for *Drosophila cis*-regulatory data containing records for empirically validated *cis*-regulatory modules (CRMs, "enhancers") and transcription factor binding sites (TFBSs) curated from the published literature. REDfly includes all sequences reported as functionally tested in a transgenic reporter gene assay regardless of whether they showed regulatory activity or have activity redundant with other, shorter regulatory sequences. Graphical views show the position of each CRM within its genomic locus, and the location of each CRM with respect to its associated gene is provided. Curation of TFBSs includes sites identified by electrophoretic mobility shift assay (EMSA, "gel shift"), DNAase I footprinting, and high-throughput yeast one-hybrid assays. REDfly currently covers more than 650 publications and contains more than 5450 records of reporter constructs regulating over 500 genes, including over 1800 "minimal" CRMs, and over 2000 TFBSs. Extensive abilities exist for database searching and results filtering. In the coming year we hope to include the ability to search based on developmental stage as well as improved, expanded download capabilities. REDfly provides a comprehensive source of *Drosophila cis*-regulatory data and is a powerful platform to facilitate high-throughput experimental and computational studies of gene regulation. REDfly is freely accessible at http://redfly.ccr.buffalo.edu.

810A

Context-dependent requirements for DNA-binding by Runt in transcription activation and repression. Michael L. Higgins[1], Lisa Prazak[2], J. Peter Gergen[3]. 1) Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, NY. 11794; 2) Graduate Program in Molecular and Cellular Biology, Stony Brook University, Stony Brook, NY 11794-5215; 3) Department of Biochemistry and Cell Biology and the Center for Developmental Genetics, Stony Brook University, Stony Brook, NY 11794-5215.

The initial metameric expression of the sloppy-paired gene is generated in response to regulatory inputs of the pair rule transcription factors Eve, Ftz, Opa, and Runt. These inputs are integrated by two distinct enhancers, the Distal and Proximal early stripe elements, DESE and PESE. The PESE enhancer is repressed by Runt whereas the DESE enhancer can either be activated or repressed by Runt depending on the absence or presence of Ftz. Here we investigate the effects of mutating binding sites for Runt in reporter gene constructs containing these enhancers. When PESE is tested as an autonomous enhancer we find that mutagenesis of Runt sites results in loss of repression in blastoderm stage embryos. However, this de-repression is not apparent in a composite reporter also containing the DESE enhancer. Similarly, mutation of Runt sites in DESE also results in loss of repression when this element is tested autonomously, but has the opposite effect of interfering with Runt-dependent activation in a composite enhancer that contains PESE. These results are discussed in the context of a model whereby Runt plays a central role in modulating competitive interactions between these two enhancers and the slp1 promoter.

811B

Decoding the transcriptional program of epidermal cell morphogenesis. Francois Payre[1,2], Delphine Menoret[1,2], Marc Santolini[3], Isabelle Fernandes[1,2], Jennifer Zanet[1,2], Yvan Latapie[1,2], Pierre Ferrer[1,2], Herve Rouault[3], Vincent Hakim[3], Philippe Besse[4], Ignacio Gonzales[4], Rebecca Spokony[5], Keven White[5], Stein Aerts[6], Serge Plaza[1,2]. 1) Centre for Developmental Biology,

University of Toulouse, Toulouse, France; 2) CNRS, UMR5547, Toulouse, France; 3) Laboratoire de Physique Statistique, ENS, Paris, France; 4) Institut de Mathematique, Toulouse, France; 5) Institute for Genomics and Systems Biology, University of Chicago, Chicago, Illinois, USA; 6) Laboratory of Computational Biology, KU Leuven, Belgium.

Developmental programs are implemented by regulatory interactions between Transcription Factors (TFs) and their target genes. How the Cis-Regulatory-Modules (CRMs) mediating these interactions are built and function during terminal differentiation remains yet poorly understood. We addressed this question in late Drosophila embryogenesis when the finely tuned expression of a Transcription Factor, Ovo/Shavenbaby (Svb), triggers the morphological differentiation of epidermal trichomes. Here we find that Svb regulates a large set of terminal effectors of trichome formation, as deduced from microarray profiling and experimental validation. Combining genome-wide approaches, computational modelling and in vivo functional dissection, we investigated the nature and logic of CRMs directing the expression of Svb-dependent effectors. We find that Svb-responsive CRMs display weak if any clustering of Svb binding sites. In addition, the in vivo function of each site relies on its intimate context, with a critical importance of adjacent nucleotides. Finally, Svb-responsive CRMs display various combinations of additional cis-regulatory elements, which contribute to different levels of activity. Together, these results show that trichome formation is underpinned by an unexpectedly flexible mode of regulation, shedding novel light on the functional organization of CRMs mediating terminal differentiation.

812C

Conserved structure of regulatory regions of the gap genes giant and Krüppel in Drosophila melanogaster and Rhodnius prolixus. Rolando V. Rivera-Pomar[1,2], Andrés Lavore[1]. 1) Centro de Bioinvestigaciones, Univ Nacional del Noroeste de Buenos Aires, Pergamino, Buenos Aires, Argentina; 2) Centro Regional de Estudios Genómicos, Universidad Nacional de La Plata. Florencio Varela, Argentina.

The insects develop in two main ways: short germ band and long germ band embryogenesis. In short germ band insects only the anterior segments are simultaneously specified, while the posterior segments are successively added later on. This mode of development is the most basal and widespread. However, most of our knowledge comes from Drosophila melanogaster, a derived insect with long germ band embryogenesis. Here we analyzed the structure, expression and function of the orthologue genes giant (gt) and Krüppel (Kr) in Rhodnius prolixus, a classical model for insect physiology that is emerging as developmental model due to the sequencing of its genome. Most of the genes that take part of the segmentation process are conserved. We have compared the expression, phenotype and regulatory enhancers of Drosophila, Tribolium and Rhodnius gt and Kr orthologs. By comparative genomic analysis, we have identified a putative regulatory region for Rp-Kr and Tc-Kr using Dm-Kr as a reference. We were able to predict two clusters of binding sites that are coincident with the empirically defined CD1 and CD2 regions. We also show the conservation of putative regulatory regions for Rp-gt. Taken together, the data suggest that the conservation of regulatory regions, at least for gap genes, is not as uncommon as we could have expected for insects largely separated during evolution and with different developmental modes. As the studies of transcriptional regulation in insects are still scarce the data presented here represent a necessary step towards the understanding of the evolution of the segmentation process. This work has been supported by grants from ANPCyT and UNNOBA.

813A

Dissecting the cis-regulatory DNA that controls the POU-domain transcription factor genes, *pdm-1* & *pdm-2*. Jermaine Ross[1,2], Thomas Brody[1], Ward F. Odenwald[1]. 1) Neural Cell-Fate Determinants Section, NINDS, NIH, Bethesda, MD; 2) Brown University, Providence, RI.

While neuroblast (NB) lineage studies have identified transcription factors (TFs) important to cell identity decisions, we currently have only an incomplete understanding of the cis-regulatory elements that control their expression. The comparative genomic programs, EvoPrinter and cis-Decoder (also see Brody et al. abstract), along with transgenic reporter assays, were used to identify, compare, and functionally test these regulatory sequences. Here, we describe the enhancers that regulate the pdm-1 & -2 genes, which encode two POU-domain TF paralogs that perform overlapping functions during CNS lineage development. Thus far, we have identified over 70 enhancers located within a 125 kb region spanning the pdm loci, and have catalogued these enhancers in an online database, cisPatterns, that documents their expression and DNA conservation. These enhancers are functionally autonomous and control different subsets of pdm expression patterns during embryonic, larval and/or adult development, including expression in overlapping but nonidentical patterns during intermediate stages of embryonic and larval NB lineage development. Further, cis-Decoder analysis of the conserved DNA within the NB enhancers identifies shared and unique conserved elements. Site-directed mutagenesis of these sequences reveals that they are important for enhancer function. One of the tested sequences includes the highly conserved 9-mer TAAAAATTG identified in both the pdm-1 & -2 NB enhancers. Based on previous work, this sequence corresponds to the DNA binding site of Castor, a zinc-finger TF that is required for proper pdm expression. We found that deletion of the 9-mer sequence triggers ectopic reporter expression in the cephalic lobes. Currently, we are testing the functional significance of other putative pdm enhancers.

814B

Spatial and temporal regulation of cell adhesion is mediated by discrete regulatory elements in the *delilah* locus. Adi Salzberg, Atalya Nachman, Naomi Halachmi, Nirit Egoz-Matia. Gen/Rappaport Fac Medicine, Technion Israel Ins Technology, Haifa, Israel.

Delilah (Dei) is a transcription factor of the bHLH family that was shown to be an important regulator of cell adhesion in Drosophila. In organs in which sub-groups of cells differentiate into more adhesive and less adhesive cell types, Dei is expressed in the stickier cells, where it is required for inducing βPS-integrin expression. In a simplistic way of thinking, Dei can be seen as a molecular switch that turns on βPS-integrin expression wherever a sticky cell has to develop. If so, it is predicted that *dei*, working as a molecular switch which is turned on in different developmental and physiological contexts, would be able to respond to various signaling pathways and transcription factors. Here we show that, as expected, the regulatory region of dei harbors multiple discrete modules that respond to different transcription factors and drive expression in distinct subsets of the *dei*-expressing cells in different developmental stages. Thus, the *dei* gene provides a molecular platform through which cell adhesion can be regulated at the transcriptional level. The cis-regulatory modules in the *dei* locus are scattered along 9.4kb of DNA located upstream to the transcription start site or within the single intron of the gene. Analyses of these regulatory elements revealed new cell types and tissues that express the *dei* gene. Additionally, the analyses revealed bi-phasic regulation of dei expression in muscles and chordotonal organs and allowed the identification of upstream regulators of dei expression. Further analysis of a specific module that drives expression in attachment cells identified the EGR protein Stripe as a direct regulator of *dei* and shed light on the common mechanism by which chordotonal attachment cells and tendon cells acquire their unique properties.

815C

Quantitative modeling of a gene's expression from its intergenic sequence. Md Abul Hassan Samee[1], Tara Lydiard-Martin[2], Angela DePace[2], Saurabh Sinha[1,3]. 1) Dept of Comp Sci, Univ of Illinois, Urbana, IL; 2) Dept of Systems Biology, Harvard Medical School, Boston, MA; 3) Institute for Genomic Biology, Univ of Illinois, Urbana, IL.

Modeling a gene's expression from its intergenic locus is a fundamental goal in computational biology. Owing to the distributed nature of *cis*-regulatory information and the poorly understood mechanisms that integrate such information, gene locus modeling is a more challenging task than modeling an individual enhancer. Here we report the first quantitative model of a gene's expression pattern as a function of its intergenic locus. We model the expression readout of a locus in two tiers: 1) combinatorial effect of transcription factor (TF) binding sites within each enhancer is predicted by a thermodynamics-based model and 2) contributions from enhancers are linearly combined to fit the gene expression pattern. The model works without any prior knowledge of the locations of enhancers. We show that the model accurately fits the expression patterns of the genes *eve, h, run,* and *gt* along the A/P axis of the *Drosophila* embryo. Our results suggest that there are sequence segments, located in inaccessible regions of the locus, that have ectopic expression readouts and were explicitly avoided by the model. We applied our model to identify the TFs forming the stripe boundaries of the studied genes. The resulting networks of TF-stripe relations show remarkable agreement with the known regulatory influences on these genes. We also developed a computational framework using the model to probe for interactions between enhancers of a gene. We found that enhancers of our studied genes tend to act autonomously to drive the respective gene expression patterns. Finally, we show that our model is able to explain the readouts from a novel set of constructs where the *eve* 3/7 and the *eve* 4/6 enhancers were fused in different orientations and with different spacers at the junctions, while existing models of enhancer function failed to do so.

816A

Transcription factor collaboration at the intersection of growth and patterning in the Hippo signaling pathway. Matthew Slattery[1], Roumen Voutev[2], Lijia Ma[1], Nicolas Negre[1], Kevin White[1], Richard Mann[2]. 1) Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL; 2) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

The Hippo pathway has recently emerged as a key regulator of cellular proliferation. A downstream effector of the pathway, the transcriptional coactivator Yorkie (Yki), is an essential mediator of Hippo-regulated proliferation and is required for cell survival and proliferation in all imaginal discs. Yki lacks a DNA binding domain and must partner with sequence-specific DNA binding proteins in the nucleus to regulate gene expression. Two well-characterized Yki binding partners are the developmental regulators Scalloped (Sd) and Homothorax (Hth), which are required for cell survival and proliferation in the wing and eye, respectively. To better understand tissue specific gene regulation by these transcription factors at the downstream end of the Hippo pathway, we performed genome-wide chromatin immunoprecipiatation experiments for each factor in both the wing and eye-antenna imaginal discs. Strong, tissue-specific binding patterns are observed for Sd and Hth, while Yki binding is remarkably consistent across these two tissues. Importantly, binding events common to the eye and wing are also present for Sd and Hth; these general binding events are associated with genes regulating cell growth and proliferation, and can account for the vast majority of Yki binding. Tissue-specific binding events for Sd and Hth are consistent with developmental roles in the given tissue. These genome-wide binding data have also led to the identification of dozens of tissue-specific enhancers. Overall these results suggest that the transcription factors Sd and Hth use distinct binding strategies - one general and associated with Hippo signaling, the other tissue-specific and associated with developmental patterning - to regulate the distinct gene sets during development.

817B

A screen for developmental enhancers targeted by the Notch effector Su(H). Elizabeth K. Stroebele[1], Andrew Brittain[1], Xin Yuan[1], Seth Brown[2], Albert J. Erives[1]. 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Carver College of Medicine, University of Iowa, Iowa City, IA.

Notch signaling regulates gene expression in cells undergoing asymmetric division and those forming borders between adjacent epithelial territories. Notch receptor-ligand interactions result in the nuclear translocation of Notch intracellular domain. A single transcription factor, Su(H), is thought to be a common transducer of NICD signaling in development. Su(H) binding sites are found in a set of neurogenic ectoderm enhancers (NEEs) characteristic of the *vnd*, *brk*, *rho*, and *vn* loci of diverse *Drosophila* species. These NEEs possess precisely-linked binding sites for Dorsal and Twist, and a separate, more loosely-linked binding site for Su(H). While the former spacing controls the dorsal extent of expression, the Su(H) site seems to control the strength of expression. To understand better how Su(H) works with different co-factors in different contexts, we wish to identify other functional enhancer families with Su(H) binding sites. We conducted a computational screen for conserved, non-coding sequences harboring Su(H) sites. We identified all such sites in 4 divergent *Drosophila* species. To detect conserved modules centered on these sites, we devised a reciprocal BLAST method optimized for regulatory regions (R-BLAST) using the NEEs as internal positive controls. R-BLAST is robust to the patterns of change seen at enhancer sequences such as frequent indels, elevated microsatellite content, and high binding site turnover that destroys serial sequence alignment. We find 1,128 regions that are conserved across the genus, and are likely to represent conserved developmental enhancers targeted by Notch signaling. We explored bioinformatic methods to determine the extent to which the NEE subset could be detected in principle given no knowledge of their binding sites other than those for Su(H). We cannot yet detect the NEEs de novo, but we identified and tested sequences from a subset of 346 modules containing an over-represented DNA-bending element.

818C

Transcriptional regulation of the *unpaired3* gene during *Drosophila* development. Yu-Chen Tsai, Hsin-Yi Huang. Dept Life Science, Tung-hai Univ, Taichung, Taiwan.

Unpaired3 (Upd3) regulates Janus Kinase/ Signal Transducers and Activators of Transcription (Jak/STAT) signaling during *Drosophila* eye development, gonad development, lymph development and in immune responses. It has been shown that *upd3* is expressed in lymph gland to maintain cell fate of prohemocytes. *upd3* is induced after septic injury and oral infection in midgut to maintain homeostasis of intestine in the larval stage. From our observation, we found the *upd3* null mutant has small eye. *upd3* is expressed in eye-antenna disc in the larval stages. In this study, we focus on the transcriptional regulation of the *upd3* gene and further analyze the upstream signaling of the *upd3* gene. The *upd3* enhancers were analyzed in 19.2 Kb genomic regions around the *upd3* gene. These *upd3* genomic fragments are cloned to pH-stinger, an enhancer-testing vector containing a GFP reporter. These enhancer-testing constructs are microinjected into fly embryos and then select for transgenic lines.The GFP reporter was examined in different developmental stages. We found *upd3* is expressed in the eye-antenna, lymph glands and midgut. Based on this study, the possible regulation of the *upd3* gene was further elucidated during *Drosophila* development.

819A

Chromatin and Transcriptional Regulation in Drosophila Salivary Gland Development. Michael B. Wells, Deborah J. Andrew. Department of Cell Biology, Johns Hopkins University, Baltimore, MD.

Salivary gland development involves an intricate balance of combinatorial signaling inputs over discrete and prolonged intervals. The molecular players that contribute to this process are known, but less is understood about how the genes encoding these proteins are regulated. Using available high-resolution data, we have begun to characterize the chromatin and transcriptional landscape of several genes important to salivary gland development at multiple developmental stages. These data strengthen previous reports that *fkh* regulation involves the balance between Polycomb and Trithorax signaling, and indicate that regulation of this balance is, in fact, critical at the loci of most components of salivary gland development. They also suggest that RNA polymerase II pausing plays a role in establishing the proper timing of transcription of several important salivary gland contributors (*crb*, *CrebA*, *hth*, *Scr*, and *tsh*). Further, these data indicate that differential enhancer utilization and nucleosome remodeling are important to proper expression of the salivary gland gene *sage*. We are currently exploring these findings in more detail using molecular biology methods including immunoFISH, RT-qPCR, and *in situ* hybridization. Finally, we are also determining whether this regulation remains important in adult flies for salivary gland maintenance as well as the extent to which salivary gland development and this chromatin and transcriptional regulation are conserved with respect to *Drosophila melanogaster* in the mosquito malarial vectors *Anopheles gambiae* and *Anopheles stephensi*.

820B

Regulatory architecture of the *Drosophila* IAB7b enhancer. Lauren N. Winkler[1], Jessica S. Kurata[1], Michael J. Nevarez[1], Lily Li[1], Jacqueline M. Dresch[2], Robert A. Drewell[1]. 1) Biology Department, Harvey Mudd College, Claremont, CA; 2) Mathematics Department, Harvey Mudd College, Claremont, CA.

In *Drosophila*, cellular identity along the antero-posterior axis falls under the control of two homeotic (Hox) gene complexes. The 330 kb bithorax complex (BX-C), which regulates cell type differentiation during development in the posterior thorax and abdomen, comprises three Hox genes: *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*). The expression of each of these genes is in turn controlled through interactions between transcription factors (TFs) and a number of *cis*-regulatory modules (CRMs) in the neighboring intergenic regions. We sought to determine how the sequence architecture of TF binding sites mediates the functional activity of a CRM.

To address this issue, we are investigating the IAB7b enhancer, which regulates *Abd-B* expression in the seventh abdominal segment (A7/PS12) of the early *Drosophila* embryo. A cross-species comparison of the IAB7b enhancer reveals a conserved motif containing two FUSHI-TARAZU (FTZ) TF binding sites in close proximity to two KRUPPEL (KR) binding sites. This signature motif is capable of driving reporter gene expression in A5, A7, and A9. We investigated the ability of the transcriptional repressor KNIRPS (KNI) to suppress reporter gene expression in A5. Additionally, we examined the functional importance of the spacing between the two FTZ binding sites using both computational and molecular genetic experimental approaches. Our studies demonstrate that the transcriptional output of the IAB7b CRM relies on a complex set of combinatorial inputs mediated by TF binding.

821C

Mechanisms Controlling *repo* Transcription. Jamie L. Wood, Bradley W. Jones. Dept. of Biology, University of Mississippi, Oxford, MS.

Expression of the glial specific gene *repo* is required for proper glial cell development in *Drosophila*. *repo* is initially activated by the transcription factor Gcm, but other factors that act to maintain *repo*expression are currently unknown. We propose one of these factors is Repo protein acting in an autoregulatory manner. We have identified a region of the *repo cis*-regulatory DNA that produces ectopic epidermal expression when tested in a *UAS-repo/Act5C-Gal4* system, indicating Repo can interact with this portion of the regulatory DNA. We have previously characterized this same fragment as producing epidermal reporter staining. We are also testing mutagenesis of putative Repo binding sites in the *cis*-regulatory DNA to determine if ectopic expression can be disrupted using the same UAS/Gal4 lines.

822A

Deciphering the *cis-trans* regulatory circuit mediating RTK/RAS signaling in visceral muscle founder cell specification. Yiyun Zhou[1,2], Emily Deutschman[1,2], Jean-Daniel Feuz[5], Korneel Hens[5], Bart Deplancke[5], Marc S. Halfon[1,2,3,4]. 1) Dept. of Biochemistry, SUNY-Buffalo, Buffalo, NY; 2) NYS Center of Excellence in Bioinformatics & Life Sciences; 3) Dept. of Biological Sciences, SUNY-Buffalo, Buffalo, NY; 4) Mollecular and Cellular Biology Depatment, Roswell Park Cancer Institute, Buffalo, NY; 5) Laboratory of Systems Biology and Genetics, Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

Receptor Tyrosine Kinase (RTK) signaling plays a crucial role in many developmental processes and diseases. In both the somatic and visceral mesoderm of the *Drosophila* embryo, the RTK/Ras/MAP Kinase signaling pathway is required to specify the muscle founder cells. These founder cells (FCs) then fuse with fusion-competent myoblasts in order to form multinucleate muscle fibers. Although the well-established Ras effector Pnt is typically assumed to be the primary transcription factor acting in FC specification, we have established that FC specification in the visceral mesoderm is mediated by a Ras-dependent but Pnt-independent pathway. Analysis of an FC-specific transcriptional enhancer for the *mib2* gene has identified putative transcription factor binding sites required for Ras-dependent FC specification. Mutagenesis of these sites leads to an expansion of FC markers throughout the trunk visceral mesoderm, suggesting a possible derepression model for RTK-based induction of FC fates. Recently-performed high-throughput yeast one-hybrid screening has yielded a number of potential transcription factors that bind to these sites as well as elsewhere on the *mib2* enhancer and on FC enhancers from additional FC-specific genes including *duf/kirre*, *org-1*, and *Hand*. We are continuing to investigate the roles of these factors in RTK/RAS signaling during visceral mesoderm development and to detail the *cis-trans* circuitry of this important developmental pathway.

823B

Genetic dissection of the *Mcp* regulatory element from the BX-C. Mario A Metzler[1], Daryl Gohl[2], Paul Schedl[3], Martin Müller[1], Markus Affolter[1]. 1) Biozentrum, Universität Basel, Basel, Switzerland; 2) Stanford University, Stanford, USA; 3) Princeton University, Princeton, USA.

Miscadestral pigmentation (*Mcp*) refers to a few dominant *Abd-B* gain-of-function alleles. Karch et al showed that they consist of small overlapping deletions defining an interval of less than 1 kb located between regulatory regions *iab4* and *iab5*. It was proposed that *Mcp* functions as a boundary or insulator element which enables *iab4* and *iab5* to function independently of each other. Over the past 20 years, *Mcp* function has been dissected using various transgene assays. Several labs have demonstrated that *Mcp* (1) contains a Polycomb Response Element (PRE), (2) can act as an enhancer blocker, (3) can mediate long-distance interactions between Mcp elements located several megabases apart or even on different chromosomes. More recently, Pirrotta and Georgiev et al have shown that the latter two activities might co-localize and be separable from PRE function. We have established a φC31-dependent assay system which allows us to easily study the function of many mutated *Mcp* fragments. They are introduced ~400bp upstream of the *apterous* promoter, separating it from its wing enhancers. Similar to the well-studied *su(Hw)* insulator, wild-type *Mcp* interferes efficiently with enhancer-promoter interaction which results in characteristic *apterous* wing phenotypes. Furthermore, this *Mcp* insert also mediates efficient long-distance interactions with other *Mcp*-containing insertions on the second chromosome. Transgenic lines for many small deletions in the context of a ~800bp *Mcp* fragment have been established. Data on their activities in the enhancer blocker and the long-distance interaction assays will be presented. We hope that these experiments may give further insights into the working mechanism of the *Mcp* insulator.

824C

Expression of Epigenetic Reporters During Wound Closure in *Drosophila* larvae. Aimee E. Anderson, Sirisha Burra, Michael J. Galko. Biochemistry and Molecular Biology, University of Texas MD Anderson Cancer Center, Houston, TX.

Epidermal wound closure is essential to reestablish a barrier between the animal and the outside environment. Proper closure requires a complex and tightly orchestrated series of events including coordinated changes in gene expression. Chromatin modification is one likely mechanism by which the expression of multiple genes can be altered simultaneously but a comprehensive and functional study of the role of chromatin modifying factors during wound closure has not been undertaken in any system. Profound chromatin modifications are mediated by two major groups of proteins: the repressive Polycomb Group (PcG) factors and the activating trithorax Group (trxG) members. In mice, certain PcG members are down regulated at the wound edge, while expression of some trxG factors is increased. These data suggest that the acute stress of wounding provokes a transcriptional response that requires regulation at the level of chromatin. To understand the role of epigenetic factors in wound closure, we undertook a reporter screen to identify histone-modifying proteins whose expression levels change in response to wounding. We screened 55 publicly available GFP- or YFP-tagged protein traps (targeting 33 distinct polypeptides) from the FlyTrap and Cambridge Protein Trap Insertion collections, for up- or down-regulation in wound-edge epidermal cells four hours after wounding. Remarkably, we identified 16 lines, representing seven distinct proteins, whose expression was sharply reduced in the nuclei of epidermal cells adjacent to the wound edge. These include proteins with known roles in both transcriptional repression (Mi-2, Sin3A, Sap130, and Mip120) and activation (Spt6, Kismet and Osa). These results suggest that epigenetic regulation of wound closure is a complex process requiring both up and down regulation of target genes, and implicates protein degradation as a regulatory mechanism in this process. We hope to be able to report on functional analysis of some of these genes, as well as whether reporter down-regulation is controlled by known wound closure pathways such as JNK and Pvr signaling.

825A

Goodness of fit: structural equation modeling methods to reconcile gene regulatory networks. Justin Fear[1,2], Daniel Campo[3], Matthew Salomon[3], Sergey Nuzhdin[3], Lauren McIntyre[2]. 1) Genetics & Genomics, University of Florida, Gainesville, FL; 2) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 3) Section of Molecular and Computational Biology, Department of Biological Sciences, University of Southern California, Los Angeles, CA.

Gene regulatory networks (GRN) have moved to the forefront of methodologies providing insight into gene regulation and transcriptional response. By building GRNs from the "bottom-up", decades molecular knowledge can be incorporated into network structure, while reducing the dimensionality of the problem. The majority of these molecular studies have used gene perturbation to painstakingly dissect regulatory networks, resulting in potential differences between hypothesized GRNs and those found in non-perturbed populations. How best to reconcile these differences and compare the topology of GRNs conducted under different experimental conditions is a complex problem, made more difficult by the number of possible comparisons among any pair of experiments. In order to address this problem a formal test for the goodness of fit is needed. Using structural equations modeling, we examine several goodness of fit statistics that have been previously developed in other fields, and test their application to GRNs. We demonstrate the utility of our approach using RNA-Seq data from a *D. melanogaster* heterozygous panel. Focusing on the InR/tor pathway, we compare the fit between virgin and mated female head tissue. We identified statistically significant differences in transcriptional regulation between these two experimental conditions. We also identified several gaps in the hypothesized network and searched for candidates to include in the GRN. This approach can be used to compare GRN topology between experimental conditions.

826B

Beyond Codon Usage Bias: The Regulation of Translation Encoded in Synonymous Sites. David S. Lawrie[1], Dmitri A. Petrov[2]. 1) Genetics, Stanford University, Stanford, CA; 2) Biology, Stanford University, Stanford, CA.

Using the signature of selective constraint from polymorphism data in the synonymous sites of *D. melanogaster*, we identify synonymous codons favored by strong purifying selection. While these favored codons often overlap with "optimal" codons as defined by canonical codon bias, "non-optimal" codons can be under greater selective constraint. Meanwhile, those codons rarely used in the *D. melanogaster*genome appear to be evolving neutrally. Evolutionary analysis over the Drosophila phylogeny supports the existence of at least two selective forces operating on synonymous sites: one force, a weak force, drives the signature of codon bias and preferentially conserves "optimal" codons; a second selective force, a strong force, favors both optimal and non-optimal codons. This strong constraint is enriched in highly expressed genes, particularly so in genes expressed in mid-late embryonic, pupal, and adult stages of development. We plan to further investigate the signal of constraint in the context of riboprofiling data detailing translationally fast and slow sites.

827C

Drosophila *Myb* represses retrotransposition and regulates DNA copy number. Juan Santana[1], Abby Long[2], Kealie Rogers[2], Stephen Butcher[2], Scott McDermontt[2], J Robert Manak[1,2,3]. 1) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA; 2) Department of Biology, University of Iowa, Iowa City, IA; 3) Department of Pediatrics, Carver College of Medicine, University of Iowa, Iowa City, IA.

c-*Myb* is a proto-oncogene associated with leukemias and lymphomas in birds and mammals. Vertebrates have three representatives of the *Myb* gene family consisting of A-, B- and c-*Myb*, all of which encode DNA-binding factors that are

important for the proper expression of large numbers of genes including those that regulate cell cycle progression and cell differentiation. *Drosophila melanogaster*contains a single *Myb* gene (Dm-*Myb*), mutants of which die before reaching adulthood. Small interfering RNA (siRNA) knockdown of Dm-*Myb* in an embryonic *Drosophila* cell line was shown to prevent the proper expression of genes with prominent roles in coordinating cell division. Along the same lines, Dm-*Myb* mutant larvae display cell cycle defects such as aneuploidy, aberrant spindle formation, and abnormal numbers of centrosomes. Dm-Myb protein is present in a complex which includes the nucleosome remodeling factor NURF. Through yeast two-hybrid experiments and genetic screens, we show that Dm-Myb is specifically interacting with the major subunit of NURF (NURF301) and that these proteins are co-regulating a large number of genes, including those involved in cell cycle control. More surprising, we show that Dm-Myb and NURF301 are working in concert to repress transcription of LTR retrotransposons, and failure to do so results in large-scale transposition events in the genome. Finally, we show that both proteins are required to repress DNA replication of a multitude of genomic regions in polytene tissue nuclei. Collectively, these data have allowed us to identify two new roles for Dm-*Myb*in controlling large-scale genomic processes which, when compromised, lead to deleterious effects on genome function.

828A

Investigating context-dependent transcription factor binding in early *Drosophila* development. Jessica L. Stringham[1], Adam S. Brown[2], Robert A. Drewell[2], Jacqueline M. Dresch[3]. 1) Computer Science Department, Harvey Mudd College, Claremont, CA; 2) Biology Department, Harvey Mudd College, Claremont, CA; 3) Mathematics Department, Harvey Mudd College, Claremont, CA.

Gene expression in the *Drosophila* embryo is controlled by functional interactions between protein transcription factors (TFs) and DNA *cis*-regulatory modules (CRMs). These interactions are mediated by the binding of TFs to specific sequences in CRMs. The binding site sequences for any TF can be experimentally determined and represented in a position-weight matrix (PWM). PWMs can then be used to predict the location of TF binding sites in other regions of the genome. Serious limitations to this approach are that often we only have a few examples of confirmed binding sites and the sites are frequently less than 10bp in length. As a result, the information content in a PWM is often less than optimal, leading to an inability to make accurate predictions.

Analysis of a large number of CRMs that control transcription of target genes along the anterio-posterior axis of the embryo reveals the presence of blocks of evolutionarily conserved sequence that extend beyond the predicted TF binding sites. In this study we are examining the function of these flanking sequences. In particular, we are using computational approaches to determine whether the flanking sequences potentially enhance the specificity of particular TF binding. Expanding PWMs to include context-dependent transcription factor binding will allow us to functionally dissect CRMs and significantly expand the information content in PWMs.

829B

An integrated image-to-mesh conversion and machine learning framework for gene expression pattern image analysis. Wenlu Zhang[1], Daming Feng[1], Andrey Chernikov[1], Nikos Chrisochoides[1], Sudhir Kumar[2,3], Shuiwang Ji[1]. 1) Department of Computer Science, Old Dominion University, Norfolk, VA; 2) Center for Evolutionary Medicine and Informatic, The Biodesign Institute, Arizona State University, Tempe, AZ; 3) School of Life Sciences, Arizona State University, Tempe, AZ 85287.

Analysis of the spatiotemporal gene expression patterns is essential for understanding the regulatory networks driving development. We made use of the in situ images from the Berkeley Drosophila Genome Project to study the gene regulations during early Drosophila embryonic development. Previous image-based methods are limited to image registration using a perfect ellipse and clustering of the embryonic locations or the genes into groups separately. In this work, we created a highly flexible mesh generation framework for performing image to mesh conversion and non-rigid image registration. Our methods provide a faithful representation of the developing embryos by incorporating the distortions on the images. Based on the common coordinate framework resulted from the non-rigid registration, we proposed a soft co-clustering machine learning method to co-cluster genes and embryonic locations with similar expression patterns. Experimental results indicate that our image registration approaches are more accurate than prior method. Additionally, simultaneous clustering of genes and embryonic locations leads to more biologically significant results.

830C

Groucho mediates a subset of Capicua repressor activities in *Drosophila*. Leiore Ajuria[1], Claudia Nieva[1], Marta Forés[1], Rona Grossman[2], Sergio González-Crespo[1], Ze'ev Paroush[2], Gerardo Jiménez[1,3]. 1) IBMB-CSIC, Parc Científic de Barcelona, Barcelona, Spain; 2) Dept. of Developmental Biology and Cancer Research, IMRIC, The Hebrew Univ., Jerusalem, Israel; 3) ICREA, Barcelona, Spain.

The HMG-box protein Capicua (Cic) is a general sensor of RTK-Ras-MAPK signaling pathways. In different patterning systems, Cic represses genes regulated by RTK signaling; following RTK activation, Cic repression is relieved and this allows the expression of responsive genes. We are investigating the mechanisms by which *Drosophila* Cic represses transcription, and their potential conservation in mammals, where Cic has been implicated in cancer and neurodegeneration. Using a combination of genetic and molecular assays, we find that Cic repression in the blastoderm embryo is strictly dependent on the Groucho (Gro) corepressor. This Cic-Gro interaction may not involve a direct physical association, since we do not observe

co-immunoprecipitation of both proteins under conditions where Gro binds efficiently to other repressors. In contrast, Cic repressor activity does not require Gro in tissues such as the wing or the ovarian follicular epithelium. In all cases, the highly conserved C1 motif of Cic is essential for repression. Thus, Cic displays both common and context-specific requirements to perform its multiple repressor functions in development.

831A

The Role of *Dbcl11* in *Drosophila* Muscle Formation. Wiley Barton, Jennifer Elwell, Erica Baca, Richard Cripps. Biology, University of New Mexico, ALbuquerque, NM.

Myoblasts, the precursors to mature muscles, undergo mitotic proliferation until specific cues initiate differentiation. From the expression pattern of *Dbcl11* within muscle specific tissue, we have identified the gene as a potential regulator for this critical developmental process. Overexpression of *Dbcl11* resulted in irregular muscle formation that was observed through the visualization of these structures in late stage embryogenesis. Electrophoretic mobility shift assay (EMSA) was used to demonstrate that *Dbcl11* protein adheres to a known binding site for the mammalian ortholog of the gene, suggesting the capacity for molecular interaction with other genes. *Dbcl11* was shown to regulate *Dap* (*dacapo*), a *Drosophila* gene that encodes an inhibitor of cyclin-dependant kinase similar to *p21*, a target of the mammalian homolog of *Dbcl11*. In situ hybridization of late stage embryos with upregulated *Dbcl11* illustrated reduced levels of *dap*, indicating its inhibition by *Dbcl11*. Our results reveal an influential role of *Dbcl11* in the process of muscle development. Due to the high conservation of these genes, our studies will yield insight into the process of human muscle development.

832B

The influence of hairpin RNA against *lawc* on the expression of overlapping *lawc* and *Trf2* genes in *D. melanogaster*. Olga B. Simonova, Roman O. Cherezov, Julia E. Vorontsova, Ilya B. Mertsalov, Dina A. Kulikova. Genetics of Morphogenesis, Koltzov Institute of Developmental Biology, Moscow, Russian Federation.

Two *Drosophila* genes - *leg-aristae-wing complex* (*lawc*) and *TBP related factor 2* (*Trf2*) - share exonic overlap and are in opposite chromosomal orientation. We used genetic system of *lawc/Trf2*gene complex as convenient model for *in vivo* researching the relationship between sense transcripts and the complementary antisense transcripts of the overlapping genes that regulate development in*Drosophila*. In order to investigate the interaction of sense and antisense transcripts we have tried to suppress an expression of the antisense *lawc*-transcripts using the method of RNA-interference gene inactivation. The transgenic constructions that express long hairpin RNA that subsequently cleaved into siRNAs and are capable to induce a knock-down of the antisense *lawc*-transcripts have been created and *Drosophila* transgenic lines have been finally generated. The activation of constructions in vivo in two-component UAS/GAL4 system led to fly death with high frequency. The comparative analysis of*lawc* and *Trf2* gene expression has yielded unexpected result: level of an expression of the antisense *lawc*-transcripts in the conditions of their suppression has increased 3 times while level of *Trf2* gene expression, on the contrary, has been down-regulated 4 times. Ectopic expression of *Trf2* cDNA rescues *lawc* RNAi-induced lethality and have confirmed result. We concluded that sense and antisense*lawc/Trf2* transcripts are discordant self-regulated. Data obtained showed that the RNAi of one of overlapping genes may cause a boomerang effect. This fact is very important for considering, as the list of human and vertebrates overlapping genes continually enlarged, and as it is predicted, "switch of" genes by using of the RNAi becomes one of approaches to treatment of some genetic diseases.

833C

Transcription factors FTZ-F1 and Blimp-1 control the pupal development and eclosion timing in *Drosophila*. Abdelrahman Sayed Sultan[1*], Hitoshi Ueda[1,2]. 1) THE GRADUATE SCHOOL OF NATURAL SCIENCE AND TECHNOLOGY, OKAYAMA UNIVERSITY, OKAYAMA, JAPAN; 2) BIOLOGY DEPARTMENT, FACULTY OF SCIENCE, OKAYAMA UNIVERSITY, OKAYAMA, JAPAN.

FTZ-F1 is a member of the nuclear hormone receptor, induced after ecdysone pulse and expressed transiently during the development. Blimp-1, an ecdysone inducible transcriptional repressor, binds to the promoter region of the *ftz-f1* gene and plays an important role in determining the precise timing of *ftz-f1* expression during prepupal period. We have shown that the temporally restricted expression of FTZ-F1 is important for prepupal development of *Drosophila*. We also have shown that FTZ-F1 is expressed throughout the pupal development, with especially high levels during late pupal stage and disappeared slightly before eclosion. However, the regulation mechanism and function of FTZ-F1 during the pupal development remain unknown. We examined effects of *blimp-1* and *ftz-f1* knockdown and over expression by using GAL4/UAS system. We found that *blimp-1* knockdown in the whole body induces premature expression of FTZ-F1 and advancing of pupal development and eclosion timing. Interestingly, we also found that *blimp-1* knockdown in fat body, an organ that retains endocrine and storage functions of vertebrate liver and adipose tissue, induces advancing of pupal development and eclosion timing. Moreover, *blimp-1* over expression in fat body induces delaying of pupal development and delaying or inhibition of eclosion. On the other hand, *ftz-f1* knockdown in fat body induces delaying of pupal development and advancing of eclosion timing. In conclusion, these results reveal that the expression of *ftz-f1* is controlled by Blimp-1 and both of them control the pupal development and eclosion timing.

834A

A novel role of transcriptional repressors on the targets of the Jak/Stat pathway, in larval hematopoiesis. Aditi Vyas,

Soichi Tanda. Dept. of Biological Sciences, Ohio University, Athens, OH.

The Janus Kinase and Signal Transducer and Activator of Transcription (Jak/Stat) pathway is known to control larval hematopoiesis in *Drosophila*. A gain of function allele of the *Drosophila* Jak gene ($hop^{Tum\text{-}l}$) causes hyperactivation of the pathway, leading to a tenfold increase in the hemocyte count and increased differentiation to a specific hemocyte type called lamellocytes. Decreasing the negative regulator, phosphatase 61F in the $hop^{Tum\text{-}l}$ background, causes a further increase in the pathway activity. The total cell count of these larvae is reduced by half and there is a four fold greater differentiation to lamellocytes, when compared to $hop^{Tum\text{-}l}$ larvae. We hypothesize that there are two sets of Stat92E target genes; low threshold genes that are turned on at moderate levels of pathway (i.e. $hop^{Tum\text{-}l}$ levels) and high threshold genes that are transcribed at higher levels of the pathway. This phenomenon could result from either of two possible mechanisms. One mechanism predicts that the Stat92E binding sites regulating the transcription of the two sets of genes have different binding affinities for Stat92E. The other mechanism assumes a greater role of transcriptional repressors in regulating the transcription of high threshold genes. To test if transcriptional repressors have a role in regulating Stat92E target genes, we screened loss of function mutants of co-repressors. We found that when larvae carried loss of function alleles of the co-repressor, C-terminal Binding Protein (CtBP) in the $hop^{Tum\text{-}l}$ background, the hemocyte counts were similar to when we increased the pathway activity levels. We then screened the DNA-binding partners of CtBP, and identified Suppressor of Hairless [Su(H)] as a repressor regulating the transcription of the high threshold genes. In another approach, we used a modENCODE dataset for CtBP binding regions and scanned it for the presence of Stat92E and repressor binding sites, using the bioinformatics tool Target Explorer. We generated a list of 32 genes, that lie within 5kb of these identified regions and are now testing them for roles in hematopoiesis.

835B

The transcription elongation factor Spt5 interacts with Pleiohomeotic to mediate Polycomb Group Repression. Barbara H. Jennings, Robert Harvey. UCL Cancer Institute, University College London, London, United Kingdom.

The checkpoint regulated by Positive Transcription Elongation Factor b (P-TEFb) in the early phase of transcription elongation is a critical control point for the expression of many genes. Spt5 interacts directly with RNA polymerase II and has an essential role in establishing this checkpoint, and also for further transcript elongation. Previously, missense mutations have been mapped to the *Spt5* genes in*Drosophila* and Zebrafish that give rise to specific developmental defects, indicating that Spt5 activity can be influenced by contextual factors. Here we demonstrate that Spt5 can physically interact with the Polycomb Group protein Pleiohomeotic in *Drosophila*, and that mutations in *Spt5* and *pho* interact during the processes of adult wing maturation and Polycomb Group (PcG) repression *in vivo*. Our results indicate that Pho interacts with Spt5 to prevent RNAP II transcribing through the P-TEFb checkpoint to maintain PcG repression.

836C

Expression and evolution of lincRNAs in Drosophila pseudoobscura using RNA-Seq. Kevin G Nyberg, Carlos A Machado. Department of Biology University of Maryland, College Park, MD 20742.

Long intergenic noncoding RNAs, or lincRNAs, are known to function in processes like dosage compensation and stem cell regulation, but little is known about how they evolve, especially among closely related species. Here, we use RNA-Seq to identify lincRNAs in Drosophila pseudoobscura and characterize their expression and the selective forces that influence their evolution. We generated sex-specific RNA-Seq libraries at four different developmental stages (1st instar larvae, 3rd instar larvae, pupae, and 7-day adults) and in adult gonads and carcasses. Transcripts were identified and screened for signatures of protein-coding ability to generate a list of putative lincRNAs. LincRNA expression was then characterized in terms of sex, developmental regulation, and tissue localization. With multiple genome lines now publicly available from the pseudoobscura subgroup, we used sequence-based neutrality tests to look for evidence of selection on lincRNAs. We found that many lincRNAs show developmental and sex-specific regulation. In addition, some lincRNAs in D. pseudoobscura show evidence of selection.

837A

A tissue-specific microRNA prevents cellular reprogramming by two master regulator transcription factors. Anna Lyuksyutova, Mark Krasnow. Dept Biochem, Stanford Univ, Stanford, CA.

Direct cellular reprogramming converts one cell type to another, bypassing the pluripotent state usually required to assume a new cell fate. Some aspects of the process, such as tissue-specific variability in direct-reprogramming potential, are not well understood. Here, we examine the reprogramming potential of nine cell types in *Drosophila melanogaster* embryos. We find that two potent reprogramming factors, the master regulators Trachealess(Trh) and Eyeless(Ey), are selectively destroyed in differentiated muscle, thus blocking direct reprogramming in this tissue. An 8bp sequence element within their 3'UTR is necessary for this tissue-specific regulation. The 8bp sequence element is part of a predicted binding site for miR-190, a microRNA expressed in muscle. When Trh is expressed in muscle lacking miR-190, the *trh* mRNA is no longer destroyed. The importance of preventing inappropriate expression of powerful master regulator genes in differentiated tissues is underscored by the significant reduction in locomotion speed in larvae expressing Trh in their muscle. This is a novel aspect of developmental regulation where cells can protect themselves against transcriptional hijacking by specifically destroying potent transcription factors and making these cells refractory to potential reprogramming. .

838B

***Drosophila* miRNA affinity purification for cell-type and tissue-specific miRNA profiling.** Amanda Thomas[1], Weimin Xiao[2], Cristian Coarfa[3], Pei-Jung Lee[1], Esther Jung[1], Gregg Roman[2,4], Preethi Gunaratne[2], Herman Dierick[1,5]. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Biology and Biochemistry, University of Houston, Houston, TX; 3) Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; 4) Biology and Behavior Insitute, University of Houston, Houston, TX; 5) Department of Neuroscience, Baylor College of Medicine, Houston, TX.

MicroRNA expression and function play a wide variety of roles throughout an organism, although little is known about the specificity of microRNAs (miRNAs) in any specific tissue or cell type. The highly heterogeneous and intermixed neuronal subtypes in the fly brain makes cell type specific miRNA analysis even more pertinent in understanding neuronal function. In miRNA biogenesis, Argonaut proteins associate with the fully mature, 21-23 nt miRNAs. A technique, called miRAP (miRNA affinity purification) has been developed in worms and mice and uses tagged components of the RNA induced silencing complex to co-immunoprecipitate mature miRNAs; however, no such system exists for cell-type specific miRNA profiling in flies. We have generated transgenic lines with random insertions of a *UAS-Ago1::GFP* fusion gene to examine the miRNA profiles of specific cell-types when combined with specific GAL4 drivers. Expression of *UAS-Ago1::GFP* with pan-neuronal driver *Elav-GAL4* showed expected tissue wide protein localization of the GFP-tagged Ago1 in the cyptoplasm. Pull down experiments from head extracts of the *Elav-Gal4>UASAgo1::GFP* flies demonstrated a highly reproducible enrichment of a subset of miRNAs in neurons. We are currently using this technique to examine miRNA populations in specific neuronal subtypes with the goal of understanding which miRNAs are important in specific neurons. This newly adapted technology can be used in combination with any GAL4 driver line to examine cell-type specific miRNA expression under different mutant, physiological, and pharmacological conditions.

839C

Visualisation of Ribosomal Subunits Interaction in Drosophila Cells. Akilu S. Abdullahi. Bioscience, University of Birmingham, Birmingham, Birmingham, W. Midlands, United Kingdom.

Abstract The nuclear membrane is a unique feature of eukaryotes. It divides the cell into two main compartments: nucleus and cytoplasm. Specific steps of the gene expression are restricted either to the nucleus or the cytoplasm. Transcription and RNA processing takes place in the nucleus, but translation is restricted to only the cytoplasm. It was therefore believed that there was no direct link between nuclear events such as pre-mRNA splicing and cytoplasmic events such as translation and mRNA degradation. This view has been challenged in recent years by reports that indicate that the nature of the nuclear mRNP also impinges on the cytoplasmic events such as translation and NMD. This research is aimed at further addressing the issue whether ribosomal subunits interact in the nucleus and whether the interaction is translation dependent. Visualization of ribosomal interactions in drosophila cells was carried out based on bimolecular fluorescence complementation technique (BiFC). To visualize association between ribosomal subunits, I have tagged pairs of Drosophila ribosomal proteins (RPs) located in different subunits with mutually complementing halves of Venus fluorescent protein or YFP. Pairs of tagged RPs expected to interact, or be adjacent in the 80S structure, showed strong BiFC fluorescence, but pairs far apart did not. The results obtained showed that translation sites are more apparent in the cytoplasm. However, some percentage of the cells showed a signal in the nucleolus. This signal was found to be enhanced by translation elongation inhibitors and the proportion of cells with nucleolar signal increased. Notably, the nucleolar signal observed was prevented by Pol II inhibition. This technique achieved 80S visualization in both cultured cells and in fly tissues in vivo.

840A

Identification of directly targeted mRNA substrates of the NMD pathway. Alex Chapin, Mark Metzstein. Human Genetics, University of Utah, Salt Lake City, UT.

Nonsense mediated mRNA decay (NMD) is an evolutionary conserved post-transcriptional regulatory pathway which, in addition to targeting mRNAs harboring premature termination codons for rapid degradation, is also necessary for normal gene expression. Mutations in NMD genes result in upregulation of numerous endogenous transcripts and in *Drosophila*, like other complex organisms, NMD genes are vital, likely due to misregulation of certain endogenous targets. However, the identity of the critical target genes is not known. Complicating the discovery of critical NMD targets, is that it cannot be easily distinguished which of the overexpressed genes are directly regulated by the NMD machinery, from those whose expression changes in response to overexpression of direct targets. To address this, we have developed a novel, kinetics-based assay to identify direct targets of NMD in whole animals. In our assay, wild-type *Upf2* (a core component of the NMD machinery) is resupplied to *Upf2* mutant larvae using a heat inducible transgene. The transcriptome of these animals is then monitored over time using microarray analysis. In this experiment, it is expected that levels of direct targets will quickly return to baseline, while levels of indirect targets are restored more slowly. Using this approach, we have identified a candidate set of direct targets. One of these is the evolutionary conserved gene *Gadd45*. *Gadd45* is activated in response to various cellular stresses to mediate growth arrest, apoptosis and transcription of stress related genes. Interestingly, defects found in animals overexpressing only *Gadd45* mirror those of NMD loss-of-function mutants, including cell cycle, and embryonic patterning. We have also found that genomic deficiencies which remove *Gadd45*, or the immediate downstream transducer of *Gadd45* activity,*Mekk1*, can suppress lethality in *Upf2* mutants. Our results suggest that *Gadd45* is one of, if not the, critical downstream effector of NMD-mediated lethality.

841B

Bicaudal-C controls the spatial and temporal expression of the nanos mRNA during Drosophila oogenesis. Chiara Gamberi[1,2], Paul Lasko[2]. 1) Institut des Recherches Cliniques de Montreal, Montreal, PQ, Canada; 2) Department of Biology, McGill University Montreal Canada.

nanos (nos) mRNA encodes a key determinant of posterior embryonic patterning in Drosophila. Expression of Nos is temporally and spatially regulated by several proteins that interact with its mRNA and control its translation and/or its localization to the posterior of the oocyte. We found that Bicaudal-C (Bic-C) interacts directly with an element in the nos 3' UTR, negatively regulating its expression in early to mid-oogenesis through effects on its polyadenylation, stability and translation. Bic-C mutations also cause ectopic accumulation of nos mRNA in the oocyte, possibly identifying an intermediate locale for posteriorly localized mRNAs. In Bic-C mutants, or when expressing nos transgenes that are mutated for the Bic-C interaction site, ectopic Nos is produced that accumulates in large structures in the cytoplasm of the nurse cells. We further show that, unlike in the pole plasm, oskar activity is not required for Nos expression earlier in oogenesis. These results further highlight the exquisite refinement of nos post-transcriptional regulation.

842C

Identification and analysis of RNAs associated with Sm proteins. Zhipeng Lu, Greg Matera. Biology, UNC at Chapel Hill, Chapel Hill, NC., NORTH CAROLINA.

Sm proteins are a family of highly conserved RNA-binding proteins present in all three domains of life. In eukaryotes, Sm proteins bind small nuclear RNAs (snRNAs) to form snRNPs, which are basic components of the pre-mRNA splicing machinery. However, little is known about other functions of Sm proteins in eukaryotic cells, given their divergent roles in regulating mRNA stability in bacteria and archaea. Our lab recently found that Sm proteins form a complex with oskar mRNA and are required to help specify the germline in Drosophila ovaries. This discovery led us to hypothesize that Sm proteins play important, but so far unrecognized, roles in RNA metabolism and fundamental cellular processes in eukaryotes. To test this hypothesis, we developed a strategy to identify Sm-associated RNAs by deep sequencing immunopurified complexes (RIP-seq). Using this approach, we discovered a subset of mRNAs and novel unannotated non-coding RNAs that associate with Sm proteins. The association between mRNAs and Sm proteins is independent of splicing. Many of the Sm-associated mRNAs encode mitochondrial and ribosomal proteins. Furthermore a number of these mRNAs colocalize with Sm proteins in the oocyte cortex, a region rich in mitochondria. Together with our previous results, these findings support the view that Sm proteins function in mRNA transport and/or localized translation. In addition to novel Sm-bound mRNA targets, one of the non-coding RNAs we identified is encoded by a newly-evolved snRNA gene. This young gene is present only within a subset of Drosophilids. We identified a putative base-pairing region with U6 snRNA, suggesting a role for this novel snRNA in splicing regulation.

843A

The NMD gene *Smg5* is required for viability independent of NMD function. Jonathan Nelson[1], Dominique Foerster[2], Stefan Luschnig[2], Mark Metzstein[1]. 1) Human Genetics, University of Utah, Salt Lake City, UT; 2) University of Zurich IMLS, Zurich, Switzerland.

Nonsense mediated mRNA decay (NMD) is a cellular quality control mechanism that targets mRNAs containing premature termination codons for degradation. The NMD pathway is tightly regulated, particularly at the transition from target recognition to decay initiation. This transition is in part regulated by phosphorylation of the key NMD factor Upf1 by the kinase Smg1. Phosphorylation of Upf1 provides platforms for the endonuclease Smg6, which initiates degradation of the target mRNA, and Smg5, which recruits a phosphatase that initiates dephosphorylation of Upf1 and release of the NMD complex from the mRNP.

Our current understanding of the role of Upf1 phosphorylation in NMD function comes from cell culture experiments. However, less is known about the NMD requirements of Upf1 modification *in vivo*. Previous work in *Drosophila* has shown that *Upf1* mutants are lethal, probably due to severe defects in NMD function, while *Smg1* mutants have only minor NMD defects and are viable. We have now found that loss-of-function alleles of *Smg5* are lethal. One possible explanation is that *Smg5* lethality is due to hyperphosphorylation of Upf1 by Smg1, caused by loss of the Smg5-recruited phosphatase, and the subsequent failure of the NMD complex to disassemble and recycle. However, we have found that *Smg1-Smg5* double mutants are still lethal. These data suggest two alternative hypotheses: 1) *Smg5*-mutant hyperphosphorylation of Upf1 can occur through another kinase, or 2) an NMD-independent function of *Smg5* is required for viability. We found that *Smg5* mutants have only weak defects in NMD function, suggesting that lethality of *Smg5* single and *Smg1-Smg5* double mutants is not due to defects in NMD function, but instead due to an unknown NMD-independent function of Smg5. We are currently working to elucidate the contribution of Smg5 to Upf1 phosphorylation status, NMD activity, as well as other NMD-independent functions of Smg5 that may be required for viability.

844B

In vivo interactions of eIF4E in Drosophila cytoplasmic foci. Rolando V. Rivera-Pomar[1,2], Carla Layana[1,2], Paola Ferrero[1,2], Ezequiel Paulucci[1], Pablo Gutierrez[1]. 1) Centro Reg Estudios Genomicos, Univ Nacional de La Plata, Florencio Varela, Buenos Aires, Argentina; 2) Departamento de Ciencias Básicas y Experimentales, Universidad Nacional del Noroeste de Buenos Aires. Pergamino, Argentina.

Eukaryotic translation initiation factor 4E (eIF4E) is required for cap-dependent initiation. In addition, eIF4E occurs in cytoplasmic foci such as processing bodies (PB) and stress granules (SG). We examined the role of key functional amino acid residues of eIF4E in the recruitment of this protein to cytoplasmic foci. We demonstrate that tryptophan residues required for mRNA cap recognition are not required for the recruitment of eIF4E to SG or PB. We show that a tryptophan residue different than the eIF4E-BP interacting domain required for protein-protein interactions is essential for the accumulation of eIF4E in granules. We conclude that protein-protein interactions rather than interactions with the mRNA are essential for the recruitment of eIF4E and for a putative nucleation function. We have also analyzed the interaction of eIF4E with the non-canonical partner Me31B -an ortholog of the helicase rck/p54, Lsm-1 and Cup. We demonstrate by in vivo FRET that the interaction occurs in the cytoplasmic foci between each of the proteins and eIF4E, but not among the interacting proteins. This suggests that eIF4E acts as a simultaneous interactor. With the method used we could not establish id the interactions are simultaneous or they represent subpopulation of interacting molecules in the foci. However, we hypothesize that the interaction may be simultaneous and propose a model for the sequential assembly of the foci in which eIF4E is the common factor for each step. This work has been supported by grants from ANPCyT and CONICET.

845C

Exploring the role of the GW182 protein Gawky during Drosophila early embryogenesis. Jing Li, Andrew Simmonds. Dept. of Cell Biology, University of Alberta, Edmonton, Canada.

The GW182 protein family is composed of multiple proteins having high percentage of glycine-tryptophan (GW) and troptophan-glycine (WG) repetitive amino acid sequences and a RNA recognition motif on their C-terminal. The Drosophila GW182 single homologue, Gawky (Gw), has been shown to participate in the microRNA (miRNA) repression pathway. A mutation in the gw gene results in an abnormal number of centrosome, missegragation of chromosomes and disrupted microtubule network in the nuclear division cycle at an early stage of embryogenesis (0-2hrs after egg deposition). Our work shows Gw interacts with centrosomal structural protein Centrosomin (CNN) and peri-centriolar protein γ-tubulin. The endogenous Gw body is adjacent to centrosome in the early embryo. A subset of transcripts related with centrosomal organization function has been found to associated with Gw and conserved miRNA-277 binding sites were predicted residing in the 3' untranslated regions of 4 transcripts. These results suggested that Gw potentially plays an important role during centrosome organization in nuclear mitosis during Drosoophila early embryogenesis.

846A

Pervasive RNA localization in Drosophila ovaries. Helena Jambor, Pavel Mejstrik, Stephan Saalfeld, Pavel Tomancak. Max Planck Institute of Molecular Cell Biology and Genetics.

Eukaryotic cells use RNA localization coupled with translational control to restrict protein activity. Localized transcripts are found in most species, but their biological role is best understood in the Drosophila ovary, where local mRNAs in the oocyte determine the embryonic body axes. Furthermore, a high number of mRNAs were shown to be asymmetrically distributed in the embryo.

To decipher the importance of prevalent mRNA localization, we started by probing mRNA distributions globally during Drosophila oogenesis. We combined quantitative methods to probe mRNA expression with fluorescent in situ hybridization (FISH) and developed a method for high-throughput egg-chamber isolation, FISH and screening. The collected 3D-datasets are deposited into a public database along with a systematic annotation of the mRNA localization to enable analysis of the data. We analyzed the distribution of over 4000 mRNAs in the ovary, of which 70 percent are expressed and >1000 show a restricted expression domain.

Among the candidates, we find many mRNAs that co-localize with the maternal determinants at the anterior an posterior pole and are now investigating whether their localization requires the known mRNA localization machinery. Importantly, we also observe instances of mRNAs that localize to equivalent subcellular sites in two ovarian cell types, supporting the current view that the RNA localization machinery and localization signals are re-used in different cell types. In addition, we discovered novel sites of subcellular mRNA enrichment, particularly in the nurse cell portion of the syncytial egg-chamber. Ongoing research is focused on determining whether localized mRNAs result in asymmetric protein distributions and discovering over-represented motifs responsible for the post-transcriptional regulation of co-clustered mRNAs.

847B

How piRNA inheritance affects endogenous gene expression across generations. Alexandra A Erwin, Michelle Wickersheim, Justin Blumenstiel. University of Kansas, Lawrence, KS.

Piwi-interacting RNAs (piRNAs) appear to have a dominant role in role in regulating transposable elements (TEs) in the germline. Recently, the role of piRNA regulation has been expanded to include endogenous gene control. We have identified unique clusters of piRNAs that target endogenous genes and that vary between two strains that cause the syndrome of hybrid dysgenesis in *Drosophila virilis*. We analyzed how the inheritance of these gene-targeting piRNAs influence gene expression across generations. This serves to test whether the requirement for piRNA priming through the maternal germline - as has been demonstrated for TEs - also applies to endogenous genes targeted by piRNAs. Preliminary studies have shown that there are diverse modes of action for gene-targetting piRNAs across generations. In addition, by filtering out the genes that may be regulated by the piRNA silencing pathway, we seek to identify genes whose expression is modified by the response to the hybrid dysgenic syndrome independent of changes in gene expression attributed to shifts in piRNA control.

848C

Mutations affecting piRNA system components alter snRNA levels in *Drosophila* ovaries. Alina P Korbut, Sergey Lavrov, Vladimir Gvozdev. Institute of Molecular Genetics, Moscow, Russian Federation.

It is well known that piRNAs silence transposons and other types of repeats in the animal germline. Several proteins play crucial roles in the process of piRNA-mediated silencing, e.g., Argonaute proteins of Piwi subfamily, RNA helicases, and others. Piwi is one of the central proteins of the namesake pathway (piRNA for Piwi-interacting RNA). Importance of Spn-E and armitage RNA helicases is also well-established, although details concerning particular functions of those proteins in the pathway remain unclear. In order to identify new targets of piRNA system components we performed microarray-based expression profiling of Drosophila mutant ovaries where one of the listed proteins was absent due to a mitation. We didn't observe significant alterations in abundance of the majority of unique transcripts in all three mutations, but the levels of all core histone transcripts increased in PiwiNT mutant ovaries. Quantities of snRNAs tested on the microarrays, namely U2, U5, U6 also increased significantly. Those effects were confirmed by real-time PCR and Nothern blotting. The amount of U7 snRNA increase as well in case of PiwiNT mutation, according to qPCR data. We assume that observed impact on histone RNAs may be indirect, via changes of U7 snRNA level, since this type of snRNA is essential for histone mRNA maturation.

849A

Paramutation in Drosophila linked to emergence of a piRNA-producing locus. Stephane Ronsseray[1], Augustin de Vanssay[1], Catherine Hermant[1], Antoine Boivin[1], Laure Teysset[1], Valérie Delmarre[1], Anne-Laure Bougé[2], Christophe Antoniewski[2,1]. 1) Biol du Developpement, CNRS/UMR7622, Paris, France; 2) Drosophila Genetics and Epigenetics, CNRS/URA2578, Institut Pasteur, Paris, France.

A paramutation is an interaction between two alleles of a locus, through which one allele induces a heritable modification of the other allele without modifying the DNA sequence. The paramutated allele retains the epigenetically-acquired properties in the succeeding generations and becomes itself paramutagenic. Although well characterized in plants, stably-inherited paramutations had not, until now, been described in animals. Using *Trans*-Silencing Effect (TSE), a homology-dependent repression mechanism discovered in the course of study of *P* transposable element repression, we have shown the existence of a fully penetrant and stable (>50 generations) paramutation in *Drosophila melanogaster*. In TSE, a *P-t*ransgene inserted in heterochromatin has the capacity to repress in *trans*, in the female germline, a homologous *P-lacZ* transgene located in euchromatin. Phenotypic and genetic analysis have shown that TSE exhibits variegation in ovaries, displays a maternal effect as well as epigenetic transmission through meiosis. We show that clusters of *P*-element derived transgenes exhibiting a strong capacity to induce TSE can convert other homologous transgene clusters stably incapable of TSE into strong silencers. This conversion is mediated by maternal inheritance of cytoplasm carrying piRNAs homologous to the transgenes. The paramutated cluster, previously unable to produce piRNAs, is converted into a piRNA-producing locus and is itself fully paramutagenic. TSE in *Drosophila* shows that paramutation can occur without pairing of the paramutagenic and the paramutated loci. Our work provides a model to analyze the emergence of piRNA loci, as well as *trans*-generational epigenetic effects observed in transposable element repression. Using a candidate gene approach, we investigate factors affecting paramutation in*Drosophila*.

850B

The Role of Piwi in Transposon Silencing and Heterochromatin Formation. Kiri Ulmschneider, Monica Sentmanat, Sidney Wang, Sarah Elgin. Dept Biol, Washington University, St. Louis, MO.

Transposable elements (TEs) and their remnants make up a large part of eukaryotic genomes. To avoid genome damage, TEs must be silenced, particularly in the germ line. Both post-transcriptional (PTGS) and transcription-based mechanisms (TGS) are utilized; in Drosophila, the piRNA pathway appears to be involved in both. Both primary piRNAs and the products of the ping pong cycle, driven by Argonaute proteins Aubergine and Argonaute 3, are used to eliminate transposon mRNAs (PTGS). piRNAs can be bound by Piwi, and this enables Piwi to enter the nucleus and silence transposons via a chromatin-based mechanism (TGS). Two proteins, Armitage and Squash, have previously been shown to be involved in the piRNA pathway, interacting with Piwi. Here we examine the effects of germ line specific knockdown (KD) of these proteins on accumulation of heterochromatic marks at transposon loci. Squash KD does not appear to affect heterochromatic marks in the germ line, while Armitage KD does have an affect at a subset of transposon loci, consistent with the idea that Armitage (a putative helicase) helps to load piRNAs onto Piwi. To test the impact of the piRNA system on heterochromatin silencing in somatic cells, we use an hsp70-white reporter, situated adjacent to a heterochromatic block and exhibiting Position Effect Variegation (PEV) in the presence of an added TE. Mutations in components of the piRNA pathway or deletion of the piRNA sequences from the TE (either 1360 or Invader4) both result in a loss of silencing, indicating that the piRNA pathway is playing a role in silencing at this site. Transcription across the reporter-associated TE is observed in the early embryo, when heterochromatin formation occurs, suggesting that piRNA sequences could recognize the site through an RNA-RNA interaction, localizing Piwi. Piwi is known to interact with HP1a in vitro, so might be able to recruit heterochromatic factors. This system enables further tests of this hypothesis.

851C

A Bioinformatic Analysis of Alternative Splicing Patterns in Metabolic Genes of Drosophila. Stacey J. Lytle[1], Alexis

Nagengast[2]. 1) Dept Biology; 2) Dept Biochemistry & Chemistry, Widener University, Chester, PA.

Alternative splicing (AS) is a fundamental mechanism responsible for the creation of biological diversity. AS in the coding region of a gene results in proteins with functions specific for different tissues, sexes or stages of development. However, the consequence of AS in the 5' or 3' untranslated region (UTRs) is less understood. Using a bioinformatic approach and publically available gene models on FlyBase, AS patterns were investigated for genes of key metabolic processes including glycolysis, the citric acid cycle, and the pentose phosphate pathway in the Drosophila melanogaster species. AS in the 5' or 3' UTRs rather than coding regions appears to be a common pattern specific to each pathway. Many glycolytic genes are alternatively spliced in the 3' UTR while those in the pentose phosphate pathway are alternatively spliced primarily in the 5' UTR. Additionally, regions of high conservation at the nucleotide level were observed in these alternatively spliced UTRs. Further analysis will focus on using motif detection programs to identify areas of high conservation in the 5' and 3' UTR sequences. The alternatively spliced UTR sequences in D. melanogaster will be compared to orthologous sequences in other Drosophilid species to see if a shared regulatory mechanism may exist through the evolution of the species.

852A

Selective translational control refines cell-type specific responses to the steroid hormone ecdysone. Robert Ihry, Arash Bashirullah. Sch Pharmacy, Univ Wisconsin, Madison, Madison, WI.

Steroid hormones elicit a wide range of biological responses by directly regulating transcription. Here we show that selective translational control plays a critical role in refining steroid-dependent transcriptional responses in *Drosophila*. We show that the DEAD box RNA helicase *belle*, directly regulates translation of the ets transcription factor *E74A*. *E74A* transcription is directly and ubiquitously induced by the steroid hormone ecdysone; in contrast, translation of *E74A* mRNA is restricted to a subset of cells. We demonstrate that the distribution of E74A protein expression is determined by cell-type specific regulation of *belle*-dependent translation. Belle protein is ubiquitously expressed, indicating that *belle* function is itself regulated in a cell-type specific manner. Using forward genetic approaches we have identified several genes required for *belle*-dependent translation, genes that have not been previously implicated in translational control. Given that the human homolog of *belle* (DDX3) plays a critical role in tumorigenesis and in HIV infections, this *belle*-dependent pathway outlines a novel and critical regulatory network.

853B

Mutations in a 5' region of the *osk* gene disrupt both Osk protein function and *osk* mRNA translational activation. Matt Kanke, Goheun Kim, Young-Hee Ryu, Paul M. Macdonald. Molecular Cell and Developmental Biology, University of Texas at Austin, Austin, TX.

Proper regulation of *oskar* (*osk*) mRNA translation is essential for axial patterning. *osk* is synthesized in the nurse cells and subsequently localized to the posterior of the oocyte in a translationally repressed state. Upon posterior localization, the two Osk isoforms, Long Osk and Short Osk, begin to accumulate. Repression of *osk* mRNA translation has been extensively characterized and relies on defined elements in the *osk* 3' UTR as well as known regulatory factors. Translational activation is less well understood. The current, long standing model proposes that a 5' regulatory element acts to override repression specifically at the posterior pole of the oocyte. The proposed element lies in the 5' part of the *osk* mRNA that is either a protein coding region (of Long Osk) or 5' UTR (for production of Short Osk). Mutations in the element may have consequences from change in an RNA element or from change in the Osk protein sequence. We have used *osk* and *osk::GFP* transgenes to evaluate the role of the 5' region. Characterization of *osk::GFP* transgenes shows that the Osk aminoterminal domain confers anchoring on the fusion protein, and that mutations in the presumed regulatory element both disrupt protein anchoring and reduce protein levels. The anchoring defect shows that a protein domain is altered; reduced protein levels could result from either protein or RNA changes. To definitively test for the presence of an RNA translational activation element we used *osk* transgenes in which Long Osk is not produced, and mutations in the proposed 5' element only affect the 5' UTR. We find that there is indeed a translational activation element in the *osk* 5' region, and that this element is essential for Short Osk expression.

854C

Role of Bruno phosphorylation in translational regulation of *oskar*. Goheun Kim[1], Keiji Sato[2], Akira Nakamura[2], Paul Macdonald[1]. 1) Molecular Cell & Developmental Biology, University of Texas at Austin, Austin, TX; 2) Laboratory for Germline Development, RIKEN Center for Developmental Biology, Kobe, Japan.

Oskar (Osk) is a posterior body patterning determinant in Drosophila and is highly concentrated at the posterior pole of the oocyte. Tight spatial and temporal restriction of the Osk patterning activity is essential for proper development of the embryo. Bruno (Bru) directly binds to the *osk* mRNA and represses translation during mRNA localization to the posterior pole. After *osk* mRNA localization, repression must be alleviated to allow accumulation of Osk protein. In one model for repression, Bru bound to *osk* mRNA recruits Cup, which in turn binds eIF4E and prevents its interaction with eIF4G. In another model, Bru promotes oligomerization of multiple *osk* mRNAs into large particles that are inaccessible to the translational machinery. The interactions of Bru with RNA and proteins must underlie its repressive activity, and may be disrupted for release from repression. We show that Bru dimerizes, and have investigated interaction of Bru with itself and with Cup. We also show that Bru is phosphorylated, and have investigated how this modification affects the binding activities of Bru. Using GST pull-down assays we show that two domains of Bru, aa1-146 and aa334-416, contribute to both interactions. Deletion of the N-terminal

domain dramatically reduces binding, and the isolated N-terminal domain binds both Bru and Cup. A small fraction of Bru in ovaries is phosphorylated. Several predicted sites of phosphorylation by PKA in Bru lie within the N-terminal domain. These could be targets for inhibition of protein interactions, and thereby mediate inactivation of repression. Phospho-mimetic mutations in these sites interfere with Bru binding to both itself and Cup, while corresponding phospho-silent mutations have no effect. The same phospho-silent mutations interfere with in vitro phosphorylation of Bru by purified PKA. We propose that local phosphorylation of Bru by PKA at the posterior of the oocyte alleviates Bru-dependent translational repression of *osk*.

855A

Nutritional control of mRNA translation in Drosophila larvae. Sabarish Nagarajan, Savraj Grewal. Souther Alberta Cancer Research Institute, University of Calgary, Calgary, Alberta, Canada.

Dietary protein is a key determinant of growth in Drosophila larvae. Several studies have described changes in gene transcription, (particularly metabolic genes) that may mediate nutrient effects on growth. In contrast the role of nutrition-dependent translational regulation is less clear. We have used polysome profiling to study global and specific translation of mRNAs in response to altered nutrition in larvae. We find that bulk translation is markedly decreased within 2 hours after protein starvation, with a maximal decrease in translation observed between 18 and 96hrs post-starvation. We next looked at translation of specific mRNAs by using qRT-PCR anlaysis of polysome fractions. We examined translation of a selected set of genes whose overall transcript levels were highly upregulated, highly downregulated or unchanged following protein starvation. With all genes, we observed decreased translation upon starvation. These translationally repressed genes included transcription factors such as Myc, hence providing a mechanism by which nutrition can alter gene transcription. Interestingly, we also found that several translationally repressed mRNAs contain specific features such as putative IRESs or decreased 5'UTR complexity, that were previously suggested to allow elevated translation upon starvation. Together our data suggest that while starvation induces strong increases and decreases in transcription, translational repression may be an additional key determinant of overall gene expression in starved animals. The current view, largely from work in mammalian cell culture, is that nutrient signaling stimulates mRNA translation by inhibiting 4EBP, a conserved translational repressor. We found that 4EBP null larvae show no change in translation in fed conditions and are unable to reverse the decrease in mRNA translation during starvation. These data argue that 4EBP-independent mechanisms must contribute to nutrition-dependent translational control in larvae. We are currently exploring other potential translational regulators that may mediate these effects of nutrient signaling.

856B

The role of Bicoid Stability Factor in *oskar* regulation. Young Hee Ryu, Paul Macdonald. Molecular Cell & Developmental Biology, University of Texas at Austin, Austin, TX.

Axial patterning of the *Drosophila* oocyte and embryo relies on asymmetric localization of body patterning determinants. One such determinant, the Oskar (Osk) protein that is responsible for posterior patterning and germ cell formation, accumulates only at the posterior pole of the oocyte. This restriction is achieved by a coordinated program of *osk* mRNA localization and translational regulation. Many of the *cis*-acting elements required for regulation lie in the *osk* mRNA 3' untranslated region (3' UTR). The *osk* mRNA also has a function in progression through oogenesis independent of its protein coding capacity. This *osk* RNA function is mediated by the 3' UTR. A short region near the 3' end of the *osk* mRNA contains both regulatory elements and *osk* RNA function elements. To identify proteins that bind to this region, and could act in regulation or RNA function, we used a streptavidin (S1) aptamer affinity purification strategy. Hybrid transcripts containing the *osk* 3' region, in either wild type or mutant forms, and the S1 aptamer were bound to streptavidin beads and mixed with ovary extracts to allow assembly of RNP complexes. Bound proteins were identified by mass spectrometry. Bicoid Stability Factor (BSF) was identified as a protein associated with the wild type RNA but not a subset of mutant RNAs. Like *osk* RNA null mutants, *bsf* mutants are arrested in oogenesis at an early stage. However, the phenotypes of the *bsf* and *osk* RNA null mutants are not identical. Moreover, not all of the *osk* RNA 3' region mutants that fail to bind BSF are defective in *osk* RNA function. Therefore, BSF appears not to act in *osk* RNA function. Reducing *bsf* activity late in oogenesis interferes with Osk protein accumulation in early embryos, as does an *osk* RNA 3' region mutant that fails to bind BSF. These results argue that BSF has a role in regulation of Osk protein expression, either directly affecting translation or indirectly reducing Osk accumulation through an effect on *osk* mRNA stability or localization.

857C

Clk mRNA turnover de-noises circadian transcription and behavior in time and space. Sebastian Kadener[1], Lerner Immanuel[1], Bartok Osnat[1], Afik Shaked[1], Friedman Nir[2]. 1) Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel; 2) Computer Sciences Department and Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel.

Most organisms use circadian clocks to keep temporal order and anticipate daily environmental changes. These clocks keep time through a complex transcriptional-translational negative feedback loop. Circadian clocks are extraordinarily robust systems, although the mechanisms that contribute to this robustness are still unknown. Here we demonstrated that Clk mRNA turnover is exceptionally high and that this is a key mechanism underlying the robustness of the circadian system. We show that while Clk is transcribed at high levels, mature Clk RNA molecules are quickly degraded in a 3' UTR-depending way through a polyA-shortening mechanism. This regulation is key to buffer stochastic changes in transcription that could result in

ectopic expression of clk in time or space. We hypothesize that clk high turnover rate restricts the translatability of Clk mRNA to few hours during the circadian cycle. This is important since we also show that although CLK protein levels are constant throughout the day, CLK protein localization is strongly regulated. In order to test the importance of this mechanism for normal timekeeping, we generated flies carrying a Clk genomic construct in which the Clk 3' UTR has been replaced with a control (SV40) 3' UTR. Indeed, a Clk genomic rescue that has lost the post-transcriptional control rescues Clk mutants at much lower degree than control genomic contructs. In addition, these flies exhibit high levels of ectopic CLK and CLK-CYC targets in non-circadian cells in the fly brain. This ectopic expression is more pronounced under perturbation (i.e. temperature changes) and leads to decrease fertility, higher mortality and developmental phenotypes. Therefore our study shows that post-transcriptional regulation of Clk is essential for proper circadian-cell determination and hence for developmental robustness.

858A

A Sensitized Screen for Genes that Interact with *Bag-of-marbles* During Definitive Hematopoiesis. Erin A.T. Boyle, Dawn W. Hopkins, Robert A. Schulz. University of Notre Dame, Notre Dame, IN.

Homozygous mutation of *bag-of-marbles (bam)* causes an overproliferation of differentiated blood cells in the lymph gland of *Drosophila* at the expense of the progenitor population. Using lamellocyte induction as an indication of aberrant differentiation, a sensitized screen of the second and third chromosomes was performed in order to identify genes that interact with *bam*. I have identified four regions that cause significant induction of lamellocytes. Deficiency 8074 induced lamellocytes in 22% of the bam-deficient trans-heterozygote animals compared to 12% for animals that contained the deficiency alone. The gene likely responsible for this induction is *CG9384*, which induces lamellocytes at a frequency of 30% in the trans-heterozygote and 21% in the mutant alone. Region 8957 induced lamellocytes at a frequency of 25% in the trans-heterozygotes and 66% for animals that contained the deficiency alone. Preliminary data suggests that the genes responsible for this phenotype are *Tctp* and *CG4820*. A third region 27346 induces lamellocytes independent of *bam* at a frequency of 30%. Smaller deficiencies in this region are currently being screened. The fourth deficiency region 7659 contains a set of two genes *Cha* and*VACht*, which induce lamellocytes in 54% of larva, independent of *bam*. Furthermore there is a reduction of the medullary zone in heterozygous *Cha* and *VACht* mutants, which indicates they may play a role in progenitor maintenance. When the genes responsible for lamellocyte induction in the four deficiency regions are confirmed, complete mutant phenotype analysis will be completed to determine their likely role in larval hematopoeisis.

859B

Yorkie and Scalloped regulate availability of Serrate signaling cells required for crystal cell differentiation in the larval lymph gland. Gabriel B. Ferguson, Julian Martinez-Agosto. University of California, Los Angeles, Department Of Human Genetics. Los Angeles, CA.

Cellular microenvironments established by the spatial and temporal expression of specific signaling molecules are critical for both the maintenance and lineage specific differentiation of stem and progenitor cells. The Drosophila Lymph Gland is an ideal system in which signaling can be studied within the highly relevant and complex process of hematopoiesis. Lymph Gland homeostasis is largely regulated by a cluster of cells known as the Poster Signaling Center or PSC. These cells secrete the signaling factors Hedgehog and PVF1 which are required to maintain the hematopoietic progenitors in an undifferentiated state. The PSC also expresses the Notch ligand Serrate which has been shown to be a critical component in the differentiation of the Crystal Cell lineage of mature hemocytes in Drosophila. While specification of Crystal Cells is known to be dependent on Serrate activity, activation of Notch signaling, and its downstream target the transcription factor Lozenge, we provide evidence that the Serrate expressing cells in the PSC are not required for this process. Here, we report that the Hippo Pathway effectors Yorkie and Scalloped are required for the expression of Serrate in interior signaling cells of Drosophila Lymph Gland. These Serrate expressing cells are distinct from the niche cells and are required for the specification of the Crystal Cell Lineage of hemocytes in Drosophila. Furthermore, we found that Yorkie expression is activated by Notch signaling specifically in Crystal Cells to promote cell survival via the anti- apoptosis factor Diap1. These findings demonstrate a role for Hippo pathway components in normal hematopoiesis and establish internal signaling cells as an alternative niche for blood progenitor cells.

860C

Loss of the nuclear lamina protein Otefin causes Checkpoint kinase 2-mediated death in female germline stem cells. Lacy J. Barton, Pamela K. Geyer. Dept Biochemistry, Univ Iowa, Iowa City, IA.

Tissue homeostasis is supported by adult stem cells. Emerging evidence suggest that compromised adult stem cell function is associated with human diseases caused by mutations in components of the nuclear lamina, such as LEM Domain (LEM-D) proteins. How defects in LEM-D proteins affect stem cell function is unclear. To gain insights into these mechanisms, we study the Drosophila LEM-D protein Otefin (Ote), which is required for female germ cell differentiation and germline stem cell (GSC) maintenance. Our recent studies have shown that progressive loss of GSCs in *ote* mutants occurs in the absence of the early germline differentiation factor, Bag of marbles, indicating that GSC loss is due to cell death. To understand this process, we have performed a genetic suppression screen, testing candidates known to have a role in cell death. Strikingly, loss of the DNA Damage Response (DDR) transducer Checkpoint kinase 2 (Chk2) fully suppresses $ote^{-/-}$ GSC loss and permits all stages of oogenesis. Despite normal morphology, $ote^{-/-}$, $chk2^{-/-}$ eggs that are fertilized with wild type sperm fail to undergo even the earliest stages of embryogenesis, suggesting that the critical defect persists. Surprisingly, immunohistochemical analysis of $ote^{-/-}$, $chk2^{-/-}$ germ cells showed normal chromatin organization, capped telomeres and a lack of DNA damage foci. In addition, $ote^{-/-}$

GSC phenotypes were not suppressed by the loss of other DDR components, including those upstream and downstream of Chk2. Together, these data suggest that DNA damage is not the cause of GSC death in *ote* mutants. We are currently investigating the initiating cause of Chk2 activation, including defects in transposon silencing, Chk2 sequestration and nuclear lamina integrity. These studies will provide needed insights into mechanism of stem cell dysfunction caused by mutations in LEM-D proteins.

861A

Notch signaling controls Drosophila female germline stem cell competitiveness for niche occupancy. Tseng Cheng-Yuan[1,2*], Hsu Hwei-Jan[1,2]. 1) Institution of Cellular and Oragnismic Biology, Academia Sinica, Taipei, Taiwan; 2) Institution of Life Sciences, National Defense Medical Center, Taipei, Taiwan.

Stem cells reside in a specialized microenvironment, or niche, which provides physical contact mediated by cell-cell adhesion molecules, and secretes factors that regulate stem cells. We have previously shown that insulin signaling directly controls germline stem cell (GSC) division via FOXO. In addition, insulin levels regulate Notch activation to maintain GSC niche via the effect of FOXO on transcription of fringe (fng), which encodes a glycosyltransferase that modulates Notch glycosylation. It is not clear, however, if insulin levels also control GSC division via the same mechanism observed in the niche. To address this, we disrupted Notch signaling in GSCs by generating fng and Notch mutant GSCs. We evidenced that their division and maintenance were not affected, indicating that insulin/FOXO-mediated signaling controls GSC division independently of Notch signaling in GSCs. Surprisingly, these mutant GSCs tended to push wild-type GSCs away from the niche, suggesting that GSCs with low Notch signaling have relative higher competitiveness for niche occupancy. The self-renewal-promoting BMP niche signal and GSC-niche adhesive molecule, E-cadherin, are the only factors known to control female GSC competition. Interestingly, we found that BMP signaling was not affected in fng and Notch mutant GSCs, while E-cadherin expression at the junction between these GSCs and their niche was significantly increased. These results indicate that Fringe-mediated Notch signaling controls GSC competiveness for niche occupancy through E-cadherin.Our results uncover a novel function of Notch signaling in stem cell competition, which may serve as a quality control to keep “good” stem cells in the niche. These results also provide insights into how stem cells communicate with each other, and how stem cells interact with their niche to ensure the integrity of tissue function.

862B

Bazooka Forms a Platform that Integrates Stem Cell Polarity and Cell Cycle Progression. Mayu Inaba[1,2], Yukiko Yamashita[1]. 1) Center for stem cell biology, Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Molecular Biology The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas.

Many stem cells are known to divide asymmetrically to balance self-renewal and differentiation. The essence of asymmetric cell division is polarization of the cell and subsequent cell division that unequally compartmentalizes cellular components that confer distinct cell fates to daughter cells. Precocious cell division before establishment of cell polarity would lead to failure in asymmetric cell division. However, it is poorly understood how cell polarity is coordinated with the cell cycle progression. In Drosophila male germline stem cells (GSCs), asymmetric division is achieved by stereotypical positioning of the centrosomes. The centrosome orientation checkpoint (COC) serves as a mechanism to coordinate correct centrosome orientation and mitotic entry. Here, we show that Bazooka (Baz)/Par-3 is an integral component of the COC. Baz forms small foci between GSC and its niche component, hub cells. We provide evidence that Baz-centrosome interaction at hub-GSC interface triggers mitotic entry, whereas lack of Baz-centrosome interaction leads to association of Baz with the spectrosome, a germline-specific organelle, leading to cell cycle delay. We propose that Baz forms a signaling platform to monitor centrosome orientation and regulate cell cycle progression.

863C

A Novel Interaction Between Stem Cell Factors FMRP and Zfrp8. William Tan, Tatyana Naryshkina, Neha Changela, Curtis Schauder, Ruth Steward. Waksman Institute, Rutgers University, Piscataway, NJ.

Fragile-X Mental Retardation Protein (dFMRP) is a ubiquitously expressed protein required for a number of developmental processes, including neural differentiation and stem cell maintenance. Drosophila FMRP is required for the proper maintenance of both neural and ovary germline stem cells. FMRP functions by selectively binding mRNA targets co-transcriptionally in the nucleus, then subsequently inhibiting the initiation step of translation at the ribosome. We have identified stem cell factor Zfrp8 as a component of the dFMRP complex. Zfrp8 is an essential stem cell protein also required in the nucleus. As in the case of dFMRP, reduction of Zfrp8 results in loss of stem cells. We have also determined a direct physical interaction between Zfrp8 and Drosophila NUFIP (Nuclear FMRP Interacting-Protein), another RNA-binding protein. These interactions bring to light a novel mechanism for FMRP regulation.

864A

pineapple eye, a putative Drosophila E3 ligase for FOXO, is required for stem cell self-renewal in three adult stem cell types, female GSC, male GSC and ISC. Yalan Xing, Manisha Thuparani, Irina Kurtz, Hannele Ruohola-Baker. Department of Biochemistry, Institute for Stem Cell and Regenerative Medicine, University of Washington, SEATTLE, WA.

Adult stem cells are key for regeneration. However, we need to understand the molecules that control this process to fully harness adult stem cell potential in future medicine. Drosophila serves as a good model for understanding adult stem cell

regulation. Three excellent models for adult stem cells in Drosophila melanogaster have been extensively investigated: female and male germline stem cells (GSCs), and intestinal stem cells (ISCs) in fly midguts. Previous studies have revealed that these adult stem cell types share similar molecular regulatory pathways, suggesting that common regulators for adult stem cells can be unraveled. One of these shared regulatory pathways is Insulin receptor (InR) pathway. Through a loss-of-function screen, we identified pineapple eye (pie), a homologue of human E3 ubiquitin ligase G2E3, as an essential factor in female GSC maintenance and division. We now show that pie is also required for male GSCs self-renewal and ISCs proliferation. These data suggest that pie has a conserved role in maintaining pluripotency. One of the negative downstream targets of InR signaling pathway is transcription factor FOXO. Phosphorylation by Akt inhibits the nuclear localization and consequently the transcriptional activity of FOXO. In this study we demonstrated that pie regulates stem cell self-renewal through mediating FOXO on protein level, but not on transcription level. FOXO protein is upregulated in pie mutant stem cells. Further, reduction of FOXO efficiently rescues loss-of-pie caused stem cell loss and division defects. Based on these data, we propose a mechanism for pie action: pie regulates FOXO levels in Drosophila GSCs and ISCs, possibly through ubiquitination-mediated protein degradation. FOXO protein level in stem cells is a rheostat that is responsible for regulating self-renewal.

865B

Systemic regulation of intestinal tissue homeostasis in Drosophila melanogaster. Arshad Ayyaz, Jason Karpac, Heinrich Jasper. Buck Institute for Research on Aging, Novato, CA.

The breakdown of tissue homeostasis is a hallmark of aging, and barrier epithelia are particularly vulnerable due to their continuous exposure to bacteria and adverse environmental factors. Intestinal stem cells (ISCs) embedded in the intestinal epithelium are capable of regenerating all known cell types of the intestine. It is well established now that under conditions of stress, such as immune challenge, cytokines secreted by damaged enterocytes are sensed by ISCs, triggering a tissue regeneration program. Interestingly, however, intestinal homeostasis is also controlled by systemic factors that influence ISC proliferation. For instance, both neuronally and muscle-derived insulin like peptides (DILPs) activate insulin/IGF (IIS) signaling in ISCs, influencing their proliferation. DILPs are also critical for maintenance of metabolic homeostasis, indicating a key role in coordinating regenerative activity with nutrient availability. It is not clear, however, whether signals from other metabolically important tissues, such as oenocytes or fatbody, also influence regeneration in the intestine, and whether such signals influence tissue homeostasis in aging animals. Furthermore, various domains of the intestine are innervated by nerve fibers, establishing an intestine-brain circuit. It is not known, however, whether regeneration of the intestinal epithelium is influenced by the activity of these neurons. We are exploring these questions by perturbing gene function in selected tissues while monitoring regenerative activity of ISCs. For this, we are developing fly strains that allow labeling stem cell clones using a newly developed gene targeting system, the Q system, thus allowing us to use temperature sensitive Gal4/UAS and Gal80 systems for tissue specific gene manipulations. We will use these flies to perform tissue specific high throughput screens. This work is expected to provide important insight into the systemic regulation of tissue homeostasis and thus into the development of age-related proliferative diseases. Progress on this work will be reported.

866C

Transcriptome profiling of *Drosophila melanogaster* midgut cell populations by mRNA sequencing. Devanjali Dutta, Bruce Edgar. CELL GROWTH AND PROLIFERATION, ZMBH-DKFZ, HEIDELBERG, BADEN-WÜRTTEMBERG, Germany.

Drosophila midgut is a rapidly proliferating tissue and is maintained by a continuous supply of differentiated cells that originate from intestinal stem cells (ISCs).ISCs proliferate throughout life, renewing and generating transient cells called enteroblasts (EBs), which differentiate into enterocytes (ECs) or enteroendocrine cells (EEs). The proper regulation of intestinal stem cell maintenance, proliferation and differentiation is critical for maintaining gut homeostasis.Notch knockdown produces tumors,however the identity of these tumors is unclear.We have developed a method for isolating the intestinal stem cells, progenitors and all the differentiated cell types using Fluorescent activated cell sorting (FACS).Analysis of our comparitive cell-type transcriptome data suggests that the genetic differences between the intestinal cell types in the adult Drosophila midgut are not completely classified or graded.Interestingly only 17 genes were found to be specific to Myo1A+ ECs ,whereas the How + visceral muscle seemed to be expressing 911 specific genes.We observed that the Dl+ISCs, Su(H)+EBs and the Rab3+EEs had a reasonable 104, 319 and 168 cell type specific genes respectively. Presence of known cell type specific genes in our filtered dataset confirms that pure populations of cells were FACS sorted and that our expression profiling of the intestinal cell populations is accurate.mRNA sequencing of sorted esg+ tumor cells revealed that ISC tumors expressed a number of ISC markers such as Dl, spdo, esg, shg & EE specific markers like ase, sc, Ast, Pros and Rab3.We also found increased expression of the EGFR receptor,spi,Krn and factors like rhomboids 1, 4, 5 and Star.We compared the whole transcriptomes of esg+ tumor cells with those of Dl+ ISCs, SuH+ enteroblasts or esg+ cells from wild-type flies, and found that esg+ tumor cells were more similar to the esg+ population (83.8%) and to SuH+ enteroblasts (76.3%)than to wild-type Dl+ ISCs (61.8%). These comparisons suggest that these tumor cells are proliferative progenitors or precursors but not identical to wild-type Dl+ ISCs.

867A

Elucidating the tissue damage-sensing mechanism that maintains Drosophila midgut homeostasis. Julieta A. Maldera, Bruce A. Edgar. DKFZ-ZMBH Alliance, Heidelberg, Germany.

Maintenance of epithelial homeostasis relies on a tightly coordinated process that involves the balance between removing damaged cells and producing new cells from resident stem cells. To ensure optimal epithelial regeneration while preventing

dysplasia, the proliferation and differentiation rate of the stem cells has to be linked to tissue needs. In *Drosophila melanogaster*, the integrity of the midgut epithelium is preserved by intestinal stem cells (ISCs), by means of their ability to self-renew and produce differentiated cells. As in the mammalian colon and small intestine, the epithelium lining of the fly midgut has a rapid turnover rate due to its constant exposure to environmental toxins and pathogens. Interestingly, ISC proliferation can be further increased in response to damage and infection. The injured midgut produces Unpaired cytokines and Epidermal Growth Factor Receptor (EGFR) ligands that promote ISC division and differentiation, leading to tissue regeneration. However, how the intestinal epithelium senses the damage in the first place and translates this signal into a regenerative response remains mostly unknown. Here we propose to extend this research by identifying and characterizing genes that act as signaling molecules to translate the damage information into mitogen production. To achieve this objective, we performed fly midgut gene expression profile by mRNA deep sequencing either in healthy tissue or after gut epithelial injury (specifically, bacterial infection, heat shock, oxidative stress, induction of apoptosis, and JNK-mediated stress). The comparative analysis of this data allowed us to determine a subset of candidate genes that may be implicated in triggering mitogen secretion upon injury. Currently, we are performing a targeted UAS-RNAi screen in order to confirm the involvement of these genes during midgut regeneration. Understanding how an epithelium senses damaged cells and thereby regulates stem cell proliferation will provide insights into the basis of tissue homeostasis and regeneration.

868B

APC loss-induced intestinal tumorigenesis in Drosophila: roles of Ras in Wnt signaling activation and tumor progression. Chen-Hui Wang, Rui Zhao, Pin Huang, Zhenghui Quan, Fu Yang, Na Xu, Rongwen Xi. NIBS, No. 7, Science Park Road, Zhongguancun Life Science Park,Beijing, China.

Adenomatous polyposis coli (APC) and *K-ras* are the two most frequently mutated genes found in human colorectal cancers. In human colorectal cancers, Wnt signaling activation after the loss of *APC* is hypothesized to be the key event for adenoma initiation, whereas additional mutations such as Ras activation are required for the progression from adenoma to carcinoma. However, accumulating data have led to conflicting views regarding the precise role of Ras in *APC* loss-induced tumorigenesis. Here, using Drosophila midgut as a model system, we show that in the absence of Ras, *APC* mutant epithelial cells cannot initiate hyperplasia, suggesting that Ras plays an essential role in tumor initiation. Conversely, activating Ras by expressing oncogenic Ras or Raf in *APC*-deficient cells led to a loss of cell polarity and to preinvasive tumor outgrowth, characteristics that are shared by advanced colorectal carcinoma in humans. Mechanistically, we found that Ras is not required for Wnt signaling activation after *APC* loss, although Ras hyperactivation is able to potentiate Wnt signaling by increasing the cytoplasmic and nuclear accumulation of Armadillo/β-catenin via mechanisms independent of JNK/Rac1 or PI3K-Akt signaling, partly owing to the downregulation of DE-cadherin. Together with the data from gene expression analyses, our results indicate that both parallel and cooperative mechanisms of Wnt and Ras signaling are responsible for the initiation and progression of intestinal tumorigenesis after *APC* loss.

869C

Age-related stem cell de-regulation by ER stress in the Drosophila intestine. Lifen Wang[1], Hyung Don Ryoo[2], Heinrich Jasper[1]. 1) Buck institute for research on aging.8001 Redwood Blvd, Novato, California. 94945, USA; 2) Department of Cell Biology, New York University School of Medicine, 550 First Avenue, New York, New York 10016, USA.

ER stress has been reported to associate with many age-related diseases, such as diabetes, cancer and inflammatory bowel disease, suggesting that loss of protein homeostasis in the ER causes tissue dysfunction in aging animals. The precise cellular basis for this dysfunction, and the impacted regulatory processes, however, remain unclear. Regeneration of the Drosophila intestinal epithelium by Intestinal stem cells (ISC) represents a powerful model system in which to address questions regarding age-related changes in tissue homeostasis. In the aging gut, ISCs become hyperproliferative, resulting in epithelial dysplasia. We have used this model to explore the effects of ER stress and of ER stress response signaling mechanisms on ISC function and on tissue homeostasis. Our results suggest that (I) excessive ER stress causes dysplasia of the intestinal epithelium. (II) Increasing the ability to overcome ER stress by over-expressing XBP1 (which regulates cellular ER stress responses) or Hrd1 (which promotes ER stress associated protein degradation) in ISCs can prevent stress-induced dysplasia. (III) The ER stress response influences ISC proliferation by controlling CncC activity. (IV) Increased ability to alleviate ER stress prevents age-related dysplasia in ISC. It can be anticipated that these studies will provide important new insight into the role of protein homeostasis in the control of stem cell function and the development of age-related stem cell dysfunction.

870A

Regulation of String during Drosophila intestinal stem cell proliferation. Jinyi Xiang, Bruce Edgar. Cell growth and proliferation, DKFZ-ZMBH Alliance, Heidelberg, Germany.

The adult Drosophila midgut is a highly regenerative organ that is maintained by intestinal stem cells (ISC). ISC proliferation and division produces new ISCs and enteroblast (EB) daughters, which differentiate into the major midgut cell types, enterocytes (EC) or enteroendocrine (EE) cells. The mono-layered epithelium of the midgut is regularly replenished by stem cells once every two weeks under normal physiological circumstances. However in response to epithelial damage and stress condition, such as bacteria infection, DNA damaging agents, oxidative stress, induced apoptosis or JNK stress signaling activation, damaged ECs will produce ligands of EGFR and JAK/STAT to activate these pathways in ISCs and EBs, thus promoting their proliferation and differentiation to compensate for epithelium cell loss. During this feedback, we find that the

String (Stg) gene, the Drosophila homologue of Cdc25 phosphatase that is the ultimate regulator of mitosis in most eukaryotic cells, is strongly increased in expression. Gain- and loss- of function studies show that Stg is necessary for midgut stem cell proliferation in response to the extrinsic growth signals from epithelium. Moreover, we find that one small fragment of the Stg enhancer is specific for its transcription in midgut stem cells. To elucidate the mechanism of how extrinsic signals regulate this key cell cycle gene, we will use yeast 1 hybrid (Y1H) to screen the candidate transcription factors that can directly bind this specific enhancer and regulate Stg expression in stem cells. With this work, we expect to find the essential link between signal transduction factors and cell cycle control in stem cells.

871B

SWI/SNF Chromatin Remodeling Complexes Regulate Stem Cell Asymmetric Division and Daughter Cell Fate Specification in Adult Drosophila Posterior Midgut. Xiankun Zeng[1], Xinhua Lin[2], Steven Hou[1]. 1) The Mouse Cancer Gen Program, Frederick National Laboratory for Cancer Research, National Institutes of Health, Frederick, MD 21702; 2) Key Laboratory of Stem Cell and Developmental Biology Institute of Zoology, Chinese Academy of Sciences, Beijing 1 Beichen West Road, Chaoyang District Beijing 100101, P.R.China.

The adult Drosophila posterior midgut is maintained by multipotent intestinal stem cells (ISCs). Asymmetric Notch signaling first regulates the transition of an ISC to an immature daughter cell enteroblast (EB) and then directs the differentiation of an EB to an enterocyte (EC) or a secretory enteroendocrine (ee) cell. However, it is not known what regulates the asymmetric Notch signaling and the signaling's function in regulating daughter cell fate determination. In a screen for genes that regulate cell lineage determination in the posterior midgut, we identified the core components of SWI/SNF chromatin-remodeling factors Osa and Snr1. Mutations of osa and snr1 resulted in ISC expansion as well as ee reduction. We further demonstrated that the Osa and Snr1 regulate ISC asymmetric division by directly controlling Delta transcription and ee differentiation by controlling asense transcription. Therefore, our data suggest that SNF/SWI chromatin-remodeling complexes can regulate both ISC intrinsic asymmetric division and ISC daughter cell fate determination by controlling Delta and ase transcription, respectively.

872C

Drosophila activating transcription factor 3 non-autonomously regulating intestinal stem cell division and differentiation. Jun Zhou, Anna-Lisa Boettcher, Michael Boutros. Signaling and Functional Genomics, German Cancer Research Center, Heidelberg, Germany.

Activating transcription factor 3 (ATF3) is a member of the CREB/ATF family of transcription factors. Previous study in mice suggest ATF3 may represent a down-regulated tumor suppressor in colon cancer however the underlying mechanism is poorly understood. In the Drosophila gut, the replacement of damaged enterocytes (EC) relies on intestinal stem cell (ISC) proliferation and their progenitor cells (Enteroblast-EB) differentiation. Recent studies show a number of signaling pathways are involved in the modulation of ISC division and EB differentiation upon aging and environmental stresses. To understand the role of ATF3 in tumor suppression, we first show that Drosophila ATF3 is expressed in ISC, EB and EC but not enteroendocrine cells (EEs). Using two independent RNAi line, we find that loss of Atf3 in midgut precursor cells stimulate ISC division and EB differentiation. We also observed an upregulation of several signaling pathways ligand or targets like JAK/STAT signaling, EGFR signaling and JNK signaling after loss of ATF3 in ISCs and EBs. We also show that RNAi dATF3 in ECs stimulate ISC proliferation and differentiation which is dependent on JNK and Yki activation. These results suggest loss of ATF3 in intestinal stem cells non-autonomous activate JNK pathway in neighboring enterocytes which secrete ligands like Upd3 and then positively feedback on ISC by activating JAK/STAT signaling to stimulate ISC proliferation and EB differentiation. We are currently using ChIP-seq and other genomic techniques to identify targets of dATF3 in regulating intestine homeostasis.

873A

Distinguishing progenitor cells from stem cells by dampening their responses to self-renewal transcription factors. Cheng-Yu Lee[1,2,3,4], Derek Janssens[4], Hideyuki Komori[1]. 1) Center for Stem Cell Biology, Life Sciences Institute; 2) Division of Molecular Medicine and Genetics, Department of Internal Medicine; 3) Department of Cell and Developmental Biology; 4) Program in Cellular and Molecular Biology, University of Michigan Medical School, Ann Arbor, MI 48109.

Tissue-specific stem cells generate functional cell types by first giving rise to progenitor cells that possess restricted developmental potential. Restricted potential allows progenitor cells to undergo limited proliferation to produce differentiated progeny. Thus, precise restriction of the developmental potential allows progenitor cells to amplify stem cell output while protecting stem cell genome However, the precise mechanisms restricting the potential in progenitor cells remain unknown. Type II neuroblasts in fly larval brain divide asymmetrically to self-renew while producing an immature intermediate neural progenitor (INP). We find that the asymmetrically inherited protein Brain tumor (Brat) and the transcription factor Earmuff (Erm) act sequentially in immature INPs to restrict their potential. This mechanism prevents INPs from reverting into neuroblasts by dampening their responses to the re-expression of neuroblast self-renewal transcription factors. Reduced erm function leads to aberrant reversion of immature INPs into neuroblasts induced by mis-expression of the self-renewal factors. Increased erm function blocks aberrant reversion of immature INPs despite mis-expressing the self-renewal factors. Our study defines a mechanism that functionally defines progenitor cells by reprograms their responses to the re-expression of the self-renewal transcription factors.

874B

Selective functions for core promoter factors in neuroblast identity. Alexandre A. Neves, Robert N. Eisenman. Dept Basic Sci, Fred Hutchison Cancer Res Ctr, Seattle, WA.

Development, homeostasis and repair of animal tissues require stem cells to balance self-renewal and differentiation. How stem cells maintain their identity through multiple rounds of division without differentiating is not well understood. Drosophila neuroblasts (NBs) have emerged as an ideal system to address this question because they can be readily identified and manipulated in vivo. NBs divide asymmetrically into a larger self-renewing daughter NB and a smaller, differentiating ganglion mother cell (GMC). Current models argue that asymmetric inheritance of cell fate determinants plays a major role in NB identity. However, the mutant phenotypes of such cell fate determinants suggest that multiple, redundant pathways are required for NB identity. To identify novel NB identity genes, we performed a focused RNAi screen using a worniuGAL4, UASGFP; UASDcr2 line to drive expression of RNAi transgenes in NBs and GMCs, and we used GFP expression as a proxy for NB fate. We focus here on genes encoding TATA -binding protein associated factors (Tafs) and TATA -binding protein related factor 2 (Trf2). In most cell types, Tafs bind to and function with Tbp, but not with its paralog Trf2. In contrast, we found that taf or trf2 knockdown (KD) brains, but not tbp KD brains, exhibited defects in NB morphology and homeostasis. The NB homeostasis function appears to be independent of survival as blocking apoptosis failed to suppress taf or trf2 phenotypes. Moreover, tafs and trf2 are required for the expression of the NB markers Asense and Cyclin E , but not Deadpan. We confirmed these results with taf MARCM clones. We further show that taf,tbp or trf2 knockdown NBs all express ectopic nuclear Prospero. Prospero is a transcription factor thought to be necessary and sufficient for GMC identity and is normally excluded from NB nuclei. However only tbp KD , but not taf or trf2 KD, suppresses supernumerary NBs that arise in a prospero background. Taken together, these results demonstrate novel, unexpected, and selective functions for core promoter factors in stem identity in vivo.

875C

Hmgcr regulates spermatogonial dedifferentiation in Drosophila male germline. CY Ason Chiang[1], Yukiko Yamashita[1,2]. 1) Cell and Developmental Biology, University of Michigan, Ann Arbor, MI; 2) Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Adult stem cell populations sustain highly differentiated but short-lived cells, such as intestinal epithelial cells and sperm. Dedifferentiation, the reversion of differentiated cells into stem cells, is a mechanism to maintain stem cell number, counteracting sporadic loss of stem cells. In spite of its fundamental importance in stem cell maintenance, the mechanisms that regulate dedifferentiation are poorly understood. In*Drosophila* testes, it has been shown that adult male germline stem cells (GSCs) are maintained at least in part by dedifferentiation. Previously, partially differentiated spermatogonia are shown to be able to migrate back to the stem cell niche to re-acquire stem cell identity. (Sheng and Matunis, (2011) *Development*.) Recently, we found that Hmgcr, which is known to direct primordial germ cell (PGC) migration during embryogenesis, is expressed in niche cells of adult testes and is required for dedifferentiation. Its expression is up-regulated in GSC niche upon X-ray irradiation, which leads to GSC loss and subsequent dedifferentiation. Our work illuminates an intriguing similarity between the migration of PGCs toward gonadal somatic cells in the embryo and the migration of spermatogonia toward the stem cell niche during dedifferentiation. Furthermore, we found that protein prenylation enzymes downstream of the Hmgcr pathway are also required for dedifferentiation against stem cell loss during aging. Our findings reveal the role of Hmgcr in GSC maintenance in adult testes, and similarity between PGC and GSC specification.

876A

Pvr is a receptor tyrosine kinase (RTK) that functions in Drosophila testis cyst stem cells. Kenneth Hammer, Kelli Johnson, Judy Leatherman. Biological Sciences, University of Northern Colorado, Greeley, CO.

The Drosophila testis niche is an excellent model for studying adult stem cells. This niche is composed of a group of somatic cells called the hub, surrounded by two stem cell populations, germline stem cells (GSCs) and cyst stem cells (CySCs). Extracellular signal-regulated kinase (ERK) signaling activated by epidermal growth factor receptor (EGFR) promotes differentiation in cyst cells, leading to differentiation of neighboring germ cells (Kiger, et al., 2000; Tran, et al., 2000). However, dpERK accumulates in both CySCs and cyst cells, and it is not understood how differentiation is prevented in CySCs with ERK signaling. CySCs still accumulate dpERK upon EGFR ligand inhibition, suggesting another functional RTK (Schulz et al. 2002). We show that Pvr (PDGF-VEGF receptor) is an RTK expressed in the testis, and its ligand Pvf1 is restricted to the hub, suggesting Pvr is active in CySCs. Dominant negative cyst lineage Pvr showed modest CySC loss, while constitutive Pvr promoted proliferation of cyst lineage cells away from the hub. Interestingly, constitutive Pvr did not promote differentiation of normal GSCs, while constitutive Ras alone did promote rapid GSC differentiation. Thus, active Pvr promotes some stem-like characteristics, suggesting that Pvr signaling is distinct from ERK signaling alone. This distinction may explain why CySCs with dpERK accumulation do not differentiate. We are also investigating if CySC-restricted Zfh-1 may influence RTK signaling. Zfh-1 is a member of the ZEB family, which promote epithelial to mesenchymal transition. ZEB factors impart resistance to EGFR-induced senescence and EGFR inhibitors, and Zfh-1 was identified in an RNAi screen as an RTK modifier (Brabletz and Brabletz, 2010, Friedman and Perrimon, 2006). In a preliminary approach, we are characterizing Zfh-1's effect on ERK signaling in cultured cells. We found that stimulated S2 cells accumulated high levels of dpERK, and Zfh-1 knockdown slightly inhibited dpERK accumulation. Future work will clarify whether Zfh-1 differentially regulates EGFR versus Pvr pathway

activation.

877B

Investigating germline stem cell abscission delay as a mechanism for stem cell coordination in the testis niche. Kari Lenhart, Steve DiNardo. Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

Tight regulation over stem cell proliferation and maintaining a consistent balance between self-renewal and differentiation are critical to ensure homeostasis in many organs. These properties of stem cells are regulated, at least in part, by the niche in which these cells reside. In the Drosophila testis, the niche contains three different cell types; the terminally differentiated hub cells that anchor the stem cells and provide self-renewing signals, along with somatic cyst stem cells (CySCs) and germline stem cells (GSCs). Both stem cell populations divide asymmetrically, with one daughter cell being retained at the hub and continuing to self-renew while the other is displaced from the niche and goes on to differentiate. Proper germ cell differentiation requires that the daughter of a GSC, the gonialblast (Gb), is associated with two somatic cyst cells, each derived from a flanking CySC; any disruptions of the interaction between cyst cells and the Gb lead to severe defects in differentiation. Thus, the production of progeny from CySCs and GSCs must be coordinated to achieve the proper 2:1 ratio of cyst cells to Gb. However, we found that CySCs and GSCs do not exhibit S-phase or M-phase synchrony, arguing against the simple model where cell cycle coordination achieves the necessary 2:1 ratio of cyst cells to Gb. We are instead focusing on a unique feature of GSC divisions as a potential means of stem cell coordination in the niche: the significantly delayed completion of cytokinesis during division of the GSC. Through a combination of genetic manipulations and live imaging, we are attempting to address whether the timing of GSC abscission is regulated by CySCs or cyst cells, as well as investigating how the block to cytokinesis is engaged within the GSCs, and what triggers its reinitiation.

878C

Ecdysone Regulation of Stem Cell Maintenance in the *Drosophila* Testis Niche. Yijie Li, Qing Ma, Erika Matunis*. Dept of Cell Biology, 725 N. Wolfe Street, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205.

The *Drosophila* testis is a good model to study stem cell biology within an intact local microenvironment, or niche. In this tissue, quiescent somatic cells called hub cells create a niche for two types of stem cells: germline stem cells (GSCs) and cyst stem cells (CySCs). Although several local signals are known to regulate stem cells in the testis, the roles of systemic signals in this tissue are less well understood. Ecdysone is critical for development and for oogenesis, but was not thought to function in the testis niche. Here, we find that the Ecdysone receptor (EcR) and Ultraspiracle (USP), the two components of the ecdysone receptor complex, are expressed and activated in the *Drosophila* testis niche. We also find that EcR and its downstream targets *ftz-f1* and *E75* are required in the CySC lineage and hub cells for GSC and CySC maintenance. Furthermore, the EcR co-activator Taiman (Tai) is also expressed in the hub and required in the hub for stem cell maintenance. However, *Tai* is not autonomously required in the CySC or GSC lineage, indicating that different co-activators likely act in hub cells and stem cells. Finally, overactivation of *Tai* in the CySC or GSC lineage leads to CySC and GSC loss, likely through apoptosis, while overactivation in the hub has no effect. Together, our work indicates that ecdysone signaling promotes stem cell maintenance in *Drosophila* testis niche by acting both autonomously within CySCs, and non-autonomously in hub cells with distinct co-activators function in the hub and CySCs.

879A

A novel niche-specific aminopepetidase regulates dedifferentiation of progenitor germ cells in Drosophila testis. Cindy Lim, Xin Chen. Biology, Johns Hopkins University, Baltimore, MD.

Understanding molecular mechanism underlying stem cells' maintenance and proper differentiation is the key to effectively advance stem cells therapy in medicine. To accomplish this aim, we have systematically analyzed transcription profile of normally developing germ cells at discrete but continuous differentiation stages in male Drosophila. One of our most exciting finding is a novel gene slamdance (sda) which expressed specifically in Drosophila testis niche. The sda gene encodes an aminopeptidase, an enzyme whose function has not been previously shown in any stem cell system. We found that loss-of-function in sda leads to dramatic abnormalities in the testis niche, including deterioration of the niche architecture and loss of stem cells. We determined that loss of germline stem cells (GSCs) in the sda mutant is caused by defects in both dedifferentiation of progenitor cells and cadherin-dependent maintenance of GSCs. The molecular mechanism governing progenitor cell dedifferentiation pathway is mostly unknown. We showed that Sda is the first niche specific factor that affect dedifferentiation pathway. Further studies using loss-of-function and gain-of-function assays showed that that Sda is both necessary and sufficient to promote dedifferentiation of progenitor germ cells through the requirement of its catalytic domain. Therefore, our findings provide in vivo evidence that a novel niche-specific aminopeptidase promotes dedifferentiation to repopulate the stem cell niche under both physiological conditions and genetically manipulated depletion of stem cells. Our results advance understanding how a niche-specific peptidase influences "differentiation versus dedifferentiation" decision of progenitor cells in an endogenous stem cell system.

880B

Impact of Wolbachia on the male stem cell niche biology. Stephanie M. Pontier[1], François Schweisguth[1,2]. 1) Departement de developement, Institut Pasteur, Paris, France; 2) CNRS URA 2578.

Stem cells are pivotal to tissue homeostasis. Understanding the biological elements controlling stem cell biology is hence

essential to the development of efficient strategies allowing their control in vivo. One of these elements has been identified as the stem cell niche, which describes a particular environment insuring both the stem cell maintenance as well as the control of their proliferation. The most precisely identified stem cell niches are found in invertebrates, in both female and male gonads. In fly testis, a group of ten post-mitotic cells forming a hub at the very tip of the testis are believed to play a niche function. This hub was shown to fulfill all the prerequisite of a niche by controlling the position, the maintenance and the proliferation of two populations of stem cells, the germinal (GSC) and the somatic stem cells (SSC). While GSC differentiation takes place all along the gonad and gives rise to sperm, SSC can differentiate into two types of somatic cells: the cyst cells essential to sperm maturation and the hub cells that SSC can replenish upon their initial embryonic specification. Few years ago, a mutualistic and endosymbiotic alpha-proteobacteria, Wolbachia pipientis (Wolbachia), was found to invade the fly gonad niches with a strong tropism. While this bacteria was recently shown to increase germ cells proliferation in ovaries and improves female egg laying, its impact on the biology of the male stem cell niche remains poorly understood. Our study aims to describe these impacts and shows that Wolbachia affects both the establishment of the niche biology as well as its evolution in either stressed or physiological conditions. Importantly, Wolbachia impairs the role of the hub as a niche and promotes a novel equilibrium of the stem cell niche that correlates with a more robust interaction between somatic and germinal stem cells and their better maintenance. We are now trying to decipher the underneath signaling network supporting Wolbachia impacts on the male stem cell niche biology.

881C

patched **Increases Cellular Proliferation and Skews Neutral Drift Among Testis Stem Cells During Niche Competition.** Marc Amoyel[1*], Benjamin Simons[2], Erika Bach[1]. 1) Biochemistry and Mol Pharmacology, New York University School of Medicine, New York, NY; 2) Department of Physics and The Wellcome Trust/Gurdon Institute, University of Cambridge, Cambridge UK.

Stem cells are critical for tissue regeneration during adulthood. However competing models exist to explain stem cell behaviour. In one model stem cells are eternal and invariantly divide asymmetrically to produce one stem cell and one differentiating offspring. In another, known as neutral drift, stem cell populations as a whole do not change, but their clonal make-up is dynamic, reflecting loss and replacement of individual stem cells. In the *Drosophila* testis, the niche maintains two stem cell populations, germ-line stem cells (GSCs) and somatic cyst stem cells (CySCs). CySCs require both JAK/STAT and Hedgehog pathway signalling to self-renew. There are ~12-14 GSCs in a wild-type testis, and it is commonly believed that there are twice as many CySCs as GSCs. Here we first established that in a wild-type testis there are ~13 CySCs, defined as Zfh1-positive cells in physical contact with the niche. Thus, unexpectedly, CySCs exist in a 1:1 ratio with GSCs. Second, we followed single neutral clones and showed that CySC clones obey neutral drift dynamics: individual CySCs are lost and replaced by their neighbours. Third, we show that increasing Hh signalling by mutation of *patched* (*ptc*) alters clone size distribution, in favour of the mutant clones. *ptc* mutant clones outcompete wild-type CySCs and GSCs for niche space. This is due to increased proliferation of *ptc* mutant cells, not altered adhesion, since reduction of the dose of *string* (*stg*), required for G2/M progression, suppresses the colonising behaviour of *ptc* mutant CySCs. Finally, we show that CySC clones mis-expressing *cyclin E* and *stg*, which accelerate the rate of mitosis, also out-compete wild-type CySCs and GSCs, as do clonal mis-expression of other proliferative factors - like *yorkie*. Thus, increased proliferation is necessary and sufficient to cause niche competition in *Drosophila*. Finally, we identify a key similarity between vertebrate and invertebrate adult stem cells.

882A

Diet controls Drosophila Follicle Stem Cell proliferation via Hedgehog sequestration and release. Tiffiney R. Hartman, Alana O'Reilly. Fox Chase Cancer Center, Philadelphia, PA.

A healthy diet improves adult stem cell function and delays aging-associated diseases such as cancer, heart disease, and neurodegeneration. Defining molecular mechanisms by which nutrients dictate stem cell behavior is a key step toward understanding the role of diet in tissue homeostasis. Here, we elucidate the mechanism by which dietary cholesterol controls epithelial Follicle Stem Cell (FSC) proliferation in the fly ovary. In starved flies, Hedgehog (Hh), is sequestered at the surface of Hh-producing cells within the ovary, preventing FSC proliferation. Ingestion of cholesterol initiates an S6 kinase-dependent signaling event within Hh producing cells, triggering Hh release and FSC proliferation. This mechanism enables a rapid, tissue-specific response to nutritional changes, tailoring ovarian stem cell divisions and egg production to environmental conditions sufficient for progeny survival.

883B

Multiple ovarian follicle stem cells reside in the germarium and contribute stochastically to follicle cell daughters. Amy Reilein[1], Ari Berg[1], David Melamed[1], Natania Field[1], Elisa Cimetta[2], Nina Tandon[2], Gordana Vunjak-Novakovic[2], Daniel Kalderon[1]. 1) Biological Sciences; 2) Biomedical Engineering, Columbia University, New York, NY.

Follicle stem cells (FSCs) provide a very interesting paradigm for how stem cells compete and function as a community. FSCs reside midway along the anterior-posterior axis of the germarium of the Drosophila ovary and give rise to epithelial follicle cells that surround developing germ cells. We used lineage analysis and live imaging to examine the behavior of FSCs. We generated ovaries with up to 6 distinguishable genotypes of FSC clones using flies of the genotype *yw hs-flp/yw; ubiGFP FRT40A FRT42B ubiRFP/tub-lacZ FRT40A FRT42B* to produce lineages that were GFP $^{+}$lacZ^{+}, GFP^{+}lacZ^{-} or GFP^{-}lacZ^{+}, each with or without RFP. We defined a FSC clone as one persisting for at least 7d after clone induction and containing a fasciclin III-

negative cell at the 2a/b border of the germarium and a clone of matching genotype in the ovariole. Single ovarioles often contained four or more distinguishable genotypes. Putative FSCs of multiple genotypes were located around the circumference of the germarium at the 2a/b border. We also counted the number of distinct lineages present in each egg chamber and found that egg chambers were comprised most often of 2 or 3 FSC lineages and that genotypes frequently changed from one egg chamber to the next. For any given lineage there was a random pattern throughout the ovariole, indicating stochastic contribution of follicle cell daughters by individual FSCs. A second population of somatic cells, Escort cells (ECs), resides in the anterior half of the germarium. ECs are postulated to renew through self-duplication (Kirilly et al., 2011). Our lineage experiments showed that the genotypes of ECs largely matched the genotypes of FSC lineages, leading us to postulate that new ECs may derive from FSCs. Live imaging and positive marking experiments support this idea. Live imaging showed that cells in the 2a/b region move in all directions, including radially around the circumference of the germarium and in posterior and anterior directions.

884C

Characterization of the Follicle Stem Cell Niche in Drosophila Ovary. Pankaj Sahai-Hernandez, Todd G. Nystul. Anatomy Dept., UCSF, San Francisco, CA.

Adult stem cells are a small group of cells within a tissue that promote homeostasis by dividing to self-renew and produce differentiated progeny. Stem cell self-renewal is thought to be enforced by a specific microenvironment, or niche, within the tissue that may promote stemness or prevent differentiation. We have studied the follicle stem cells (FSCs) in the Drosophila ovary as a model to better understand how an epithelial niche functions. First, we determined which cells produce the signaling ligands important for FSC maintenance. We used cell-type specific Gal4 drivers and RNAi to knockdown Wingless and hedgehog ligands from different regions of the germarium and assayed for defects in the FSC lineage. We find that the Wingless ligand relevant for the FSC lineage comes specifically from escort cells whereas the hedgehog ligand comes from multiple cell types in the germarium including cap cells and escort cells. Next, we characterized the shape and distribution of escort cells near the follicle epithelium to determine which escort cells form adherens junctions with FSCs. We find that FSCs form adherens junctions with multiple posterior escort cells, which have a diversity of shapes and positions. We conclude that the posterior escort cells create the niche for the FSCs by acting in aggregate to provide a stable source of signaling ligands and cell adhesion important for FSC maintenance.

885A

Identifying target genes for the stem cell transcription factor Zfh1 in the *Drosophila* testis. Qi Zheng[1,3], Stephen DiNardo[2,3]. 1) Department of Biology, School of Arts and Sciences, Univ Pennsylvania, Philadelphia, PA; 2) Dept Cell & Developmental Biol, Perelman Sch Med, Univ Pennsylvania, Philadelphia, PA; 3) Penn Institute for Regenerative Medicine, Philadelphia, PA.

Understanding how stem cells are maintained in their microenvironment (the niche) is vital for their application in regenerative medicine. Studies of *Drosophila* male germline stem cells (GSCs) have served as a paradigm in niche-stem cell biology. Recent work from our lab identified the transcription factor Zfh1, which is necessary and sufficient for cyst stem cell (CySC) self-renewal (Leatherman and DiNardo, 2008). Interestingly, sustained zfh1 expression in the cyst lineage can also nonautonomously cause neighboring germ cells to become GSCs (Leatherman and DiNardo, 2008). Therefore CySCs function as part of the niche, in a role governed by Zfh1. Since we find that neither STAT nor BMP pathway activation intrinsically in germline cells, nor both STAT and BMP together, is sufficient to generate GSCs, there must exist an unidentified GSC renewal signal(s) from CySCs that is controlled by Zfh1. Here we report our progress in attempting to identify Zfh1 target genes in CySCs using a ChIP-Seq approach.

886B

Mechanism of Silver Nanoparticles Action on Insect Pigmentation Reveals Intervention of Copper Homeostasis. Atanu Duttaroy[1], Najealicka Armstrong[1], Malaisamy Ramamoorthy[2], Delina Lyon[2], Kimberly Jones[2]. 1) Dept Biol, Howard Univ, Washington, DC; 2) Department of Civil and Environmental Engineering, Howard University, Washington, DC.

Silver nanoparticles (AgNPs), like almost all nanoparticles, are potentially toxic beyond certain concentration because past that concentration survival of the organism is compromised due to scores of pathophysiological abnormalities. But the mechanism of AgNP toxicity remains undetermined. Instead of applying a toxic dose, we attempted to monitor the effects of AgNPs at a nonlethal concentration on wild type Drosophila melanogaster by exposing them throughout their development. All adult flies raised in AgNP doped food shows that up to 50 ppm (125 µg/gm of food) concentration AgNP has no negative influence on median survival however, these flies appeared uniformly lighter in body color due to the loss of melanin pigments from their cuticle. Additionally, fertility and vertical movement ability were compromised due to AgNP feeding. Determination of the amount of free Ag+ led us to claim that the observed biological effects have resulted from the AgNPs and not from free Ag. Biochemical analysis suggests that the activity of copper dependent enzymes, namely tyrosinase and Cu-Zn superoxide dismutase are decreased significantly following the consumption of AgNPs, despite the constant level of copper present in the tissue. Consequently, we propose a mechanism whereby consumption of excess AgNPs in association with membrane bound copper transporter proteins cause sequestration of copper, thus creating a condition that resembles copper starvation. This model also explains the cuticular demelanization effect resulting from AgNP since tyrosinase activity is essential for melanin biosynthesis. Finally, we claim that Drosophila, an established genetic model system, can be well utilized for further

understanding of the biological effects of nanoparticles.

887C

Constructing a Synthetic Gene Network to Model and Understand Signaling Interactions in Drosophila melanogaster. Ashley Jermusyk, Gregory Reeves. Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.

A complex system of gene regulatory circuits controls the signaling processes involved in systems patterning. These circuits act to buffer the developing pattern against noise, thereby minimizing mistakes in gene expression and preventing patterning defects. Despite their importance to patterning and development, hypotheses regarding these gene regulatory circuits have been difficult to test experimentally due to their complexity and high interconnectivity. Therefore, to better understand the fundamental processes involved, we created a synthetic gene network in Drosophila that utilizes genes from yeast and E. coli, namely, gal4, gal80, and lacZ. We expressed gal4 in a graded fashion along the anterior-posterior axis of the embryo, mimicking the endogenous Bicoid gradient. Gal4 activates expression of UAS-linked gal80 and lacZ, while Gal80 inhibits activation by Gal4, creating a negative feedback loop in our system. By using exogenous genes, all interactions within the network can be more fully understood, and their effects can be definitively determined. Our goal was to measure the location and variability in the position of the lacZ domain both with and without the negative feedback loop. This information was combined with model equations of the network to suggest changes that were used to optimize our experimental network. This system provides a direct experimental test of whether negative feedback loops in muticellular systems such as Drosophila, can provide robustness to noisy, diffusive systems.

888A

Mining ChIP data for evidence of mechanisms underlying transcription factor DNA-occupancy. Qiong Cheng[1], Majid Kazemian[1], Hannah Pham[2], Charles Blatti[1], Michael Brodsky[2], Saurabh Sinha[1]. 1) Department of Computer Science, UIUC, IL 61801; 2) Department of Molecular Medicine, University of Massachusetts Medical School, MA 01655.

ChIP-based genome-wide assays of TF occupancy have emerged as a powerful, high throughput method to understand transcriptional regulation. This has led to great interest in the underlying biochemical mechanisms that direct TF-DNA binding, with the ultimate goal of computationally predicting a TF's occupancy profile in any cellular condition. In this study, we examined the influence of various potential determinants of TF-DNA binding on a much larger scale than previously undertaken. We used a thermodynamics-based model of TF-DNA binding, called "STAP", to analyze 45 TF-ChIP data sets from Drosophila embryonic development. We built a cross-validation framework to use a baseline model that takes into account only the ChIP'ed ("primary") TF's motif and compare it with more complex models where binding by secondary TFs are hypothesized to influence the primary TF's binding. Candidate interacting TFs were chosen based on RNA-SEQ expression data from the time point of the ChIP experiment. We found widespread evidence of both cooperative and antagonistic effects by secondary TFs. We were able to identify multiple classes of interactions, including long-range interactions between primary and secondary motifs, suggestive of indirect effects such as chromatin remodeling, short-range interactions with specific inter-site spacing biases, suggestive of direct physical interactions, and overlapping binding sites suggesting competitive binding. We found evidence that the TFs ZELDA and TRL may synergistically promote the binding of several primary TFs in early and mid-stage embryonic development respectively, while EXD, RETN, JIGR1 and TTK can antagonistically influence TF-DNA binding in many cases, relying on both accessibility-mediated and accessibility-independent mechanisms. Finally, we conducted in vitro pull-down assays to test model-based predictions of short-range cooperative interactions, and found that seven of the nine TF pairs tested physically interacted.

889B

Direct Quantification of Transcriptional Regulation at an Endogenous Gene Locus. Heng Xu[1], Anna Sokac[1], Ido Golding[1,2]. 1) Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX; 2) Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL.

Gene regulation by sequence-specific transcription factors underlies the diversity of cell fates and behavior observed during development and homeostasis. Our quantitative understanding of this process is limited by the fact that most experimental methods involve averaging over many individual cells, while single-cell studies typically require the use of reporter fusions which perturb the function of the system under study. Here we present a direct quantification of gene regulation by a transcription factor at the level of a single, genetically unmodified gene locus, at single-molecule resolution. By combining single-molecule fluorescence *in situ* hybridization (smFISH) with quantitative immunofluorescence, and introducing novel image analysis algorithms, we are able to simultaneously measure the numbers of endogenous Bicoid (Bcd) transcription factors and the nascent mRNAs from the *hunchback* (*hb*) gene they regulate, in individual nuclei of the early *Drosophila* embryo. We measure the binding of Bcd at the gene locus and correlate this binding with the transcriptional activity of the *hb* gene. Our measurements allow us to derive a detailed stochastic model of *hb* regulation by Bcd and extract the *in vivo* biochemical parameters governing the process. The experimental and numerical procedures described here can be directly applied to the study of transcriptional regulation in other genes gene networks and organisms.

890C

Phylogenetic footprinting and comparative analysis of related cis-regulatory modules reveals structural constraints

on enhancer evolution and function. Thomas Brody[1], Alexander Kuzin[1], Mukta Kundu[1], Jermaine Ross[1], Amar Yavatkar[2], Ward F. Odenwald[1]. 1) Neural Cell-Fate Determinants Section; 2) Information Technology Program, NINDS/NIH, Bethesda, MD.

We have developed web-accessible alignment algorithms and a genome-wide database of *Drosophila* conserved sequence clusters to explore the structural and functional constraints on the regulatory genome. Analysis using the comparative genomics tool EvoPrinter reveals 1) that enhancers consist of clusters of conserved sequence blocks (CSBs); 2) flexibility of non-conserved inter-clustal sequences can be used to define functional limits of enhancers; 3) CSBs are often organized into tightly associated groupings referred to as 'super-blocks,' multiple CSBs connected via invariant spacing length, and 4) insertions between CSBs can be used to resolve semi-autonomous sub-modules. The alignment tool *cis*-Decoder reveals three aspects of enhancer structure: 1) conserved clusters contain overlapping repeat motifs, suggesting that cooperative interactions among multiple factors are required for enhancer activity; 2) most developmental enhancers contain multiple binding sites for a signature factor(s) that functionally defines their cis-regulatory behavior, and 3) coordinately regulated enhancers can often be identified based on their shared conserved elements present in the same proportionate balance. Functional tests reveal 1) that dynamic gene expression is carried out by multiple sub-pattern enhancers that drive expression in overlapping, non-identical subsets of cells; 2) many enhancers are multipurpose, functioning in embryos, larvae and/or adults; in evolutionary terms, it might be easier to incorporate novel functions into pre-existing enhancers than to create enhancers anew, and 3) most conserved sequence clusters function as cis-regulatory modules, suggesting that the multiplicity of enhancers, on the order of 70,000 in the entire genome, represents an added dimension to the regulatory complexity required for organism development. These studies highlight the advantages of using evolutionary conservation as a guide to the analysis of cis-regulatory sequences.

891A

GeneSeer: A Flexible, Easy-to-Use Tool to Aid Drug Discovery by Exploring Evolutionary Relationships Between Genes Across Genomes. Douglas D. Fenger, Matthew Shaw, Philip Cheung, Tim Tully. Bioinformatics Dept, Dart NeuroScience, San Diego, CA.

Homologous relationships are useful in drug discovery because they facilitate the mapping of gene/protein function between and within species, allowing functional predictions of novel or unknown genes. Early knowledge of a gene's paralogous family is also important when designing the safety screens associated with a drug discovery project. If the target has related paralogs, it is important that the drug discovery team understand the potential effects of an off target interaction. A drug discovery program might want to include a selectivity screen as part of their assay cascade to ensure that their drug is not binding close paralogs, resulting in undesirable off target effects. Now that genomic sequences for many species are readily available, bioinformatic algorithms can perform entire genome comparisons to identify these same relationships.

GeneSeer (**http://geneseer.com**) is a publicly available tool that leverages public sequence data, gene metadata information, and other publicly available data to calculate and display orthologous and paralogous gene relationships for all genes from several species, including yeasts, insects, worms, vertebrates, mammals, and primates such as human. GeneSeer calculates homology relationships by performing a full proteome BLAST calculation between two species. The GeneSeer interface is designed to help scientists quickly predict important drug discovery attributes such as selectivity and safety. It is a useful tool for cross-species translational mapping and enables scientists to easily translate hypotheses about gene identity and function from one species to another. Besides describing GeneSeer's underlying methods and user-friendly interface, we will also present a validation study of GeneSeer versus Homologene, the homolog prediction tool from NCBI. The underlying scientific data for GeneSeer has been validated to be as good as, if not better than, Homologene. Finally, a comparison of features shows GeneSeer to be the most feature rich when compared to alternative orthologing tools.

892B

myFX: Turn-key software for laboratory desktops that analyzes spatial patterns of gene expression in Drosophila embryos. Sudhir Kumar[1,2], Ivan Montiel[1], Qian Sun[1,3], Michael McCutchan[1], Bremen Braun[1], Adam Orr[1], Stuart Newfeld[1,2], Jieping Ye[1,3]. 1) Center for Evolutionary Medicine and Informatics, Arizona State Univ, Tempe, AZ; 2) School of Life Sciences, Arizona State Univ, Tempe, AZ; 3) School of Computing, Informatics, and Decision Systems Engineering, Arizona State Univ, Tempe, AZ.

Practical bioinformatics tools for data-driven analysis of images containing spatial patterns of gene expression are needed to effectively harness the power of existing knowledge when analyzing one's own data. We present a biologist-centric, turn-key software platform (myFX) for use in experimental laboratories actively collecting such data. It accepts images in multiple formats and contains new computational methods to automatically process images and standardize and align them. Among the new computational methods are programs that semi-automatically annotate anatomical view (e.g., lateral or dorsal) and asses developmental stage for embryos, information required for biologically meaningful image comparisons. myFX also provides approaches for creating digital descriptions of spatial expression patterns in images that enable measurements of gene pattern similarity and synthesis across images from one or genes. myFX contains facilities to generate Genomewide-Expression-Maps (GEMs) for new images acquired in the investigator's laboratory that illuminate transcriptionally active regions of the embryo by capturing the spatial expanse of expression in all embryos within a given set of images with similar stage and anatomical view. Using GEMs and direct image comparison, users can identify all images (and, thus, genes or genetic constructs) showing similar patterns of expression in their own dataset. myFX is programmed to interact directly with FlyExpress, which enables

searching for images with similar patterns of expression in the wealth of community-generated data captured in FlyExpress. myFX is a cross-platform application with a user-friendly interface, optimized for use on laboratory desktops that provides a multi-user environment with an integrated image management system, and is available free of charge (http://www.flyexpress.net).

893C

Sinbad Fly: A resource for functional variant discovery in *Drosophila melanogaster*. Kjong-Van Lehmann, Paul Marjoram, Ting Chen. University of Southern California, Los Angeles, CA.

Functional variant prediction from biological data has become a common complementary approach to many genome wide screens. With the advent of genome wide association studies in model organisms, organism specific prediction of functional variants is desirable. Here we present SInBaD Fly, an extension of our functional variant detection program SInBaD to *Drosophila melanogaster*. SInBaD Fly is based on using nucleotide sequence conservation across species to distinguish phenotype associated variants from synonymous variants. High accuracies have been achieved and sensitivity/specificity trade-offs are comparable to our human model. A web server is being made available providing predictions for functional variants under different false positive rates in coding regions.

894A

Advances in the FlyExpress Platform Facilitate the Integration of Gene Expression Spatial Patterns with Associated Regulatory Sequence. Michael E. McCutchan[1], Sudhir Kumar[1,2]. 1) Center for Evolutionary Medicine and Informatics, Biodesign Institute, Arizona State Univ, Tempe, AZ; 2) School of Life Sciences, Arizona State Univ, Tempe, AZ.

The overarching goal of the FlyExpress project is to develop computational methods and practical bioinformatics resources for data-driven integrative analyses of expression pattern images and sequence data to discover functional, genetic, and regulatory interactions among genes and genomic elements. FlyExpress contains a large dataset of spatial expression patterns from high throughput studies and from peer-reviewed journal articles. We enriched the existing data by standardizing and annotating images such that they are aligned and contain information on developmental stage and anatomical view. From these data scientists can now visualize temporal sequences of gene expression (Time-course embryos) for individual genes, informative global views of stage- and view-specific expression patterns (Genomewide-Expression-maps, GEMs), and search for co-expressed genes via spatial expression pattern matching. We have now evolved the FlyExpress platform by adding new functions. First, genome sequence data is now integrated with results from expression pattern studies, such that researchers are able to explore common motifs found in the genomic region of a query image and thus identify candidate trans-acting regulators of that images gene expression pattern. In refined stage-based image searches for matching expression patterns and for time-course based embryonic gene expression displays, we employ our new automated within stage predictions that affords a more biologically grounded basis for proposing a set of co-expressed genes. In addition, a new sketch-and-find utility enables researchers to paint desired patterns on selected GEMs to identify all co-expressed genes in those locations. The updated FlyExpress platform can be accessed at http://www.flyexpress.net .

895B

Recent advances in NCBI's Eukaryotic Genome Annotation Pipeline and expansion to process RNA-seq data. Terence D. Murphy, Alexander Souvorov, Francoise Thibaud-Nissen, Eyal Mozes, Wratko Hlavina, Eric Engelson, Olga Ermolaeva, Alex Astashyn, Craig Wallin, David Managadze, Kim Pruitt, Paul Kitts, Michael DiCuccio. NCBI, NIH, Bethesda, MD.

Recent advances in sequencing technology are resulting in an explosion of genome sequences for a wide variety of taxa. Making use of this sequence barrage will require accurate and efficient methods for genome annotation, especially for protein-coding and non-coding genes. The NCBI eukaryotic genome annotation pipeline has been substantially redesigned in the last few years to help meet this need. The pipeline produces evidence-based models using transcript and protein alignments combined with *ab initio* prediction, and is being extended to include use of RNA-seq data. It is largely automated from the initial retrieval of genome, transcript and protein sequences from NCBI archival databases, to calculating and interpreting sequence alignments, providing validation and QA reports, and providing final genome annotation results that are integrated with the RefSeq and Gene databases. The pipeline utilizes assembly-assembly alignments to track gene annotations from one annotation run to the next, thus maintaining identifiers even when the assembly is updated. The final annotation product can include transcripts and proteins for which the sequence has been modified relative to the draft genome assembly in order to correct a truncating mismatch or frameshift and represent a more complete protein. The pipeline has been designed to annotate multiple organisms in parallel, with run times in the range of 1-5 days. This presentation will provide an overview of the annotation pipeline, our approach to integrate RNA-seq data, and an analysis of annotation results for test cases including *D. melanogaster* and human. We will be working with FlyBase to help re-annotate the genomes for 11 Drosophila species, which should greatly improve the cross-species analyses possible in this important genus.

896C

Impact of P278A mutation conferring Breast Cancer susceptibility in the p53 DNA-Binding Core Domain interacting partners. Yeguvapalli Suneetha, Chitrala Kumaraswamy Naidu. Department of zoology, Sri venkateswara University, Tirupati, India.

Breast cancer is the second most common cancer faced by the women around the world. Both genetic and non genetic factors

constitute risk for breast cancer. Several studies have showed that TP53 constitute a high risk factor to Breast Cancer [1, 2]. TP53 is known to interact with several proteins [3]. Among the several domains present in TP53 DNA binding core domain is known to show interaction with Hypoxia inducible factor-1 alpha (HIF-1a), Heat shock protein 90 (Hsp90), Rad51, 53BP2/ASPP2 and Bcl-XL/Bak protein [4]. In our previous study, we have analyzed the deleterious phenotypic effect conferring to breast cancer by rs17849781 (P278A) and further we have analyzed the structural effect of the P278A mutation on the p53 DNA-binding core domain[5]. In the present study, we aim at analyzing the impact of P278A on p53 DNA-binding core domain interacting partners using computational approaches. Key words: Computational analysis, Protein-Protein interactions, Breast Cancer, TP53, Deleterious mutations References: 1.Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M: Genetic susceptibility to breast cancer. Mol Oncol 2010, 4:174-191. 2.Gasco M, Shami S, Crook T: The p53 pathway in breast cancer. Breast Cancer Res 2002, 4:70-76. 3.Keller DM, Zeng SX, Lu H: Interaction of p53 with cellular proteins. Methods Mol Biol 2003, 234:121-133. 4.Fernandez-Fernandez MR, Sot B: The relevance of protein-protein interactions for p53 function: the CPE contribution. Protein Eng Des Sel 2011, 24:41-51. 5.Y Suneetha, Naidu CK: Structural effect of P278A mutation conferring breast cancer susceptibility in the p53 DNA-binding core domain. BMC Proceedings 2012, 6:P50.

897A

Leveraging a knowledge base of *Drosophila cis*-regulatory modules for regulatory element discovery in diverged insect species. Kushal Suryamohan[1], Majid Kazemian[2], Jia-Yu Chen[2], Yinan Zhang[2], Marc Halfon[1,3,4], Saurabh Sinha[2]. 1) Department of Biochemistry and Center of Excellence in Bioinformatics and Life Sciences, State University of New York at Buffalo; 2) Department of Computer Science, University of Illinois Urbana-Champaign, IL; 3) Department of Biological Sciences, State University of New York at Buffalo; 4) Molecular and Cellular Biology Department, Roswell Park Cancer Institute, Buffalo, NY.

Although growing numbers of insect genomes are being sequenced, defining the sequences involved in transcriptional regulation within these genomes remains a challenge. Most effective methods for *cis*-regulatory module (CRM) discovery rely either on empirical assays or computational models that rely on sequence alignment to closely related species and knowledge of CRMs or transcription factor binding sites for the organism being studied. The lack of well annotated databases for regulatory regions of DNA for insect species outside of the well-studied *Drosophila* genus makes such approaches intractable. We previously demonstrated success at computational CRM discovery in *Drosophila* using a supervised learning approach in which experimentally validated CRMs are used to train a CRM prediction algorithm, with as low as a 10% false-positive rate. We demonstrate here that these same *Drosophila* CRM training data can be leveraged to identify CRMs in diverged species such as the emerging model insects *Nasonia vitripennis*, *Tribolium castaneum*, *Anopheles gambiae*, and *Apis mellifera*. Examination of 16 predicted CRMs for regulatory activity *in vivo* in transgenic *Drosophila* showed positive regulatory activity in 12 of the 16 CRMs with 75% clearly associated with the expected gene and about 50% regulating gene expression in the expected pattern. Our results indicate that the extensive experimental CRM data that exists for *Drosophila* can be used to facilitate CRM discovery in distant insect species with sequenced genomes but little functional data, and suggests that core regulatory strategies have been conserved despite the lack of any clear non-coding sequence alignment.

898B

Automated Annotation of Developmental Stages of Drosophila Embryos by Image Analysis. Jieping Ye[1,2], Lei Yuan[1,2], Qian Sun[1,2], Cheng Pan[1,2], Michael McCutchan[1], Stuart Newfeld[3], Sudhir Kumar[1,3]. 1) Center for Evolutionary Medicine and Informatics, Biodesign Institute, Arizona State University, Tempe, AZ; 2) Computer Science and Engineering, Arizona State University, Tempe, AZ; 3) School of Life Sciences, Arizona State University, Tempe, AZ.

Images capturing spatial patterns of Drosophila gene expression are being produced at a higher throughput than ever before. Automated and efficient tools for analyzing these images are a prerequisite for generating biological insights into gene function, interactions and networks. These analyses are the most biologically meaningful when images from a similar time point during development are compared. We present a computational method to automatically annotate the developmental stage of Drosophila embryos displaying gene expression images. The method is based on our observation that image texture changes as embryonic development progresses. Our system is able to accurately determine the development stage of embryos de novo with high accuracy (79%) employing the Campos-Ortega and Hartenstein stage demarcations. The method can also predict ages within the standard developmental stages (e.g., Early versus Late age for a given stage). The within stage information is value in making evaluations about absolute timing of gene expression initiation or alteration. From an analysis of high throughput image data, we found that the Genomewide-Expression-Maps (GEMs) generated using images from embryos in with highly refined stages illuminate global gene activity and transitions better that those employing the standard stages alone. A more precise knowledge of developmental stage also improves the investigator's ability to predict interacting genes when embryonic expression patterns matches are discovered. We will also present results from additional new computational methods that automatically orient, align and annotate expression images with a specific suite of embryo characteristics (e.g., early stage and lateral view).

899C

MiMIC-TIFF: A Method for Making Gene-specific Gal4 lines from MiMIC Insertions into Coding Introns. Fengqiu Diao[1], Feici Diao[1], Sarah Naylor[1], Holly Ironfield[2], Matthias Landgraf[2], Benjamin White[1]. 1) Laboratory of Molecular Biology, NIMH, NIH, Bethesda, MD 20892; 2) University of Cambridge, Dept. of Zoology, Cambridge, UK.

The viral T2A sequence can promote translation of two proteins from a single mRNA, a feature that can be exploited to generate transgenic driver lines that express Gal4 in the same pattern as an endogenous gene of interest (Diao & White, 2012). This technique (called T2A-Gal4-In-Frame-Fusion, or T2A-GIFF) requires insertion of the T2A-Gal4 coding sequence into the reading frame of the endogenous gene by homologous recombination, or by recombineering suitable P[acman] clones and re-introducing them into the fly. But both of these methods are labor intensive. We show here that T2A-Gal4 (and other T2A-transgenes) can be readily introduced into genes of interest that contain a MiMIC insertion in a coding intron. Because more than 50% of intragenic MiMIC insertions lie within such introns (Venken KJT et al, 2010), this method, which we call MiMIC-T2A-mediated In-Frame-Fusion (i.e. MiMIC-TIFF), should be widely applicable. To implement MiMIC-TIFF, we have adapted protein-trap plasmids that allow the T2A-Gal4 sequence to be introduced in-frame into MiMIC insertions by ΦC31-recombinase-mediated cassette exchange. To confirm the efficacy of the approach, we have generated transgenic lines by inserting T2A-Gal4 into multiple genes, including $Rdl^{MI02620}$, $Shaw^{MI01735}$ and $GluRII\ B^{MI03631}$ each of which requires a T2A-Gal4 fusion in a different reading frame. When crossed with a UAS-EYFP reporter, each of these lines shows strong expression in a pattern that matches that of the endogenous gene. Additional constructs, which allow creation of Gal80 and Split Gal4 hemidriver lines have also been made. To simplify the conversion of MiMIC lines into Gal4 driver lines, we are also working on dual-recombinase system using both cre and φC31 that allows Gal4 drivers to be made *in vivo*, by a series of crosses. The utility of MiMIC-TIFF can be expected to increase together with the number of MiMIC insertions generated by the Drosophila Gene Disruption Project (Bellen et al., 2011).

900A

Transposon-based forward and reverse genetics in Anopheles mosquitoes. David A. O'Brochta[1,2], Kristina L. Pilitt[1], Robert A. Harrell, II[1], Channa Aluvihare[1], Robert T. Alford[1]. 1) Institute for Bioscience and Biotechnology Research, University of Maryland, College Park, MD; 2) Department of Entomology, University of Maryland, College Park, MD.

The growing abundance of insect genome sequence data is creating a large demand for functional genomics tools, methods and technologies that can be used in these non-model systems. Transposon-based technologies such as gene- and enhancer-traps are particularly powerful functional genomics technologies and the purpose of this study was to develop these technologies for *Anopheles stephensi* mosquitoes, which are important vectors of human malaria. The mobility properties of integrated *piggyBac* elements in *An. stephensi* were tested by crossing *piggyBac*-containing lines with *piggyBac* transposase-expressing 'jumpstarter' lines. *piggyBac* was found to be efficiently remobilized and conducive to being used to create gene- and enhancer-trap systems. A *Gal4*-based enhancer-trap system was created consisting of six transgenic lines of *Anopheles stephensi*, each with a single *piggyBac-Gal4* element in a unique genomic location, six lines with a single *piggyBac-UAStdTomato* element and two lines, each with a single *Minos* transposable element containing the *piggyBac*-transposase gene under the regulatory control the *hsp70* promoter from *Drosophila melanogaster*. From five genetic screens for larval- and adult-specific enhancers 314 progeny were recovered from 24,250 total progeny (1.3%) with unique patterns of *tdTomato* expression arising from the influence of an enhancer. The frequency of *piggyBac*remobilization and enhancer detection was 2.5-3 fold higher in female germ-lines compared to male germ-lines. A small collection of enhancer-trap lines are described in which *Gal4* expression occurred in adult female salivary glands, midgut and fat body, either singly or in combination. These three tissues play critical roles during the infection of *Anopheles stephensi* by malaria-causing *Plasmodium* parasites. This system and the lines generated using it will be valuable resources to ongoing mosquito functional genomics efforts.

901B

Tools to Facilitate Circuit-Mapping Using the Split Gal4 System. William C Shropshire[1], Haojiang Luan[1,2], Benjamin White[1]. 1) Section on Neural Function, NIH, NIMH, Bethesda, MD; 2) Janelia Farm Research Campus, Ashburn, VA.

To identify neurons that function in behavioral circuits, Gal4 enhancer trap lines are used to drive UAS-effector transgenes that alter neuronal activity. If manipulation of neural activity results in a behavioral change, all or part of the circuit must lie within the Gal4 expression pattern. Refining such patterns to determine which cells specifically belong to the circuit typically requires use of intersectional methods: e.g. Gal80, or the Split Gal4 technique, in which the Gal4 DNA Binding Domain (i.e. Gal4DBD) and a transcription activation domain (e.g. VP16AD) are independently targeted to different subsets of cells. The incompatibility of Gal80 and the Split Gal4 system has limited their combined use to achieve further refinement. Here we introduce an inhibitor of Gal4DBD function, called the "Killer Zipper", which functions like Gal80 in the Split Gal4 system. In addition we introduce an enhancer trap Gal4 construct (i.e. "GG") that can be used with Gal80 and then converted in vivo to a Gal4DBD construct using cre recombinase. The "Killer Zipper" consists of the Gal4DBD fused to the same heterodimerizing leucine zipper that is fused to the VP16AD. It therefore inhibits transcriptional activity by competing with VP16AD for binding to the Gal4DBD and also by promoting formation of Gal4DBD homodimers, which can, in theory, bind UAS sites and prevent the binding of productive Gal4DBD-VP16AD dimers. We have demonstrated the efficacy of the Killer Zipper expressed in a group of neurons that express the neuropeptide CCAP. We have also generated enhancer trap lines with the GG construct and shown that the floxed Gal4 component can be excised in vivo using a heat-shock cre recombinase. Three GG lines have been converted from Gal4 to Gal4DBD expressing lines. We anticipate that the Killer Zipper and GG systems will be useful in improving the resolution of circuit mapping neuronal screens.

902C

Developing a quantitative, cellular resolution morphology and gene expression atlas for *Drosophila* embryogenesis: towards a digital 'Campos-Ortega and Hartenstein'. Soile V E Keränen[1], Jonathan T Barron[2], Pablo Arbelaez[2], Jitendra Malik[2], Mark D Biggin[1], David W Knowles[1]. 1) Life Sci Div, Lawrence Berkeley Natl Lab, Berkeley, CA; 2) Electrical Engineering and Computer Science, UC Berkeley, Berkeley, CA.

Animals comprise dynamic 3D arrays of cells, differing in histological type, shape, size, location, etc. We are extending our VirtualEmbryo (http://bdtnp.lbl.gov/Fly-Net/) of the cellular blastoderm to create a quantitative, digital, cellular resolution atlas of morphology and gene expression for all of *Drosophila* embryogenesis. Because late-stage embryos have some 40,000 cells, 70 cell types and major organs, we have had to develop new strategies to analyze these complex morphologies. Using stage 16 embryos as a model, we have developed an analysis pipeline to automatically find whole tissues or organs in embryos stained to label nuclei. First, target tissues are hand annotated in images of embryos on nuclear stain channel. Annotation accuracy is confirmed by comparison with tissue specific mRNA stains. The annotations are then converted into 3D tissues models, which are used to train support vector machine analysis that automatically locate the tissues in subsequent test images, i.e. images that were not used for model training. We have currently built classifiers that accurately detect six different tissue types based on embryo morphology. Our current detection accuracy ranges between 57% and 87% compared to hand annotations. For example, in the pharyngeal muscles the classifier finds ~80% of the estimated 89±14 cells. Repeating such analyses for all tissues will allow us to create an average morphological map and quantitate the differences between individual embryos. Our computational embryology goal is to create maps of each stage of embryogenesis, annotating all tissues, and to link these via computational analysis of fixed and live cell images.

903A

Ultrastructural analysis of Drosophila melanogaster using Helium Ion Microscopy. Dennis R. LaJeunesse[1,3], Adam Boseman[1], Kyle Nowlin[1], Jijin Yang[2]. 1) Dept Nanoscience, Joint School of Nanoscience and Nanoengineering, Greensboro, NC; 2) Carl Zeiss NTS, LLC, Peabody, Massachusetts; 3) Department of Biology, UNCG, Greensboro, NC 27402.

Scanning electron microscopy (SEM) has been the traditional method used to image the micro and nanoscale surface topology of biological samples. Here, we present a new ultrahigh resolution particle beam microscopy technology, called Helium Ion Microscopy (HIM), which is exceptional for imaging surface structures on biological samples at the nanometer scale. While operationally similar to SEM, HIM uses a beam of helium ions to probe a surface. This offers several unique advantages. HIM eliminates the need to use a conductive metal coating on the sample, thereby allowing the imaging of biological samples in their natural state. This allows the resolution of surface details that were concealed beneath a sputtered layer. Additionally, the low mass of the helium ion yields a small footprint for the generation of secondary electrons in biological materials. This permits imaging at high magnification without decreasing beam energy, thus resulting in higher resolution. The result is an increased depth of field, allowing three-dimensional surfaces to be imaged with uniform clarity across the field of view. In this study we use HIM to characterize cuticular nanostructures in the epicuticle of wild type and mutant Drosophila melanogaster. Of note, we identified novel linear arrays of 55nm nanoribs that decorate the macro and microchaetes of the adult body. In this presentation we will also demonstrate the utility of HIM for imaging cells and cellular components, such as the actin cytoskeleton and polytene chromosomes. HIM provides a powerful new tool for characterization of biological samples at the nanoscale. The identification and characterization of nanoscale structures in Drosophila melanogaster allows the use of genetic and molecular tools to facilitate the functional characterization of these structures, as well as the identification and characterization of the composition and mechanisms involved in the formation of these nanostructures.

904B

A high-throughput template for optimizing Drosophila organ culture with response surface methods. Jeremiah J. Zartman[1,2], Simon Restrepo[2], Konrad Basler[2]. 1) Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN; 2) Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.

The Drosophila wing imaginal disc is a key model organ for molecular developmental genetics. Wing disc studies are generally restricted to endpoint analyses of fixed tissues. Recently several studies have relied on limited data from discs cultured in uncharacterized conditions. Systematic efforts toward developing Drosophila organ culture techniques are becoming critical for further progress. Here we designed a multi-tiered, high-throughput pipeline employing design-of-experiment methods to design a culture medium for wing discs. The resulting formula sustains high levels of proliferation for more than twelve hours. This approach results in a statistical model of proliferation as a function of extrinsic growth supplements and identifies synergies that improve insulin-stimulated growth. A more dynamic view of organogenesis emerges from the optimized culture system that highlights important facets of growth: spatio-temporal clustering of cell divisions and cell junction rearrangements. The same approach may prove useful in improving culture conditions for other organ systems.

905C

Accounting for systematic error in RNA-seq based analysis of allele-specific expression. Rita M. Graze[1,4], Luis G. Léon-Novelo[2], George Casella (posthumous)[3,4], Justin M. Fear[1,4], Lauren M. McIntyre[1,4]. 1) MGM, UF, Gainesville, FL; 2) Mathematics, UL-LFT, Lafayette , LA; 3) Statistics, UF, Gainesville, FL; 4) Genetics Institute, UF, Gainesville, FL.

Genetic differences in transcript regulation can arise from sequence variation in the regulatory regions of a gene itself (*cis*) or in regulatory or coding regions of *trans* acting factors or through indirect or epistatic effects. In diploid organisms expression

from two, potentially different, copies of each gene can contribute to transcript abundance and to subsequent protein production. Alleles expressed in a common cellular environment can differ in *cis* regulatory sequence, but share a common pool of *trans* acting factors. For this reason a common method of identifying *cis* regulatory differences is to perform an analysis of allele-specific expression (ASE) and identify cases where alleles are expressed at different steady-state transcript levels within an outbred or F1 genotype (allelic imbalance, AI). RNA-seq is the technology most frequently used to analyze ASE. Error variance and systematic error are important issues in RNA-seq based analysis of ASE and the binomial test is insufficient to address these issues. DNA controls are an excellent solution to these issues, but are not practical in large scale experiments. We show that regions of sequence similarity in the genome result in mapping ambiguity and explain map bias found in simulations. We find that these regions are associated with detection of AI. This results in an inflation of estimates of the prevalence of *cis* variation if no control is used. Ambiguity is not the only source of bias identified by DNA controls. We propose a flexible Bayesian model, applicable to a wide variety of experimental designs. The model can use information from different sources, such as DNA controls or simulations, to correct for systematic error. The proposed model performs well compared to the standard binomial test. We use performance of the improved model, plus our increased understanding of the role of genome ambiguity, to optimize analysis plans for ASE studies.

906A

Forward genetic screen to identify genes functioning in winner cells during cell competition. Chang Hyun Lee, Gerard Rimesso, Nicholas Baker. Genetics, Albert Einstein College of Medicine, Bronx, NY.

Cell competition is a phenomenon that results from cell-cell interaction that drew interest ever since its first discovery. However, mechanisms that are responsible for this interaction or how otherwise loser cells are distinguished from winner cells in order to trigger it are still poorly defined. A systematic approach to find genes required for this feature should unveil mechanisms that are responsible during this event. We have performed an EMS screen in pursuit genes on chromosome 3R that are required in winner wild type cells to eliminate loser Minute cells. As a result, 26 candidate mutations in 16 complementation groups were isolated. Comparisons of whole genome sequences between mutants and controls is underway to identify the variants responsible for effects on cell competition.

907B

Global analysis of the Dorsal-ventral patterning regulatory network in the wasp Nasonia vitripennis. Jeremy A. Lynch[1,2], Thomas Buchta[2], Orhan Özüak[2], Siegfried Roth[2]. 1) Molecular, Cell, and Developmental Biology, University of Illinois at Chicago, Chicago, IL; 2) Institute for Developmental Biology, University of Cologne, Cologne, Germany.

Gene regulatory networks underlie developmental patterning and morphogenetic processes, and changes in the interactions within the underlying GRNs are a major driver of evolutionary processes. One of the most thoroughly characterized GRNs is the dorsal-ventral (DV) patterning system of the Drosophila embryo. Using this as a starting point, we have endeavored to characterize the DV system of the wasp Nasonia vitripennis. This wasp has convergently evolved a mode of embryonic development similar to that of the fly, and it is of interest to know whether the similarity at the gross level also extends to the molecular level. Taking advantage of our ability to produce dorsalized and ventralized embryos, and combining this with quantitative next-generation sequencing, we have identified a set of over 200 genes that appear to be differentially regulated along the DV axis of the wasp embryo. This set includes many of the genes identified in a similar experiment performed in Drosophila. We have also identified a set of Nasonia genes with distinct expression patterns that are not expressed in the fly embryo. We propose that at least some of these genes were recruited in the wasp to carry out the unique morphogenetic movements that occur in the wasp embryo at gastrulation.

908C

Vienna Tiles (VT) GAL4 driver lines: New resources at the Vienna Drosophila RNAi Center (VDRC). Lisa A. Meadows[1*], Dickson Group[2], Stark Group[2], VDRC Team[1], Alexander Stark[2], Barry Dickson[2]. 1) Vienna Drosophila RNAi Center, Campus Science Support Facilities, Vienna, Austria; 2) IMP - Research Institute of Molecular Pathology, Vienna, Austria.

The Vienna Drosophila RNAi Center (VDRC) maintains and distributes resources for the Drosophila research community. In addition to our transgenic RNAi stocks, covering more than 93.8% of Drosophila genes, we have recently expanded our resources to include a new collection of GAL4 driver lines, known as the Vienna Tiles (VT) library. This complementary resource has been generated by the groups of Barry Dickson and Alex Stark (IMP, Vienna). It currently consists of >6,000 transgenic Drosophila lines containing short fragments of genomic DNA controlling GAL4 expression. The constructs are integrated at a single defined genomic position (attP2) and each carries a distinct candidate cis-regulatory DNA fragment, typically around 2kb in size.

These lines have been constructed according to the methods developed by the Rubin group at Janelia Farm Research Campus and form a complementary resource to the Janelia "FlyLight" collection. The precise expression patterns of reporters driven by the VT lines have been extensively documented and annotated in the Drosophila adult brain (Dickson group, BrainBase) and all lines have been spatio-temporally characterised in embryos (Stark group, www.starklab.org). A database of the expression patterns and their annotations will be available, linked directly to the VDRC website (www.vdrc.at).

909A

***dm*FUCCI - a novel tool for studying cell proliferation in complex tissues.** Norman Zielke, Jerome Korzelius, Monique van

Straaten, Hanna Reuter, Katharina Bender, Gregor Schuhknecht, Juliette Pouch, Bruce Edgar. DKFZ/ZMBH Alliance, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

The development of complex tissues often involves strictly orchestrated lineages, in which only certain cell types proliferate at a time. The decision to proliferate is highly dependent on signals from surrounding cells and hence one of the future challenges is to study cell proliferation within its microenvironment. The recently introduced FUCCI system (Fluorescent Ubiquitination-based Cell CycleIndicator) allows the monitoring of cell cycle phasing in living cells. In the original FUCCI system the sensor-constructs were ubiquitously expressed, limiting its usefulness because the analysis of cell proliferation in complex tissues requires specific labeling of small subpopulations of cells. To overcome these limitations we have generated a fly-specific FUCCI system (*dm*FUCCI), whose expression can be spatially and temporally controlled. The *dm*FUCCI system is based on E2F1 and Cyclin B, which are sequentially degraded by the ubiquitin ligases $CRL4^{Cdt2}$ and APC/C. Simultaneous expression of both*dm*FUCCI probes allows a distinction of all categories of interphase cells; the E2F1-based probe will mark cells in G1 phase; cells in S phase will be labeled by the Cyclin B-based sensor and cells residing in G2 phase will be positive for both markers. To allow maximum flexibility we have generated a series of fly lines expressing GFP/RFP or CFP/YFP versions of the *dm*FUCCI probes under control of UASt, UASp and QUAS promoters. We demonstrate that the *dm*FUCCI system is capable of recapitulating the developmentally programmed cell cycle patterns in developing eye and wing discs. Furthermore, we have applied the *dm*FUCCI method to the stem cell lineage of the adult midgut, which revealed that intestinal stem cells (ISCs) reside either in G1 or G2 phase instead of being halted at a specific cell cycle stage. Altogether, our work demonstrates that the *dm*FUCCI system is a valuable tool for visualizing cell cycle phasing during development and tissue homeostasis.

910B

A *cytosolic Superoxide dismutase* mutant allele and it's metabolism: Investigating the metabolic profile of a mutant fly using Liquid Chromatography - Mass Spectrometry. Jose M. Knee[1], Teresa Rzezniczak[1], Kevin Guo[2], Thomas Merritt[1]. 1) Chemistry & Biochemistry Department, Laurentian University, Sudbury, Ontario, Canada; 2) Bruker Daltonics Inc., Billerica, MA.

We use liquid chromatography-mass spectrometry (LC-MS) to describe and quantify the impact of knocking out the *superoxide dismutase* gene in *Drosophila melanogaster*. Further we compare this metabolomic fingerprint with that of chemically-induced oxidative stress to test the hypothesis that *Sod null* mutants exist in a state of chronic oxidative stress. LC-MS analysis can detect and quantify a broad array of small molecule metabolites and is quickly developing into a powerful tool for researchers in all fields of the life sciences. We have developed a general protocol to quantify the metabolome and assessed the applicability of this technique to *D. melanogaster* by comparing a *Sod1 null* mutation line with a transgenic control under benign conditions and under paraquat exposure. The SOD1 protein is involved in reactive oxygen species scavenging and *Sod1* mutants accumulate both ROS and products of ROS damage. Paraquat induces oxidative stress by chemical production of the superoxide ion. By evaluating the levels of a large set of metabolites, it was determined that over 100 metabolites were present at a significantly different level between the nulls and the controls. The LC-MS protocol used in this study not only aids in understanding the metabolic consequence of a *Sod1* mutation and oxidative stress, but also highlights the applicability of LC-MS analysis to future studies using different systems.

911C

CG3533 plays an important role in axonal targeting and circuit formation in the olfactory system of Drosophila. Arzu Çelik[1], Thomas Hummel[2], Mustafa Talay[1], Kaan Apaydin[1], Selen Zülbahar[1]. 1) Dept Mol Bio and Genetics, Bogazici Univ, Istanbul, Turkey; 2) Dept Neurobiology, Univ Vienna, Vienna, Austria.

The olfactory system of Drosophila represents an interesting example of how a large repertoire of neuronal cell types are specified and assembled into functional circuits. Olfactory sensory neurons express one olfactory receptor from a large number of receptors in the genome to ensure proper sensory perception. Olfactory sensory neurons expressing specific types of receptors connect to second order neurons in the antennal lobe in discrete regions called glomeruli. The specification and patterning of projection neurons appears to be independent of sensory input. In contrast to the mammalian system an involvement of Drosophila ORs in orchestrating the establishment of class-specific connections in the brain have been excluded. The question of how a precise olfactory map can be established is thus of major interest. Neurons and glia largely depend on each other for their role in the nervous system, and their interaction has a distinct role in the functioning of the nervous system. Glial cells act as guiding cells and are usually located at choice points where they send out signals to which growth cones and axons respond. In an enhancer-trap screen for genes that are expressed in subsets of olfactory neurons we identified CG3533, a novel cell-adhesion molecule. This gene is primarily expressed in glia in olfactory organs as well as in the antennal lobe, mainly in glial cells. Cell adhesion molecules are known to have important roles in axonal targeting. Thus, the involvement of this cell adhesion molecule in wiring of the olfactory circuit was investigated using genetic tools. Analysis of mutants revealed severe targeting defects in all ORN classes that were analyzed and defects in the formation of the commissure, pointing to a critical role of this cell adhesion molecule in OR targeting and circuit formation. We are currently performing rescue experiments and are trying to identify interacting partners of CG3533 using genetic and biochemical experiments.

912A

Establishing new roles of Daughterless in the Drosophila melanogaster central nervous system. Mitchell D'Rozario[1], Tina Hu[1], Mohammad Nayal[1], Kaveesh Kutty[1], Daniel Marenda[1,2]. 1) Department of Biology, Drexel University, Philadelphia, PA; 2) Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA.

Proper neurodevelopment in all animals depends on basic helix-loop-helix (bHLH) proneural transcription factors that regulate how an undifferentiated cell develops into a neural precursor cell. TCF4, a vertebrates type I bHLH proneural protein has been linked to several neuropsychiatric diseases such as Pitt-Hopkins Syndrome (a disease characterized by severe mental and motor retardation) and schizophrenia.Daughterless (da), the only type I bHLH in flies, is an ubiquitously expressed protein with an established function in sex determination, differentiation of mesoderm, and as a proneural gene for establishing the central and peripheral nervous system. Consistent with its role as a transcription factor, Da has been previously localized in the nucleus of multiple cells in the CNS; however, we show that Da is also localized outside the nucleus within the axons of multiple neurons. We also have evidence that Da functions to regulate axonal development in post-mitotic cells, a novel function for this proneural bHLH factor. We suggest that the analysis of daughtlerss in post-mitotic neurons can complement and expand upon the existing studies for this disease, allowing a better understanding of the novel role of Daughterless in post-neurogenesis, neural development and TCF4 in Pitt-Hopkins Syndrome pathogenesis.

913B

Cross-talk between cellular identity specification and axon growth cone guidance in the developing Drosophila embryonic nerve cord. Mary Ann Manavalan, Gaziova Ivana, Bhat Krishna M. Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX.

Guiding axon growth cones towards their synaptic targets is one of the fundamental processes in a developing nervous system. Several major signaling systems have been known to guide axons along the way to their synaptic targets. Mutational disruptions in these signaling systems cause axon guidance defects. However the role of cellular identity specification within the nerve cord in influencing growth cone guidance has never been examined in detail apart from the specification of neurons that send out pioneering axons. Our analysis of axon guidance defects in mutations that alter cellular identity specifications and division patterns indicates that mis-specification of neuroblasts (NBs) that lie in the path of pioneering axons can stall or misroute axons across the midline or even send axons out into the periphery/peripheral system in the Drosophila embryonic CNS. This occurs without altering the expression of some of the major guidance molecules such as Slit or Roundabout. In this work, we will define type of changes in cellular identity that causes such misrouting using mutational tools. We will also show that while expression of certain transcription factors in ectopic sites can induce expression of such axon guidance molecules as roundabout or slit, it plays little, if any, role in regulating the expression of these genes in cells where they are normally expressed.

914C

Transcriptome analysis of *unfulfilled*-dependent gene expression in the mushroom body neurons. Janos Molnar, Karen Bates, Steven Robinow. Biology, University of Hawaii, Honolulu, HI.

The mushroom body (MB) of *Drosophila* is required for memory, learning, and other complex behaviors. The adult MB consists of 5 lobes that are the result of the sequential development and differentiation of three subtypes of neurons, the γ neurons, the α'/β' neurons, and the α/β neurons. The transcription factor UNFULFILLED, a nuclear receptor, is required for the development of all three neuronal subtypes although the role in each subtype is likely to be unique. While hundreds of genes are known to be expressed in the MB and many have been shown to be involved in various aspects of development and differentiation, the genetic hierarchies that regulate MB development and differentiation are only partially understood. To better define the genetic hierarchies that regulate MB development and differentiation, we are identifying genes that act in the MB downstream of *unfulfilled*. We are taking both classical genetic (see abstract by Bates *et al.*, this volume) and comparative transcriptomic approaches to this problem. The RNA of hand-dissected mutant and wild type MBs from second instar larvae was purified and sequenced. Sixteen million paired end reads were mapped and processed with Tophat and Cufflinks. We have detected the expression of 66% of the transcriptome with read counts >1 read per kilobase per million reads (RPKM), with an additional 11% of the genes showing marginal expression below 1 RPKM indicating good coverage of the transcriptome. 837 genes showed significant differential expression ($p \leq 0.05$). Included in this list are genes of the ecdysone biosynthetic pathway, ubiquitination-related genes, axon-guidance genes, and neuronal fate determination genes. These findings are being validated by *in situ* hybridization and genetic screening. We are now analyzing data of the transcriptomes from the developmental stage when the wild type and *unfulfilled* mutant γ and α' /β' neurons are first morphologically distinguishable.

915A

Cell death influences structural reorganization of the larval nervous system during metamorphosis. Soumya Banerjee, Matthew Siefert, Marcus Toral, Joyce Fernands. Zoology, Miami Univ, Oxford, OH.

In keeping with the shift of locomotor control to the thorax, the thoracic ganglion expands during metamorphosis, while the abdominal ganglion is much smaller in proportion. Another significant structural change occurs in the posterior abdominal nerves, which can be visualized as the formation of the terminal nerve trunk. In the larva, eight abdominal nerves directly exit the abdominal ganglion; whereas in the adult, the first three pairs of nerves, A1-A3 exit directly, and nerves A4-A8 are bundled into a terminal nerve trunk (TNT). Examining the abdominal segmental nerve fusion during early metamorphosis indicated that the larval to adult transition becomes evident at the onset of the second day of metamorphosis. To investigate the role of

cell death on the restructuring of ganglion we examined reaper null mutants (H99/XR38). Instead of three pairs of abdominal nerves, four or five pairs of nerves exit directly from the abdominal ganglion (80%; n=25), indicating a defect in segmental nerve fusion. In addition, instead of one terminal nerve trunk, two terminal nerve trunks are observed in reaper null adults (90%; n=25). Examining the peripheral innervation indicated that significantly more ($p<0.005$; n=10) secondary axonal branches and boutons are present in reaper null mutant flies as compared with controls. Studying the fate of dHb9 expressing motor neurons in wild type flies indicated that almost 30% of these neurons undergo programmed cell death during the larval to adult transition. Currently, we are following the fate of this subset of neurons in reaper null mutant flies during this period of development.

916B

Characterization of developmental expression pattern and identification of associated genes in Gal4 enhancer traps. Christopher R Dunne, Devin T Gordon-Hamm, Elizabeth C Marin. Biology, Bucknell University, Lewisburg, PA.

The mushroom body (MB) of *Drosophila melanogaster* is an important brain structure for associative learning and memory and in the adult contains five distinct lobes composed of three different kinds of neurons: γ, α'/β' and α/β. In order to effectively study and analyze MB function and development one must be able to specifically label and genetically manipulate each of these neuronal subtypes throughout development. The Gal4/UAS system is commonly used for this purpose; however, it is important to first ensure a stable and specific expression pattern of the Gal4 enhancer trap.

With this in mind, our lab analyzed the developmental expression pattern of several Gal4 enhancer traps previously reported to be expressed in specific MB neuronal subtypes in the adult brain. We found that none of these lines had stable and specific expression in only one neuronal subtype throughout development. Unexpectedly, we found that the expression pattern of MB247-GAL4 did not include all MB neurons in the adult brain as previously reported. In addition to acquiring expression pattern data, we also determined the genomic location of the Gal4 insert through inverse PCR, thereby identifying flanking genes with a likely role in MB development.

917C

Expression of the Calcium-independent Receptor of α-Latrotoxin (Cirl) in developing *Drosophila melanogaster*. Steve M Saylor, Mark FA VanBerkum. Biological Sciences, Wayne State University, Detroit, MI.

Latrophilins are a family of G-protein coupled receptors (GPCRs) that interact with α-Latrotoxin, a component of black widow spider venom, to stimulate exocytosis. They have been implicated in axon guidance, neural connectivity, and synaptogenesis. Here we characterize the gene structure and expression of the *Drosophila* homolog, *cirl* (CG8649), as a first step in understanding its function. There are eight splice variants of the *cirl* gene encoding two distinct proteins that share a common extracellular N-terminus, but having either one transmembrane domain (short isoform) or the seven commonly associated with GPCRs (long isoform). RT-PCR confirmed the expression of at least five cirl isoforms in embryonic tissue including long and short isoforms. Unexpectedly, none of these isoforms are predicted to have a canonical signal sequence, a feature also seen in the cirl gene of eight *Drosophila* species. Flybase identifies a full-length clone, RE25258, but it actually contains a fortuitous, in frame, signal sequence from an unrelated gene (CG42238). We have subcloned the native *cirl* cDNA and RE25258 into an expression vector and are verifying the membrane localization and topology of Cirl in S2 cells. Using *in situ* hybridization, the expression of both the long and short variants were determined in embryos and third instar larvae CNS and imaginal discs. The long isoform exhibits dynamic expression especially in tissues actively undergoing invagination and/or morphogenesis. This includes expression in the embryonic foregut, midgut, and hindgut, then spreading to the ventral nerve cord, and dorsal pouch, as well as in larval CNS, wing, leg, and eye imaginal discs. The short isoform joins the long form in a subset of these tissues; the embryonic foregut, hindgut, dorsal pouch, and larval leg/eye discs. It is notably absent from the nervous system. To begin assessing *cirl* function we are examining embryos of a P-element insertion line, KG03335, and mobilizing this P-element to create additional alleles. UAS constructs are being developed for overexpression studies. Funded by the NICHHD.

918A

Manipulation of dHb9-expressing Motor Neurons Results in Eclosion Defects. Marcus A. Toral, Soumya Banerjee, David Conway, Joyce Fernandes. Miami University, Oxford, OH.

As the fruit fly develops from a larva into a morphologically distinct adult, existing neural circuits must be remodeled during a period of metamorphosis to allow for the acquisition of new behaviors. Corresponding to this behavioral switch in motor control from the abdomen to the thorax, there is a dramatic reduction and expansion, respectively, to the abdominal and thoracic segments of the central nervous system. In this context our lab has been investigating the involvement of dHb9 expressing motor neurons (MNs) in eclosion behavior. Work in our lab has revealed that the this subset of MNs includes two that innervate larval muscle fibers (MFs) 12 and 13, which are present in abdominal segments A1 and A2 and persist through metamorphosis to aid in eclosion (Kimura and Truman, 1990). To test the role of these dHb9 expressing MNs in eclosion, we have selectively manipulated them during metamorphosis. Through GAL4-UAS targeted activation of the cell death gene Reaper (rpr), Diphtheria Toxin (DT1), and Shibire (shiTS), we initiate apoptotic pathways and functional blockades in dHb9 cells, subsequently analyzing the effects at both the behavioral and cellular levels. We have determined thus far that the dHb9 subset plays a significant, but non-essential role in eclosion. In 100% of flies that failed to eclose, we observed no dHb9-positive innervation to MF13 in A2 (n=20). From this, we have determined that innervation of adult MF13 in A2 is the most

important contribution of the dHb9 subset to proper eclosion. Additionally, preliminary data suggests that the early time period prior to the formation of the adult innervation pattern (0h-48h after pupation) is most critical to the formation of this neural circuit. Next, we will be employing the use of another functional blocking mechanism, Tetanus Toxin (TTX), more closely monitoring eclosion behavior defects with video recordings, and utilizing the extensive descriptions of dHb9 cellular identity gained from our previous work to link observed defects to the absence of individual dHb9 cells and their corresponding muscle targets.

919B

The Novel Zinc-BED transcription factor, *bedwarfed*, is essential for dendritic growth and scaling. Eswar P R Iyer, Srividya C Iyer, Luis Sullivan, Yukting Lau, Shaurya Prakash, Vihitha Thota, Farheen Shaikh, Daniel N Cox. School of Systems Biology, Krasnow Institute for Advanced Study, George Mason Univ, Fairfax, VA.

Dendrites are the primary points of sensory and synaptic inputs to a neuron, and play an important role in synaptic integration and neural function. Despite the functional importance of dendrites, relatively less is known regarding the underlying molecular mechanisms regulating cell-type specific dendritic patterning. From a large-scale screen for dendritic regulators, we identified a previously uncharacterized and evolutionarily conserved zinc-finger BED-type transcription factor, which we have named *bedwarfed* (*bdwf*), that contains a DNA-binding domain found in chromatin boundary element binding proteins and transposases. Here we report our systematic characterization of *bdwf* function in regulating cell-type specific dendrite development. Both loss-of-function and gain-of-function studies reveal *bdwf* regulates dendritic outgrowth, branching and scaling in *Drosophila* dendritic arborization (da) sensory neurons. Expression analyses indicate Bdwf protein is localized to both the nucleus and punctate granules in the cytoplasm of da neurons. Furthermore, we demonstrate that *bdwf* genetically interacts with the homeodomain transcription factor, *cut*, in morphologically complex da neurons where Bdwf and Cut are co-expressed and mutually regulate each other's expression. We also show that Bdwf co-localizes and interacts with ribosomal proteins suggesting this molecule may function in a ribonucleoprotein complex to direct dendrite arborization. Finally, we demonstrate that Bdwf and its ribosomal interactors are essential for regulating cytoskeletal protein expression by promoting actin-rich dendritic processes, while restricting tubulin expression in da neuron subclasses. Taken together, these results provide novel mechanistic insight into complex combinatorial and multi-functional roles of transcription factors in determining class-specific dendrite development.

920C

Ubuiquitin proteasome system regulates dendrite pruning of *Drosophila* sensory neuron. Tzu Lin, Yi-Ping Wu, Sih-Hua Chen, Hsiu-Hsiang Lee. Institute of Molecular Medicine, College of Medicine, National Taiwan University.

During development, the nervous systems often require remodeling to refine their neuronal circuitry to achieve the mature neuronal connection. Neuronal pruning, one of the remodeling mechanisms in the nervous systems, selectively eliminates specific parts of neurites in the absence of cell death. Class VI dendritic arborization (C4da) neurons, a subset of *Drosophila* larval peripheral sensory neurons, which undergo a large-scale dendrite pruning during metamorphosis, provide an ideal model system to study the cellular and molecular mechanisms of neuronal pruning. It is known that the function of ubuiquitin proteasome system (UPS) is essential for neuronal pruning in the developing nervous systems of both vertebrates and invertebrates. Recently, we have identified on uncharacterized mutant that showed a defective dendrite pruning phenotype in *Drosophila* C4da neurons. The dendrite pruning defects in C4da cells of this uncharacterized mutant showed genetic interaction specifically with *Rpn2*, which encodes a non-ATPase subunit of the regulatory 19S proteasome, but not with *Rpn6*, which encodes another non-ATPase subunit of 19S proteasome. Moreover, depletion of Rpn2 proteins in C4da neurons by RNAi also showed impaired dendrite pruning phenotype. To further study the regulation of proteasomal activity during dendrite pruning, we generate Dendra2-based reporter lines to monitor live proteasomal activity in *Drosophila* neurons. Currently, we are characterizing the reporter lines and expect to monitor the proteasomal activity in wild type and mutant neurons during dendrite pruning in the near future.

921A

Analysis of Dendrite Patterning Dynamics in Novel Self-Avoidance Mutant. Marvin Nayan, Jay Parrish. Dept. of Biology, University of Washington, Seattle, WA.

Dendrite morphology is a hallmark of neuronal cell-type and plays an important role in neuronal function. Underscoring the relationship between dendrite form and neuronal function, defects in the spatial arrangement of dendrites are a pathological correlate of numerous diseases of cognition. Using genetic screens, we have been dissecting genetic pathways that underlie the formation and maintenance of dendritic arbors? Here we report on a novel mutation that is defective in dendrite self-avoidance. Using time-lapse confocal microscopy and quantitative analysis, we found that this mutation leads to progressive, late-onset dendrite branching defects and intermingling of dendrites in Class IV dendritic arborization (ddaC) neurons. Early stages of dendrite patterning are not affected by this mutant, suggesting that the mutant perturbs a genetic program that normally ensures maintenance of dendrite self-avoidance. We are currently investigating how broadly this mutation affects dendrite self-avoidance by assaying defects in other classes of sensory neurons. Finally, we have mapped the mutant to a small interval on Chromosome III and will report on our progress in identifying the gene(s) affected by this mutation.

922B

Nejire, a CBP/p300 family transcription factor, regulates dendritic development by modulating the localization of the Krüppel-like transcription factor Dar1 in *Drosophila* da neurons.Myurajan Rubaharan, Srividya C Iyer, Eswar P R Iyer, Daniel N Cox. School of Systems Biol., Krasnow Inst. Adv. Study, George Mason University, Fairfax, VA.

The *Drosophila* PNS has emerged as an excellent model system for studying molecular mechanisms underlying class specific dendrite development. Dar1, a Krüppel-like transcription factor, has been previously identified as an essential regulator of dendrite development via microtubule modulation. Interestingly, while Dar1 is equivalently expressed in all da neurons at the embryonic stage, Dar1 exhibits differential localization in da neuron subclasses at the larval stage. Dar1 is primarily nuclear in the morphologically simple class I neurons as opposed to the largely cytoplasmic localization observed in the complex class IV da neurons. Here we provide novel insights on the mechanism of differential localization of Dar1 protein, and its corresponding effects on dendrite development. Via an RNAi-based approach, we screened the predicted Dar-1 protein-interaction partners for the ability to regulate Dar1 localization as well as the effects of perturbing its differential localization on dendrite development. Through this approach, we have identified *nejire* as a novel regulator of dendritogenesis that acts in modulating Dar1 localization. Nejire is expressed in a differential pattern among da neurons of varying complexity that is consistent with the larval Dar1 expression pattern. Moreover, we have conducted detailed structure-function studies using domain specific deletions of *nejire* that have provided further insights into the specific role of different protein domains in mediating distinct aspects of dendritic growth. Finally, we have investigated the regulatory role of Prospero in modulating expression levels of Nejire to affect dendrite morphology. Collectively, these analyses contribute to our understanding of molecular mechanisms of combinatorial transcription factor activity at a class-specific level and how this regulation contributes to specification of distinct neuronal morphologies that underlie the establishment of complex neural networks.

923C

Survival of glia in optic lamina is maintained by EGFR signal provided by photoreceptors in adult Drosophila visual system. Yuan-Ming Lee[1,2], Y. Henry Sun[1,2]. 1) N415, Inst Molecular Biology, Taipei, Taiwan; 2) Institute of Genomic Science, National Yang-Ming University, Taipei, Taiwan.

In the Drosophila visual system, the photoreceptor neurons send their axons into the optic lobe of the brain. The R1-6 neurons terminate in the lamina and make synaptic contacts with lamina neurons. In the lamina, the axons are surrounded by multiple types of glia. We found that when the endocytosis in glia is blocked in adult flies, the lamina glia will degenerate due to lysosome accumulation. The degeneration is progressive and irreversible, and accompanied by impaired phototransduction and motor activity. EGFR signaling in the glia is required and sufficient to maintain their survival. The survival signal is the EGFR ligands provided by the photoreceptors. These results suggest that the photoreceptors actively maintain the survival of glia in the adult visual system. Whereas many studies have shown that neurons and their target cells mutually maintain each other's survival, our findings show that neurons actively maintain the microenvironment integrity of their target field.

924A

Basigin maintains glial wrapping of axons. Lindsay Petley-Ragan. Zoology, University of British Columbia, Vancouver, BC, BC, Canada.

Drosophila axons are ensheathed by multiple layers of insulating glial cells and an outer layer of extracellular matrix (ECM). Glial-ECM, glial-glial and glial-axonal adhesion are crucial for maintaining the structure and function of nerves. We are investigating a novel role for basigin, a transmembrane protein containing two Ig domains, in glial-axonal adhesion. In vertebrates, basigin is known to be highly expressed on the surface of tumours where it induces the expression of matrix metalloproteinases to mediate tumour metastasis. However, basigin has also been suggested to bring monocarboxylate transporters to the membrane, mediate neuronal adhesion, and genetically interact with integrin. The function of basigin in glia has not yet been investigated. We have demonstrated that in 3rd instar larvae, basigin is expressed by both neurons and glia in the central and peripheral nervous systems (CNS and PNS). Of particular interest is the high concentration of basigin in axons in the CNS at the CNS/PNS transition zone and apparent lack of basigin in PNS axons. Basigin is uniformly expressed in the insulating CNS and PNS glial layers. Knockdown of basigin in glia is lethal and resulted in death and fragmentation of the innermost wrapping glial layer as well as larval locomotion defects. Larvae homozygous for basigin mutations demonstrate similar phenotypes in addition to disrupted CNS glial morphology. We hypothesize that basigin is involved in maintaining adhesion between glia and axons in the central and peripheral nervous systems.

925B

Manipulating the remodeling of glial ensheathment of peripheral nerves during metamorphosis. Matthew Siefert, Soumya Banerjee, Sayantan Mitra, Jack Wilber, Joyce Fernandes. Zoology, Miami University, Oxford, OH.

Larval peripheral nerves are surrounded by 3 layers of glia (Stork et al., 2008). During metamorphosis these layers are reorganized in 5 pairs of posterior abdominal nerves (A4-A8) which come together to form a terminal nerve trunk (TNT). The most external glial layer, the perineurial layer, makes up a large proportion of the total glia, 81.2%% (n=6) along the A3 nerve, 83% (n=12) along the A4 nerve, 85.2% (n=8) along the A5 nerve, 72% (n=6) along the A6 nerve. To probe the role of this glial layer during the formation of the TNT, we manipulated it by selectively targeting UAS-reaper using c527Gal4. When reaper is expressed during the first 24 hours of pupal development period, 66% (n=12) of animals showed defects in TNT formation. Posterior sections of the TNT remain unfused, and more anteriorly, the patterns of defasiculations become altered. There is a significant reduction in the number of glial cells by 24h APF along segmental nerves. Analysis of peripheral nerves A3 through

A5 has revealed a 32.8% (n=5, p value = 0.0002), 11.2% (n=7, p value = 0.046), and a 15.1% (n=6, p value = 0.013) decrease in glial cells along A3, A4, and A5 nerves, respectively. These data suggest a role for perineurial glia in remodeling of peripheral nerves during metamorphosis. To ensure a more efficient elimination of glial cells, we are in the process of targeting Diphtheria Toxin using c527gal4. We also plan to prevent remodeling of the perineurial layer by targeting dominant negative shibire. Results from both sets of ongoing experiments will be presented.

926C

Regulating neuronal composition in the *Drosophila* mushroom body through hormonal extrinsic cues. Daniel I Fritz, Abigail Lubin, Alper Dincer, Jaspinder Kanwal, Elizabeth Marin. Biology, Bucknell University, Lewisburg, PA.

The Drosophila mushroom body is composed of thousands of intrinsic neurons, which are generated by only four original neuroblasts. At least three major neuronal subtypes (γ, α'/β', and α/β) are born sequentially during development, but the mechanism that regulates fate switching in these cells at the appropriate time is unclear. Through the use of sucrose starvation to uncouple mushroom body neuroblast divisions from organismal growth in newly hatched larvae excess γ neurons can be generated. This result strongly suggests that neuronal composition is plastic and can be regulated by an extrinsic developmental cue.

Ecdysone, insulin, and juvenile hormone (JH) would appear to be the most logical candidates for this extrinsic cue. In prior experiments, the corpora allata (CA), the source for JH, was ablated in males resulting in total and non-γ class neurons being reduced in count. Conversely, dietary pyriproxifen, a JH mimic, increased number of non-γ and total neurons in males. In females, CA ablation alone did not affect total or non-γ neuron numbers, however the introduction of pyriproxifen with or without the CA ablation increased total and non-γ neuron numbers. We are currently performing additional genetic experiments to further explore the regulatory effects JH has on mushroom body neuronal fate determination.

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927A

A screen for suppressors of *unfulfilled* reveals novel roles for genes in *Drosophila* mushroom body development. Karen E. Bates[1], Carl Sung[2], Joshua Meldon[1], Liam Hilson[1], Steven Robinow[1]. 1) University of Hawaii Department of Biology 2450 Campus Road Honolulu, HI 96822; 2) University of Chaminade Department of Natural Sciences and Mathematics Honolulu, HI 96822.

The *Drosophila* mushroom body (MB) is an organized collection of interneurons that is required for learning and memory. Over 200 genes are known to be expressed in the MB, yet the genetic hierarchies that control MB development are relatively unknown. Given the sequential birth order and the unique patterning of γ, α'/β', and α/β neuron projections, it is likely that specific gene cascades are required for the different guidance events that form the characteristic lobes of the MB. Previously, we have shown that the nuclear receptor UNFULFILLED (UNF), a transcription factor, is required for the differentiation of all MB neurons. However, the requirement of *unf* differs among the three subtypes of MB neurons. These subtype-specific regulatory roles further emphasize the need to identify interacting genes in the MB. We have developed and utilized a classical genetic suppressor screen, which takes advantage of the fact that ectopic expression of *unf* causes lethality, to identify candidate genes that act downstream of UNF. We hypothesized that reducing the copy number of *unf*-target genes will suppress the *unf*-induced lethality. We have identified 22 genomic regions on chromosome 3 that may encode *unf*-interacting genes. To test whether candidate genes in these regions impact MB development, we performed a secondary screen in which the morphologies of the MBs of doubly heterozygous animals were examined. To date, defects were observed in four genes found within four different genomic regions of seven regions that were tested, *;unf/+;failed axon connections/+, ;unf/+;axin/+, ;unf/+;dopamine R2/+*, and*;unf/+;tailless/+* flies. These results document a previously undescribed role for *tailless*, an unexpected morphological defect for *dopamine R2*, and has identified two genes, *failed axon connections* and*axin*, that were not previously known to be expressed in the MB.

928B

Regulation of dendrite morphogenesis by Nanos and Pumilio. Balpreet Bhogal, Elizabeth Gavis. Department of Molecular Biology, Princeton University, Princeton, NJ.

The organization of dendritic arbors dictates how effectively a neuron can receive and integrate extracellular signals. Although many factors have been identified that are essential for establishing the size and complexity of dendritic arbors, much less is known about how the branching patterns are subsequently refined and maintained during neuronal development. Dendritic arborization (da) neurons in the*Drosophila* larval peripheral nervous system have provided a powerful genetic system to identify factors required for dendrite morphogenesis. The translational repressors Nanos (Nos) and Pumilio (Pum) are required to maintain dendritic arborization during late larval development in the highly branched class IV da neurons. Recent genetic analyses from our lab suggest that proper dendrite morphogenesis of class IV da neurons requires regulation of the apoptotic pathway by Nos and Pum. We found that genetically reducing apoptotic gene function suppresses the dendritic defects observed in class IV da neurons with reduced *nos* function. Time-lapse confocal microscopy revealed that suppression of the dendritic defects observed in *nos*-deficient class IV da neurons is most likely due to a restoration of branch growth and a

reduction in branch retraction. A fluorescent reporter is currently being utilized to determine whether caspase activity is increased in *nos* and *pum* mutant larvae. We are concurrently determining if Nos and Pum directly regulate the pro-apoptotic factor Head involution defective (Hid). Hid expression is elevated in *nos* and *pum* mutant larvae and genetic analysis revealed that reducing Hid expression suppresses the dendritic arborization defects observed in *nos*-deficient class IV da neurons. We have also demonstrated that Pum binds to the 3' untranslated region (UTR) of *hid* mRNA *in vitro*. We propose that Nos and Pum modulate nonapoptotic caspase function through regulation of *hid* in order to maintain dendritic complexity of larval class IV da neurons prior to metamorphosis.

929C

Regulation of metamorphic neuronal remodeling by *alan shepard (shep)*.. Dahong Chen, Randall Hewes. Department of Biology, University of Oklahoma, Norman, OK.

The nervous system undergoes substantial postembryonic remodeling in order to support developmental transitions, responses to environmental changes, and recovery from injury and disease. As a model for understanding the mechanisms governing these changes, we are studying neuronal remodeling during Drosophila metamorphosis. We found that *alan shepard (shep)* plays an important role in remodeling of peptidergic neurons. *shep* encodes multiple RNA binding protein isoforms, and it is expressed primarily in the central nervous system. *shep* mutants displayed multiple defects, including lethality accompanied by failure to evert the head at pupation, blocked wing expansion, reduced lifespan, and sexual rejection of males by virgin females. Based on the wing expansion phenotype, we examined the morphology of the neuropeptidergic neurons that synthesize bursicon, which is an essential regulator of wing expansion behavior. In *shep* mutants, the bursicon neurons displayed normal cellular morphology in the larval stages, but smaller somata as well as reduced branching and fewer boutons in pharate adults. Expression of a wild-type *shep* cDNA rescued all of the cellular phenotypes. Interestingly, we found that several other behaviors that appear unrelated to the bursicon neurons (e.g., general locomotion), were largely normal in larvae but often severely impacted in adults. Therefore, *shep* may play a general role in promoting growth of diverse neurons during metamorphosis. In order to identify the signaling pathway and downstream targets for *shep*, we analyzed RNA-seq data that were generated by inducing $shep^{RNAi}$ in S2 cells. Six genes, including 2 endopeptidases, a ribosomal protein, and a lectin, displayed significant changes in expression levels following the loss of *shep*. To verify the RNA-seq results biologically and identify other candidate interacting genes, we have initiated a genome-wide screen with 777 deficiency stocks for modifiers of the wing expansion defects triggered by $shep^{RNAi}$ in the bursicon neurons. To date, we have isolated several strong suppressor and enhancer deficiencies.

930A

***Drosophila* Tempura, a putative protein prenyltransferase, regulate synaptic growth and synaptic transmission.** Kuchuan Chen[1], Wu-Lin Charng[1], Shinya Yamamoto[1], Nele Haelterman[1], Guang Lin[2,4], Hugo Bellen[1,2,3,4]. 1) Program in Developmental Biology; 2) Dept of Molecular and Human Genetics; 3) Dept of Neuroscience; 4) Howard Hughes Medical Institute; Jan and Dan Duncan Neurological Research Institute, Baylor College of Medicine, Houston, TX.

Neurons require efficient and specialized vesicle trafficking for their proper development and function. Small Rab GTPases are key regulators of this vesicular transport system. Mutations in Rab proteins or their regulators have been shown to be associated with several neurological diseases. In a forward genetic screen of the *Drosophila* X chromosome, we isolated 8 alleles of an uncharacterized gene that we named *tempura*. Our current data (see abstract by Wu-Lin Charng et al.) indicate that *tempura* is a α subunit of geranylgeranyl transferase II (GGT II), an enzyme involved in the covalent attachment of 20-carbon geranylgeranyl groups to Rab GTPases. This lipid modification functions as a membrane anchor for Rabs and is paramount for the function and subcellular localization of the Rab proteins. To investigate the role of *tempura* in the nervous system, we examined the larval neuromuscular junction (NMJ) in these mutants. Interestingly, loss of *tempura* causes a severe morphological defect in the larval NMJ. The axon terminal boutons are less branched and more clustered, suggesting that *tempura* plays an important role in synaptic growth. To determine if *tempura* is required for proper synaptic function, we performed FM1-43 assays and electrophysiological recording. The size of the excitatory junction potential (EJP) in *tempura* mutants is comparable to controls, suggesting normal exocytosis. However, FM1-43 fluorescence is reduced in *tempura* mutants, indicating impaired endocytosis of synaptic vesicles. To examine the expression pattern of *tempura*, we created transgenic fly with a genomically tagged gene. Surprisingly, *tempura* is highly expressed in glial cells in L3 instar larvae. These data suggest that *tempura* may play a role in glial cells that regulate neuronal development and function via non-autonomous pathway.

931B

PPR-proteins, which are implicated in the neurodegenerative disease Leigh Syndrome, reveal a role for mitochondria in attenuating BMP-signaling. Nele Haelterman[1], Manish Jaiswal[2], Berrak Ugur[1], Hector Sandoval[2], Ke Zhang[3], Taraka Donti[2], Brett Graham[2], Vafa Bayat[1], Shinya Yamamoto[1,2], Hugo Bellen[1,2,3,4]. 1) Program in Developmental Biology; 2) Department of Molecular and Human Genetics; 3) Structural and Computational Biology & Molecular Biophysics Graduate Program; 4) Howard Hughes Medical Institute, Neurological Research Institute, Baylor College of Medicine, Houston, TX 77030.

In a forward genetic screen designed to isolate novel loci whose loss causes neuronal demise, we identified loss-of-function mutations in *dPPR*, a homolog of the human leucine-rich pentatricopeptide repeat containing protein (LRPPRC). Mutations in LRPPRC result in mitochondrial (mt) dysfunction and Leigh Syndrome, a childhood neurometabolic disorder. LRPPRC is a

member of the pentatricopeptide repeat-containing (PPR-)protein family that is involved in regulating mt-RNA-polyadenylation and -stability. Bicoid stability factor (*bsf*) and *dPPR* are the two *Drosophila* homologs of LRPPRC. Both proteins localize to mitochondria. We found that *dPPR* is required to maintain complex I and IV activity within the mt respiratory chain, whereas loss of *bsf* severely impairs the function of complexes I and III. Our data suggest that dPPR and Bsf coordinately regulate respiratory chain activity. This hypothesis is supported genetically, since simultaneous loss of *bsf* and *dPPR* leads to enhanced lethality. To understand how PPR-proteins affect neuronal function and maintenance, we assessed the effects of loss of *dPPR* or *bsf* in the adult eye and the third instar larval neuromuscular junction (NMJ). In the eye, homozygous mutant *dPPR* or *bsf*clones display severe degeneration upon aging. At the NMJ, loss of either protein leads to severe overgrowth, a phenotype that seems to be due to hyperactivation of the BMP pathway. We are currently investigating how mt proteins can modulate the activity of this neuronal growth-regulating pathway. Our results would help define the role of mitochondria in intercellular signaling during neuronal development and uncover novel insights into the molecular and cellular aspects of Leigh Syndrome.

932C

Adult brain compartment formation requires proper scaffolding by secondary neuronal lineages. Jennifer K Lovick, Volker Hartenstein. MCDB, UCLA, Los Angeles, CA.

The Drosophila brain is a highly ordered, compartmentalized structure. Central brain lineages (~100 paired stereotyped neural elements), mapped at the larval stage on the basis of the characteristic axon bundle formed by each lineage, constitute the fundamental units of brain circuitry. Each lineage is formed by a stem cell (neuroblast) that undergoes two phases of proliferation. An early, embryonic phase produces 10-20 primary neurons. After that neuroblasts become quiescent and subsequently reactivate at different time points during the second larval instar to initiate a second, longer phase of proliferation that produces secondary neurons (sNs); these differentiate during metamorphosis and form the vast majority of neurons of the adult brain. In the current study, we undertook systematic application of hydroxyurea pulses (chemical ablation of mitotic cells) to staged larvae, which resulted in selective loss of sN lineages (visualized in late larvae and adults with lineage-specific GAL4 reporter lines and markers for sNs). Ablation in discrete temporal windows in early larvae reveals that NBs exit quiescence at very specific times to produce sNs during larval development, thus allowing us to assign each sN lineage a "birth date." Furthermore, selective ablation of different sets of lineages, resulting from HU pulses at different time intervals, showed specific effects on the volume of brain compartments at the adult stage. We see that many compartments are reduced in size or missing when a lineage or sets of lineages are ablated. Combining our knowledge of secondary lineage and compartment morphologies in the adult brain allowed us to determine the primary or scaffolding lineage for compartments. Our goal is to show the precise order in which post-embryonic lineages are born in the larvae and how this may influence the manner in which compartments develop during metamorphosis.

933A

A Drosophila Cobblestone Lissencephaly model reveals Dystroglycan is buffered by microRNA-310s via its alternative 3'UTR. Halyna R Shcherbata, Andriy S Yatsenko, April K Marrone. MPRG of Gene Expression and Signaling, Max Planck Institute, Goettingen, Germany.

Dystroglycan (Dg) is a conserved, critical extracellular matrix protein that is responsible for general housekeeping processes relevant to total organism health. We show that in Drosophila the Dg mRNA is subject to alternative 3'UTR processing making it to some extent visible to miRNA targeting and consequential degradation, which offers an extra layer of expression precision. This precision of Dg levels is especially important in the nervous system, where misexpressed Dg causes high levels of lethality, deformed brain shape and neuroblast cell cycle defects. ECM-dependent regulation of cell-cycle progression can be mediated by mechanical stress sensors and since Dg is a mechano-signaling transmitter, we propose that Dg is involved in a signal-transduction mechanism through which the extracellular matrix regulates the speed of neuronal stem cell division. Interestingly, levels of Dg per se are critical to coordinate proper formation of the 3D architecture of the developing brain. Dg misexpression affects localization of multiple ECM proteins, resulting in formation of tumor-like structures that outgrow the normal contour of the ECM-defined brain space. This phenotype is similar to the brain cortex abnormalities associated with dystroglycanopathies in humans, implying that Drosophila Dg mutants can serve as a model for cobblestone lissencephaly. We found that misexpression of Dg affects the distribution of major cell adhesion proteins, which affects the ECM composition consequently resulting in abnormal tissue assembly. Since multiple aspects of neuron maturation involve rigorous rearrangements of cell shape and form, and ECM receptors, and particularly Dg is activated by mechanical stress due to cell shape reorganization. After the completion of cell shape rearrangements baseline Dg levels have to be reestablished and our data suggest that the miR-310s are induced in response to higher Dg levels to manage the amount of this ECM receptor.

934B

Role of cell death in the development of the adult *Drosophila* optic lobe during metamorphosis. Hidenobu Tsujimura[1], Tatsuya Sudo[1], Yusuke Hara[1,2], Yu Togane[1], Hiromi Akagawa[1,2], Ayano Ishitsuka[1], Masashi Iwamura[1], Ryo Iizuka[1], Ayaka Tsutsumi[1]. 1) Dept Dev Biol, Tokyo Univ Agric & Tech, Fuchu-si, Tokyo, Japan; 2) Dept Bio Pro Sci, Tokyo Univ of Agric & Tech, Fuchu-si, Tokyo, Japan.

In the *Drosophila* optic lobe many cells die during development. We have found that the cell death is caspase-dependent apoptosis and mainly occurs in neurons during the formation of neural circuits. It is observed in some specific clusters of cells

in the developing optic lobe and includes ecdysone-dependent and independent ones. However, physiological meaning of the cell death is remained to be defined. Here, to define the meaning, we analyzed by immunostaining the structure of optic lobes where the cell death was inhibited with *p35*, a baculovirus cell death inhibitor. *p35* was overexpressed in neurons, glia or both of them and structural defect was examined. The structure of the optic lobe was almost the same as that in the wild type when *p35* was overexpressed in glia. In contrast, many defects were observed at various structural levels when *p35* was expressed in neurons. Most prominent ones were newly emerged abnormal nerve tracts and abnormal neurite lumps in the cortices that were surrounded by glia. We also observed defects in the separation and rearrangement of neuropils and loss and disorder of some columns in medulla neuropil . These results show that the cell death is essential for normal optic lobe development. Frequency of abnormal neurite lumps and nerve tracts reduced as development proceeded when *p35* was expressed in neurons. However, the defects persisted into later stages when *p35* was expressed both in neurons and glia. These facts suggest that glia may be involved in the removal of the abnormal structures and caspase activity in gia may be required for the removal.

935C

Regulation of Axonal Branch Refinement by EGF-Receptor Signaling. Marlen Zschaetzsch, Bassem Hassan. VIB, Leuven, Belgium.

Neuronal circuit function requires correct development of appropriate connectivity. In mammalian systems a common mechanism to establish circuits is refinement of excessive projections during development. However, mechanisms of exuberant neurite refinement are largely unknown. Invertebrate nervous systems are thought not to require developmental refinement. Contrary to this view, we show that an adult specific neuronal circuit in the central visual system of Drosophila shows excessive axon branch formation and subsequent pruning during development. We find that dosage critical activation of asymmetrically distributed Epidermal Growth Factor Receptor (EGFR) by ligand released from retinal axons is necessary for branch pruning. Live imaging of the developing brain shows that EGFR signaling is required for branch growth and retraction dynamics during, likely through regulation of the actin cytoskeleton. These observations establish the first Drosophila model for developmental neuronal circuit refinement and identify non-canonical localized EGFR signaling as a novel refinement mechanism.

936A

Sexual identity affects the development and mature function of a defined neural circuit in *Drosophila melanogaster*. Parag Bhatt, Selma Advagic, Harsha Swamy. Pharmcological and Physiological Science, Saint Louis University School of Medicine, St Louis, MO.

In the fruit fly, *Drosophila melanogaster*, serotonin (5-HT) functions both as a neurotransmitter to regulate larval feeding, and as a trophic factor in the development of the stomatogastric feeding circuit. We have shown an inverse relationship between developmental 5-HT levels and the complexity of the 5-HT axonal fibers projecting from the brain to the foregut, which correlates with perturbations in feeding, the functional output of the circuit (Neckameyer, 2010). These effects are distinct from the actions of 5-HT as a neurotransmitter. We have also shown that although dopamine (DA) neurotransmission does not modulate feeding, perturbed levels of neuronal DA during development affect 5-HT innervation of the gut as well as larval feeding behavior (Neckameyer and Bhatt, 2012). Although feeding does not differ between male and female larvae, it is differentially sensitive to the trophic, or developmental, actions of neuronal 5-HT and DA. We have identified a subset of neurons, using Gal4 drivers, that are at least partially responsible for the sexually dimorphic effects in the feeding response. Using transgenic approaches, we have manipulated the sexual identity of the brain during CNS development, and have established that it is sensitive to the actions of DA and 5-HT function.

937B

The transcriptional code of adult motoneuron identity in Drosophila. Jonathan Enriquez[1], Myungin Baek[2], Richard Mann[1]. 1) Department of Biochemistry and Molecular Biophysic, Columbia University Medical Center , New York, NY; 2) NYU School of Medicine Neuroscience Program 522 First Avenue SML504 New York, NY 10016.

Drosophila offers the possibility to study how sophisticated movements are coordinated by a small number of neurons. In each fly leg, around 50 motoneurons, generated from 11 neuroblast lineages, are used for walking and grooming. Two major lineages, called Lin A and Lin B, generate about 28 and 7 motoneurons respectively. Our goal is to understand how these neurons obtain their individual identities. Our hypothesis is that each adult leg motoneuron expresses a specific combinatorial code of transcription factors (TFs) that controls its identity. To identify candidate TFs, positively labeled Lin A and Lin B MARCM clones in L3 larvae were stained with antibodies directed against a large number of TFs. Because it contains fewer motoneurons, our initial focus was on Lin B, thus simplifying the analysis. The TFs identified are Empty spiracles (Ems), Kruppel (Kr), Zinc Finger Homeodomain Factors 1 and 2 (ZFH1/2), the Hox protein Proboscipedia (Pb), and Twin of Eyeless (Toy). Double and triple immunostains reveal a complex combinatorial code, consistent with our hypothesis. Subsequent genetic experiments reveal the consequences of genetically removing or ectopically expressing these TFs during motoneuron development. Both dendritic morphology and axon branching patterns were characterized. These observations support the idea that subsets of TFs regulate different aspects of motoneuron identity, depending on the context, in particular, which other TFs are present. In some cases, dendritic morphology was regulated independently from axon targeting, while in other cases they were coordinately regulated.

938C

***Antp* regulates segment-specific survival and morphology in the postembryonic ventral nervous system.** Ginna E. Freehling, Danielle R. Alaimo, Kirstin T. Rudd, Anthony R. Cillo, Elizabeth C. Marin. Bucknell University, Lewisburg, PA.

In the postembryonic ventral nervous system, 25 persisting neuroblasts give rise to the adult-specific secondary neurons, which have been observed to exhibit segment-specific survival and morphology. Several of the Hox genes have been reported to be expressed in the appropriate time and place to regulate these differences: *Scr, Antp, Ubx, abdA,* and *AbdB*,. Our previous experiments showed that *Ubx* is expressed in a segment-, lineage-, and hemilineage-specific manner in the thoracic and anterior abdominal segments. Loss of *Ubx* function in posterior neuroblast lineages via mitotic recombination at the beginning of larval neurogenesis resulted in transformation to the neuron morphology and survival patterns of their anterior thoracic counterparts. Gain of *Ubx* function through ectopic expression in anterior thoracic segments caused the neurons to exhibit a posterior transformation phenotype, often but not always leading to cell death. The distinct outcomes we observed implied that *Ubx* has been co-opted for different and sometimes opposite roles dependent upon the neuronal hemilineage.

Antp is expressed in an adjacent and at least partially overlapping domain anterior to *Ubx* in the ventral nervous system. We have characterized the expression pattern of *Antp* in the 25 lineages and performed gain and loss of function experiments to define the role of this gene in anteroposterior patterning in the ventral nervous system. We are in the process of examining genetic interactions between *Ubx*and *Antp* to determine whether any of the earlier effects of manipulating *Ubx* expression were actually due to changes in *Antp* expression or activity.

939A

Actin associates with bHLH proneural proteins in nucleus and positively regulates neural precursor gene expression. Yun-Ling Hsiao[1,2], Yu-Ju Chen[1,2], Hsiao-Fong Yeh[2], Haiwei Pi[1,2]. 1) Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan; 2) Department of Biomedical Sciences, College of Medicine, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan.

Proneural proteins of basic helix-loop-helix (bHLH) transcription factors play a central role in neurogenesis. However, little is known about how proneural proteins interact with the transcriptional machinery to activate downstream target gene expression. Drosophila proneural proteins Achaete (Ac) and Scute (Sc) induce external sensory (ES) organ formation. Through co-immunoprecipitation and mass spectormetric (MS) analyses, we found that nuclear actin but not cytoplsmic actin associated with proneural proteins Ac and Sc in Drosophila S2 cells. Daughterless, the Drosophila homologue of E12 and E47 proteins, also associated with nuclear actin through heterodimerization with Ac. Overexpression of NLS-actin that localized predominantly in nuclei elevated proneural protein downstream target gene expression in S2 cells in a Ac/Da-dependent manner. Reduction in actin and ac sc gene activity resulted in a synergistic loss of some ES organs, and decrease in actin5C and actin42A expression level reduced phyllopod transcription in neural precursors. Taken together, our analyses suggest an actin-mediated regulation of bHLH proneural activity in promoting gene expression and precursor specification.

940B

Thinking hard for Scarecrow, the NKX2.1 homolog of *Drosophila*. Crystal L. Maki, Albert J. Erives. Dept. of Biology, University of Iowa.

Homeobox-containing genes are important regulators of development and encode tissue- and cell-specific transcription factors that bind DNA via their homeodomains. The *Drosophila* genes for *ventral neurons defective* (*vnd*), *bagpipe* (*bap*), *tinman* (*tin*), and *scarecrow* (*scro*) encode a conserved subfamily of homeobox genes. The genes *bap* and *tin* pattern the mesoderm and specify the founder cells that give rise to the musculature. In contrast, *vnd* and *scro* are involved in neuroectoderm-specification and CNS development. While much is known about *vnd* regulation in the early embryo and its role in the patterning, much less is known about *scro*. Its vertebrate homolog *nkx2.1*is well studied and is known to function in brain, lung, and thyroid development. Accordingly, in humans *nkx2.1* disruption leads to developmental pleiotropic brain-lung-thyroid syndrome.

During *Drosophila* embryogenesis, *scro* is expressed similarly in the pharyngeal primordia, pharynx, segmental neuronal precursors in the ventral nerve cord, and procephalic neuroblasts that give rise to distinct regions in the brain (Zaffran et al. 2000). We conducted a computational screen for conserved Nkx2.1-like binding sites in *D. virilis* and *D. melanogaster*, which represent divergent lineages as old as the genus. We identified all enhancer-size sequences centered on Nkx2.1-like binding sites located in each genome, and used a reciprocal regulatory BLAST method (R-BLAST) to identify conserved modules. We optimized R-BLAST by using the neurogenic ectoderm enhancers (NEEs) of *vnd* and other loci as positive internal controls. Here, we identified 76 putative enhancer sequences, which we then analyzed for enrichment of Gene Ontology categories in nearby genes. We found over-representation of loci encoding factors involved in cell fate determination and regulation of development. We report on enhancers located at *pumilio* (*pum*), *neuralized* (*neur*), *midline* (*mid*), and other loci. To test *scro* involvement at these enhancers, we are constructing mutant alleles of *scro*, which is located in the heterochromatic region of 3L.

941C

Salt Inducible Kinases in Drosophila Neural Development. Bahar H. Sahin[1], Sercan Sayin[1,2], Nic Tapon[3], Arzu Celik[1]. 1) Molekuler Biyoloji ve Genetik Bolumu, Bogazici Universitesi, Istanbul, Turkey; 2) Max Planck Institute of Neurobiology,

Munchen, Germany; 3) London Research Institute, Lincoln's Inn Fields Laboratories, London, UK.

Salt inducible kinases (SIKs) are Ser/Thr kinases from AMPK-RK family, and involved in insulin metabolism. Upon regulation by PKA signal, SIKs regulate CRTC activity. SIKs are target of a master tumor suppressor kinase Lkb-1; accordingly many instances of cancer progression exist. Here we model the highly conserved SIK homologs in Drosophila. Like the mammalian ortholog, Drosophila SIK2 interacts with the FGF pathway in the course of insulin metabolism; while SIK3 is poorly characterized. Recently SIK3 was shown to affect glucose/lipid homeostasis, skeletal and adipocyte development. Yet the molecular interactions SIK3 goes through remain largely unknown. We started to characterize the role of SIK2 and SIK3 in nervous system development using the Drosophila compound eyes as a model. We generated the SIK3 null mutants and used the conventional Drosophila genetic techniques to elucidate function of SIKs. SIK modulation causes tumors, showing a role in cancer as well. We have also shown that both SIK2 and SIK3 are involved in eye development, regulating eye size and cell specification events. Using scanning electron microscopy, we have seen that bristles and lenses are disrupted in mutants. To understand the defect in more detail, we analyzed late larval and early pupal expression of SIK3. Behind the morphogenetic furrow, SIK3 is expressed in the nuclei of neural cell, as well as other cells that are currently under investigation. Midpupal eyes will soon be dissected to understand SIK3 role on the specification. Meanwhile, we have seen that SIKs interact with the Notch pathway. Currently, we are trying to identify the pathway member SIKs are interacting with, and the mode of interaction by the genetic approach. Once SIK partner is identified, the role of SIKs in cell proliferation and apoptosis will be investigated as well. Thus, in this project, our aim is to attribute a defined role in neural development and cell specification to Drosophila SIK genes.

942A

The larval-to-pupal onset of *let-7-Complex* microRNAs regulates *chinmo* to specify neuronal temporal identity. Nicholas S. Sokol, Yen-Chi Wu. Dept Biol, Indiana Univ, Bloomington, IN.

Drosophila neural progenitors generate lineages composed of distinct neuronal subtypes. We find that the *let-7-Complex* (*let-7-C*) locus promotes the transitions in the production of these distinct subtypes in the post-embryonic mushroom body (MB) lineage, and are investigating whether *let-7-C* expression is linked to the embryonic temporal transcription factor (TTF) cascade in additional lineages throughout the nervous system. The *let-7-C* locus encodes three microRNAs (*miR-100*, *let-7* and *miR-125*) and is transcriptionally activated in post-mitotic MB neurons during the larval-to-pupal transition, when three distinct MB subtypes (α'β', pioneer αβ, and αβ neurons) are born. Loss or increase of *let-7-C* delays or accelerates the rate of transitions between these three subtypes, respectively, and lead to cell fate transformations. We speculated that *chinmo* may be a target of *let-7-C* microRNAs, since specification of these subtypes involves the post-transcriptional repression of *chinmo*. Consistent with this hypothesis, we find that the *chinmo* 3'UTR contains functional *let-7* and *miR-125* binding sites and detect elevated levels of Chinmo protein in *let-7-C* MB cells. Furthermore, genetic interactions indicate that*let-7-C* mutant MB phenotypes are caused by elevated *chinmo*. Chinmo expression is modulated throughout the nervous system by the transient redeployment of two TTF genes, *castor* and *seven-up*, suggesting *let-7-C* may generally function as an intermediary for TTF mediated-*chinmo* regulation. To investigate the relationship between *let-7-C* and the TTF cascade, we have been characterizing in detail the onset of *let-7-C* in the adult lineages of the ventral nerve cord. Current experiments are directed at testing whether *let-7-C* onset is affected by the loss or overexpression of TFF components, including Hunchback, Kruppel, Pdm, Castor, Grainyhead, and Seven-Up. Taken together, these studies identify *let-7-Complex* microRNAs as critical components of a regulatory pathway that controls temporal identity during Drosophila nervous system development.

943B

Mitosis in neurons: Roughex and APC/C maintain cell cycle exit to prevent cytokinetic and axonal defects in Drosophila photoreceptor neurons. Nick Baker[1], Robert Ruggiero[1], Abhijit Kale[1], Barbara Thomas[2]. 1) Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Laboratory of Biochemistry, National Cancer Institute, Bethesda, MD.

Neural function is facilitated by the stable, post-mitotic status of the neurons. Little has been known about the mechanisms of neuronal cell cycle withdrawal, and there are few reports of mutations that affect it. We find three genes where mutations lead to cell cycle re-entry during terminal differentiation by a particular class of developing photoreceptor neurons in the fly retina. Strikingly, these neurons do not complete cell division but only divide their nuclei, failing to develop a contractile ring or to perform cytokinesis. Most of the resulting bi-nucleated neurons retain one nucleus in its normal location in the cell body, while transporting the other nucleus towards the growth cone in a kinesin-dependent manner resembling anterograde axonal transport. Our findings identify Cyclin A regulation as crucial to maintaining cell cycle exit by these neurons, and a neuron-specific defect in cytokinesis as a further barrier to neuron proliferation. Because defects in transporting axonal material have been implicated in multiple neurodegenerative diseases, our findings raise the possibility of a connection between defective cell cycle regulation in neurons and neuronal cell death related to defective axonal trafficking.

944C

Characterization of neuronal death and degeneration upon cell cycle re-entry in *rux* and APC/C mutants. Adriana De La Garza, Nicholas E. Baker. Albert Einstein College of Medicine, Bronx, NY.

Roughex (*rux*) is a cyclin dependent kinase inhibitor that is required to maintain cell cycle exit in the Drosophila eye. In *rux* mutants R8 photoreceptor neurons re-enter the cell cycle as well as non-neuronal cells. Cycling R8 cells produce two distinct nuclei; however, they do not complete cytokinesis and one of the nuclei is often mobilized into the developing axon.

The characteristics of this phenotype match those observed in mutants of universally conserved components of the Anaphase Promoting Complex. Since both R8 cells and other photoreceptor classes are missing from the rux mutant eye, it will be important to determine how these neurons are lost and whether there are autonomous or non-autonomous survival consequences of neuronal cell cycle entry. Genetic and labeling studies of *rux* are being used to identify the times, mechanisms, and locations where retinal cells are lost, and how this depends on the abnormal cell cycle events. If neuronal cell cycle re-entry is linked to neurodegenerative disease, as has been suggested many times previously, then these mechanisms may have some relevance to disease therapy.

945A

Function of JAK/STAT and Hippo signaling pathways on *Drosophila* mushroom body neuroblast maintenance and cell proliferation. Lijuan Du[1], Jian Wang[1,2]. 1) Molecular and Cell Biology Program, University of Maryland, College Park, MD; 2) Entomology Department, University of Maryland, College Park, MD.

Mushroom bodies are a pair of prominent structures in the brains of all insects and other arthropods, which play important roles in olfactory learning and memory. Therefore, they are analogous to mammalian hippocampus. Through a forward genetic screen, we found that mutations in genes of the Janus Kinase (JAK) / Signal Transducer and Activator of Transcription (STAT) pathway components*domeless* (*dome*) and *hopscotch* (*hop*) cause precocious disappearance of mushroom body neuroblasts at early 3rd instar larval stage, which results in fewer number of mushroom body neurons. Surprisingly, ectopic expression of Yorkie (Yki), the downstream effector of Hippo signaling pathway, efficiently rescues *dome* mutant phenotypes. And overexpression of Yki target genes *cycE* or *diap1* partially rescues the γ-only phenotype of lacking JAK/STAT action. Further studies indicate that loss of Yki function caused the similar γ-only phenotype in adult mushroom bodies, and this phenotype could be rescued by dominant activation of JAK/STAT. We conclude that both JAK/STAT and Hippo pathways, which are key regulators of cell proliferation and normal growth in flies and mammals, are required for the normal development of mushroom body neurons, and they can complement each other. By examining the STAT-binding sites at *cycE* promoter region via transgenic studies, our data demonstrates that *cycE* is a direct target of STAT92E. Knowing that *diap1* is a direct target of STAT92E, our studies firstly show that JAK/STAT and Hippo signaling pathways, which have opposite effects on cell proliferation, are integrated to control development of different tissues in *Drosophila* by regulating common transcriptional targets, such as *cycE* and *diap1*.

946B

Muscle associated *Drosophila* adducin regulates *Drosophila* larval neuromuscular junction (NMJ) development and the localization of Draper to the synapse. Mannan Wang[1], Simon Wang[2], Charles Krieger[1], Nicholas Harden[2], Wade Parkhouse[1]. 1) Department of Biomedical Physiology and Kinesiology, SFU, Burnaby, BC, Canada; 2) Department of Molecular Biology and Biochemistry, SFU, Burnaby, BC, Canada.

Adducin, the cross linker of actin and spectrin, has important regulatory roles in the remodeling of actin-spectrin cytoskeleton which permits modification of the membrane at sites of cell-cell contact during synaptic plasticity. Cell-cell contact is affected in neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) where loss of synaptic contact between motor neurons and muscle is associated with motor neuron degeneration. Adducin was found hyperphosphorylated in nervous system tissue in patients with ALS, suggesting its potential association with the etiology of ALS.
In *Drosophila*, *Drosophila*adducins, encoded by *hu-li tai shao (hts)*, are localized to both pre-synaptic and post-synaptic larval neuromuscular junction (NMJ). Here we specifically examined the effects of post-synaptic (muscle-associated) Hts manipulation on NMJ development. Results show that in animals with muscle-specific knock-down of Hts, NMJs are underdeveloped, whereas overexpression of Hts in the muscle results in NMJ overgrowth. Draper, a transmembrane engulfment receptor, has also been shown to regulate *Drosophila* larval NMJ development. Our results show that Draper is co-localized with Hts to the postsynaptic NMJ, suggesting potential interaction between Draper and Hts. Interestingly, we found that in animals with muscle-specific knock-down of Hts, the immunoreactivity of Draper was tighter localized to the synapse, whereas overexpression of Hts caused delocalization of Draper immunoreactivity from the synapse. The effects of muscle-associated Hts on the targeting of Draper to the synapse highlights a new avenue by which Hts may be exerting its influence on NMJ development and opens up worthwhile possibilities for future studies.

947C

Variation in larval locomotion and NMJ among melanogaster sibling species. Emma Yang, Mirela Belu, Claudia Mizutani. Case Western Reserve University, Cleveland, OH.

Closely related Drosophila species are ideal models for studying evolutionary processes given the genomic resources available and the expected small number of genes responsible for the emergence of new phenotypes. A first step to begin to explore these questions is the identification of phenotypic variation among species. Here we describe clear phenotypic variations in the locomotion patterns and Neuromuscular Junction (NMJ) within the sibling species D. melanogaster, D. simulans and D. sechellia. By using a video-tracking analysis, we extracted parameters of larval forward crawling of speed, rate of contraction and dispersal efficiency. These analyses revealed that D. melanogaster and D. simulans have similar locomotion patterns, while the more recently diverged species D. sechellia has a lower contraction rate, speed and dispersal. The similar locomotion patterns observed in D. melanogaster and D. simulans correlate well their NMJ anatomy, with similar numbers of active synaptic boutons and branching points per muscle area. In contrast, D. sechellia contains a smaller number of active

boutons and branchings. In addition, D. sechellia is the only species with octopamine+ type II boutons in muscle fibers 6/7, an inhibitory neurotransmitter. To test the genetic contribution to these phenotypes, we generated and analyzed the locomotor patterns of hybrid larva between these species. These experiments indicate that some parameters of locomotion and the formation of type II boutons of D. sechellia are X-linked. Based on these results, we are using bioinformatics tools to search for fast evolving genes located on the X chromosome that are most conserved between D. melanogaster and D. simulans, but most divergent in D. sechellia. Currently, we are testing five candidate genes obtained through the searches, and exploring their roles in NMJ development.

948A

Nemo is a core proneural target gene and a feedback inhibitor of Atonal in the Drosophila eye. Vilaiwan Fernandes[1], Lorena Braid[1,2], Esther Verheyen[1]. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada; 2) Defence Research and Development Canada - Suffield, Biotechnology Section, Medicine Hat, Alberta, T1A 8K6, Canada.

During neurogenesis a field of cells competent to take on neural fate is established by conserved tissue-specific proneural transcription factors. These factors trigger the expression of 'core' target genes, which aid in refining the proneural domain by up-regulating proneural gene expression in the presumptive neuroblast while simultaneously down-regulating it in the surrounding cells via lateral inhibition. The eye is extensively used to study neurogenesis through the reiterative specification of R8 photoreceptor neurons. The proneural factor Atonal (Ato) is required for R8 specification; its expression is progressively refined from broad expression to intermediate groups (IGs, ~10 cells), to equivalence groups (~3 cells) and finally to single presumptive R8s. Nemo (Nmo) and Nemo-like kinases (Nlk) are conserved serine/threonine kinases, which regulate diverse developmental processes. We show that *nmo* is transcriptionally regulated by multiple proneural factors (Ato, Achaete and Scute) in multiple tissues. Furthermore, we describe a specific role for Nmo in R8 specification through inhibition and refinement of Ato. In *nmo* mutant eye discs Ato expression does not refine into IGs resulting in frequent presumptive R8 doublets, while over-expression of *nmo* in clones results in loss of IGs and greater spacing between individual R8s. These phenotypes suggest a role for Nmo during Notch-mediated lateral inhibition. Indeed, our genetic analyses reveal that Notch targets are reduced with loss of *nmo*. Hyper-activating Notch in *nmo*-deficient clones suppresses Ato refinement defects. Thus, Nmo is a novel component of the neuronal specification program in the eye downstream of Ato. Additionally, our data suggest that *nmo* may be a novel 'core' proneural target, which may globally promote refinement of proneural domains through Notch-mediated lateral inhibition.

949B

The transcription factor Escargot is involved in neuronal differentiation. Anne Ramat, Michel Gho. Laboratory of Developmental Biology CNRS/UPMC, Paris, France.

The properties of the nervous system rely on the quality of its neuronal network. How the complex arborization of axons and dendrites is determined, is still poorly understood. Cues provided by neurons themselves play a crucial role for neuronal projections. Indeed, each type of neuron has its own shape. In order to analyze how neuronal morphogenesis is intrinsically controlled, we have focused our attention on the evolutionarily conserved transcription factor Escargot (Esg) that is widely expressed in neural precursor cells in diverse model systems. In order to analyze the role of Esg in neuronal differentiation, we studied neuronal processes in the embryonic and adult Drosophila peripheral nervous system under esg mutant conditions. Using clonal analysis, our study revealed that neurons inside esg clones present thinner axons than normal and frequently harbour altered projection trajectories. Interestingly, these defects were also observed when we expressed, an RNAi directed against esg transcript exclusively in neurons. These data show that esg is involved, cell autonomously, in neuronal differentiation in both embryonic and adult PNS. In addition, these defects were correlated with a reduction in the expression of several identity factors such as those encoded by elav and prospero genes. Since it is known that axonogenesis is impaired in elav and prospero mutants, we are currently studying whether esg acts through these factors to control axonogenesis. We anticipate that this study will shed light on the mechanism by which esg regulates neuronal differentiation and axonogenesis.

950C

Sanpodo controls sensory organ precursor fate by regulating Notch trafficking and interaction with gamma-secretase. Fabrice Roegiers[1], Alok Upadhyay[1], Vasundhara Kandachar[1], Diana Zitserman Zitserman[1], Xin Tong Tong[1,2]. 1) Fox Chase Cancer Ctr, Philadelphia, PA; 2) Dept. of Molecular & Integrative Physiology University of Michigan Medical School.

In Drosophila peripheral neurogenesis, Notch controls cell fates in sensory organ precursor (SOP) cells. SOPs undergo asymmetric cell division by segregating Numb, which inhibits Notch signaling, into the pIIb daughter cell following cytokinesis. In contrast, in the pIIa daughter cell, Notch is activated and requires sanpodo, but its mechanism of action has not been elucidated. As Sanpodo is present in both pIIa and pIIb cells, a second role for Sanpodo in regulating Notch signaling in pIIb cells has been proposed. Here we demonstrate that Sanpodo regulates Notch signaling levels in both pIIa and pIIb cells via distinct mechanisms. In the pIIa cell, Sanpodo interacts with Presenilin, a component of the γ-secretase complex, promoting Notch activation. In contrast, Sanpodo suppresses Notch signaling in the pIIb cell by driving Notch receptor internalization. Together, these results demonstrate that a single protein can regulate Notch signaling through distinct mechanisms to either promote or suppress signaling depending on the local cellular context.

951A

Synaptic localization of iGluR complexes is regulated by the modulation of Neto extracellular domain. Young-Jun Kim, Mihaela Serpe. NICHD, National Institute of Health, Bethesda, MD.

Neurotransmitter receptor subunits are recruited at the opposite of the presynaptic active zones to make postsynaptic clusters upon the arrival of the axon terminals. Juxtaposition of active zones and postsynaptic densities is tightly regulated to make functional synapses. Previously we found Neto is an essential component of iGluR complexes which moves together with the receptor subunits at Drosophila NMJ. Here, we report that extracellular domain of Neto is essential for the regulation of iGluR cluster formation and is regulated by the proteolytic processing. Lethality of neto genetic null mutants could be rescued by the introduction of neto transgene which contains extracellular domain only. Drosophila Neto contains the highly conserved stretch of -RXXR- sequences just before the first CUB domain which is cut by Furin-1 protease. Inhibition of proteolytic activities by expressing Furin-1 RNAi construct both in the muscle and the neuron caused the decrease of iGluR cluster formation and pMad clusters. Two mutant lines were generated; PM (processing mutant)-Neto which contains uncleavable prodomain, and CA (constitutively active)-Neto of which prodomain was removed. According to the rescue analyses, both PM- and CA-Neto could rescue the embryonic lethality of neto null mutants. However, the animals rescued by PM-Neto couldn't make to the adult stages and showed abnormal iGluR and clustering, which means proteolytic processing of Neto prodomain is an important step for the maintainence of iGluR complexes at the synapse.

952B

Two Neto isoforms are required for proper synapse assembly at the Drosophila NMJ. Cathy I Ramos, Oghomwen Igiesuorobo, Mihaela Serpe. NICHD, NIH, Bethesda, MD.

Synaptogenesis requires the recruitment of neurotransmitter receptors, which comprises of their assembly, trafficking and stabilization at specific membrane locations. The Drosophila neuromuscular junction (NMJ) shares common features with mammalian central synapses. Notably, neurotransmission is sustained by ionotropic glutamate receptor (iGluR) at postsynaptic densities (PSDs). Drosophila NMJ iGluRs are heterotetrameric complexes composed of three essential subunits - GluRIIC, GluRIID, GluRIIE - and either GluRIIA or GluRIIB. Our lab has identified and characterized the Drosophila neto (neuropilin and tolloid-like) as an essential gene required for clustering of both iGluRs types at the NMJ. Our previous studies revealed that Neto is an essential component of the iGluR complexes required for iGluR clustering, organization of PSDs, and synapse functionality. Now, we have found alternative splicing in the neto locus encoding for a second Neto isoform, Neto-B. The Neto-A and Neto-B isoforms share similar extracellular and transmembrane domains but contain different intracellular parts. These two isoforms are conserved in Drosophilidae. We have confirmed that both isoforms are expressed in the larval body-wall muscles in D. melanogaster. Furthermore, either isoform could rescue the iGluRs clustering defects and embryonic lethality of neto null mutants. Using isoform specific mutants and rescue constructs, we are investigating the role of the two Neto isoforms in iGluRs clustering. Preliminary morphological analyses suggest that Neto-B specifically influences the synaptic accumulation of the type-A iGluR complexes. Furthermore, electrophysiological recordings in an allelic series of neto-B mutants revealed quantal sizes that correlated with GluRIIA levels. These findings indicate that the two Neto isoforms may have distinct, non-redundant functions in synapse assembly at the Drosophila NMJ.

953C

BMP signaling is required for synapse assembly at the *Drosophila* neuromuscular junction. Mikolaj J Sulkowski[1], Young-Jun Kim[1], Bing Zhang[2], Mihaela Serpe[1]. 1) Program in Cellular Regulation and Metabolism, NICHD, NIH, Bethesda, MD, USA, 20892; 2) Department of Zoology, University of Oklahoma, Norman, OK, USA, 73019.

Formation of neural circuits begins with physical assembly of the synapses and stabilization of postsynaptic densities, followed by synapse maturation and growth, and finally elimination of synapses during development. The coordinated growth of synaptic structures requires communication between the pre- and postsynaptic compartments and relies on anterograde and retrograde signals, and on the activity of the neural circuit itself. Drosophila NMJ is a glutamatergic synapse similar in composition and physiology to our central synapses. We have recently discovered that the *neto* gene is absolutely required for the initial clustering of glutamate receptors (iGluRs) at the *Drosophila* NMJ. *neto* null mutant embryos are completely paralyzed and lack iGluRs clusters at their NMJs. *neto* hypomorphs have diminished levels of synaptic receptors, and have smaller synapses with reduced quantal size, and no compensatory increase in quantal content. The reduced synapse growth and lack of presynaptic compensation are reminiscent of defects in the BMP retrograde signaling. At the *Drosophila* NMJ, Glass bottom boat (Gbb), a BMP-type ligand secreted by the muscle, provides a retrograde signal essential for coordinating the growth of synaptic structures. Retrograde BMP signaling induces phosphorylation of Mad (pMad) at synaptic terminals and in neuron nuclei. We found that at suboptimal Neto levels, pMad is completely absent from the NMJ, but it is not affected in the motor neuron nuclei. We will review our recent data that suggest that BMP signaling is required for synapse assembly at the Drosophila NMJ.

954A

Pk17e regulates Drosophila NMJ synapse development and function. Guoli Zhao[1], Li Du[2], Qifu Wang[1], Yongqing Zhang[1]. 1) Institute of Genetics and Developmental Biology, Beijing, Beijing 100101, China; 2) College of Life Science, Hubei University, Wuhan, Hubei 430062, China.

Synapses are highly specialized intercellular junctions that transmit information between neurons and their targets. Studies

on the formation and development of synapses will shed light not only on the physiological neurodevelopment but also the pathogenesis of related neurological diseases. S6K is a family of protein kinases involved in cellular and developmental processes. But its function in neuronal system is not well understood. In this study, we analyzed the function of one member of the S6K family in Drosophila, pk17e, in synaptic development and function, and found that PK17E restrains neuromuscular junction (NMJ) growth and decreases neurotransmission efficiency. pk17e mutants are viable but display distinct NMJ morphological abnormalities, including more synaptic boutons and satellite boutons. pk17e mutations caused significantly reduced synaptic endocytosis at NMJ synapses, as detected using the fluorescent dye FM 1-43 uptake assay. Electrophysiology analysis showed increased mEJP (spontaneous miniature potentials) amplitudes in pk17e mutants. The NMJ overgrowth was caused by enhanced BMP (bone morphogenetic protein) signaling as phosphorylated Mad staining intensity in NMJ synapses was increased in pk17e mutants. Genetic analysis showed that reducing the dose of wit by half in pk17e null background rescued the NMJ overgrowth phenotype. Furthermore, the bouton number was significantly increased in dad and pk17e trans-heterozygotes. Finally, the BMP receptor, Tkv level was increased in pk17e mutants compared with wild-type and the Tkv physically interacts with PK17E in S2 cells. Together, our results demonstrate that pk17e is required for synaptic development via inhibiting BMP signaling.

955B

Nora virus Transmission in *Drosophila melanogaster*: An Investigation to Teach Virulence and Pathogenic Prophylaxis to Biology Students. Darby J. Carlson[1], Wayland Weatherred[1,2], Kimberly A. Carlson[1]. 1) Biology Department, University of Nebraska at Kearney, Kearney, NE; 2) Aspen High School, Glenwood Springs, CO.

Proper hand hygiene accompanied with environmental surface disinfection provides a comprehensive approach to control and prevent respiratory and gastrointestinal illness in schools, hospitals, work environments and the home. The persistent non-pathogenic Nora virus common in *Drosophila melanogaster* is a horizontally transmitted virus that students can research and design experiments testing prophylaxis techniques for virulence and pathogenic diseases. In this study, we demonstrate Nora virus can be successfully removed by surface decontamination with 10% bleach, which was verified by reverse transcription polymerase chain reaction. The purpose of this project was to outline a laboratory investigation that allow students to use inquiry-based methods to perform an experiment, analyze data, and draw conclusions on viral inactivation from disinfectant use on Nora virus in *D. melanogaster*. This experiment provides students a practical investigative opportunity to apply the scientific method, reinforcing concepts introduced in lecture, and provide an opportunity for scientific research, which could ignite a spark of interest in scientific investigation unknown to them previously. This work was made possible by Grant Number P20GM103427 from the National Institute for General Medical Science, a component of the National Institutes of Health.

956C

The Genomics Education Partnership (GEP): Bringing Genomics Research into Undergraduate Classrooms. SCR Elgin[1], M Burg[2], J DiAngelo[3], A Haberman[4], C Jones[5], L Kadlec[6], SCS Key[7], J Leatherman[8], GP McNeil[9], H Mistry[10], A Nagengast[10], DW Paetkau[11], S Parrish[12], L Reed[13], S Schroeder[14], S Smith[15], M Wawersik[16], L Zhou[17], D Lopatto[18]. 1) Washington U MO; 2) Grand Valley St MI; 3) Hofstra NY; 4) Oberlin OH; 5) Moravian PA; 6) Wilkes PA; 7) NC Central NC; 8) Northern Colorado CO; 9) York/CUNY NY; 10) Widener PA; 11) St Mary's IN; 12) McDaniel MD; 13) Alabama-Tuscaloosa AL; 14) Webster MO; 15) Arcadia PA; 16) William & Mary VA; 17) U Pittsburgh PA; 18) Grirnnell IA.

An effective method for teaching science is to engage students in doing science. The GEP, a consortium of ca. 100 primarily undergraduate institutions, provides students with opportunities to participate in genomics research during the academic year. Current research centers on the evolution of the Muller F element (dot chromosome) in Drosophila, unusual because it exhibits both heterochromatic and euchromatic properties. Drosophila community resources - the 20 sequenced fly genomes, fosmid libraries, and FlyBase - provide the starting materials. Students improve the quality of the sequence and carefully annotate genes and other features. Most students are confused at the start but enthusiastic at the end of their GEP project. They find this hands-on approach a good way to learn about genes/genomes, and a good way to experience research. GEP students report gains on the CURE survey similar to gains reported by summer research students. Students also show knowledge gains that correlate positively with CURE. Success by both measures correlates positively with time on task, but shows no significant correlation with institutional characteristics. Faculty report that the central organization provides critical help in initial training, project organization, community-generated curriculum and technical support. They find this approach enhances both student learning and research opportunities, and is rewarding for both faculty and students. We invite additional faculty to join us (see http://gep.wustl.edu; next workshop June 2013). Support: HHMI grant 52005780 & NIH grant R01 GM068388 to SCRE.

957A

Learning Outcomes in a Required Biology Majors Genetics Course Using Two Different Pedagogies: Modified Team Based Learning Compared to Traditional Lecture. Susan R. Halsell, Timothy A. Bloss, Kimberly H. Slekar. Dept Biol, James Madison Univ, Harrisonburg, VA.

At James Madison University, all sophomore Biology and Biotechnology majors are required to take a four credit Genetics and Development course (BIO 224). The course includes a three hour laboratory and three hours of class lecture each week. Three different instructors teach the course. Recently, two of the instructors converted to using a modified team based

learning approach while the third instructor maintained a traditional lecture during the class hours; the laboratory remained unchanged for all. Learning outcomes were evaluated by administering Pre- then Post- Tests using a Genetics Concept Assessment (GCA) developed by M.K. Smith, W.B. Wood, and J.K. Knight (CBE-Life Sciences Education. 2008. 7:422-430). A total of 476 students participated in the study. Also at the end of the semester, BIO 224 students ranked their perceptions of the teaching approach they experienced, including amounts of pre-class preparation, how the teaching approach meshed with their learning style, and impact it had on developing group working skills. In addition, 145 graduating seniors were also administered the GCA to analyze any potential long term retention effects of the pedagogy. This presentation will describe the modified team based learning approach used in this class, report on the learning outcomes of the two approaches, the student perceptions and the instructor insights on utilizing a modified team based learning pedagogy.

958B

An Investigative Genetics Lab Course Using Drosophila Neurologic Mutants. Pat C. Lord, Cole Crowson, Erik C. Johnson. Dept Biol, Wake Forest Univ, Winston-Salem, NC.

We have developed and tested a new genetics lab required of all biology majors that integrates transmission genetics and molecular biology. The lab is more question driven and indicative of how we use genetics and molecular biology in our research. The lab uses wild type and several different neurologic mutants. In the first weeks of lab, students identify their mutant based on its performance in multiple different behavioral assays. Once they have identified the mutant behavior, they use deficiency mapping, and bioinformatics to identify candidate genes. Next, using RNA isolated from their wild type and mutant flies and primers they have designed, we generate cDNA using RT-PCR. In addition, we provide sequence data for wild type and mutant cDNAs. Students then use the PCR fragments and sequence data to determine the actual mutation in their gene using bioinformatics. Finally, they predict how the DNA mutation affects the function of the protein and explains the defective behavior in mutant fly. In our first test of this lab, students' struggles with DNA sequence manipulations in designing forward and reverse primers, building sequence contiqs, and analyzing DNA sequences files. We will describe strategies that we have used to improve students' abilities to manipulate sequence data and assessment of learning outcomes.

959C

Annotation of Fosmid 60 of *Drosophila erecta* and of DMAC 47 of *Drosophila mojavensis* as control sequences in the comparative genomic analysis of the *Drosophila* dot chromosome.Carolina Marques dos Santos Viera, Tiara Tirasawasdichai, Susan Parrish. Biology Department, McDaniel College, Westminster, MD 21157.

Annotation of a DNA sequence allows for the identification of genes and sequence hallmarks within a genome. In this work, we annotated DNA sequences from two *Drosophila* species, *D. erecta* and *D. mojavensis*, for a comparative genomics study of the *Drosophila* dot chromosome, a tiny chromosome that is unusual in that it displays euchromatic and heterochromatic properties that can differ between species. In this project, we annotated chromosome 3L as a euchromatic chromosome control for the dot chromosome. First, *Drosophila erecta* Fosmid 60, a 40, 000 bp sequence from chromosome 3L, was annotated. Fosmid 60 was shown to have two putative genes that are orthologous to *Drosophila melanogaster*: CG33234 and CG43375, both of which have two protein isoforms in *D. melanogaster*. Next, *D. mojavensis* Fosmid DMAC 47, a 43,870 bp sequence from chromosome 3L, was annotated and determined to contain five putative genes that are orthologous to *D. melanogaster*: CG43444, which has three protein isoforms in *D. melanogaster*, pgant6, mRpS35, CG34455, and Klp67A, each of which have only one protein isoform in *D. melanogaster*. The features annotated in this project included the translation start and stop codons, the splice junctions, and the coding exons. The overall goal of this project is to compare the higher order chromatin structure of the dot chromosome of different*Drosophila* species in relation to sequence content. This project was completed in collaboration with the Genomics Education Partnership (GEP), an educational initiative that allows undergraduate students to participate in original genomics research projects.

960A

An Interdisciplinary Approach to Molecular Bioscience Content in the Undergraduate Curriculum. Alexis Nagengast[1,3], Shirley Fischer-Drowos[1,3], Robert W. Morris[1,2], Hemlata Mistry[1,2]. 1) Dept Biochemistry; 2) Dept Biology; 3) Dept Chemistry, Widener University, Chester, PA.

Often undergraduate students do not recognize the related content in their science courses. Traditional departmental boundaries can emphasize the focus on one subject, further distracting students from the interdisciplinary nature of science. Like many Primarily Undergraduate Institutions (PUIs), Widener University offers small classes that integrate research experiences into science coursework. We recognized that a small group of students taking overlapping courses including Cell Physiology, Developmental Neurobiology, Molecular Biology, Analytical Chemistry and Biochemistry II, retained greater content knowledge than previous classes and were better able to relate that content between classes. Furthermore, students who had participated in independent research projects were better prepared to understand the primary literature. These observations motivated us to integrate molecular bioscience courses in our curriculum thereby allowing students to design and conduct multiple experiments to address one overarching experimental problem throughout multiple courses. We introduced a biomedical research problem during the sophomore year and continued the study through the upper-division electives in the junior and senior years. Continued investigation of a question enabled students to use different approaches to address a research topic. We initially addressed the control of lipid metabolism by alternative RNA splicing mechanisms and currently are examining the role of Dis3 during Drosophila development. This integrated method overcomes the apparent

disjointed nature of undergraduate laboratory experiences, increases content knowledge and promotes greater recognition of the interdisciplinary nature of molecular bioscience.

961B

A comparative genomic analysis of the Drosophila dot chromosome. William G Neutzling, Melissa Jones, Genomics Education Partnership. Biology, McDaniel College, Westminster, MD.

The process of annotation provides biological meaning and organization to the genome of an organism. In this project, we analyzed DNA sequence from two Drosophila species to identify genes and gene coordinates for subsequent comparative genomic analysis of the Drosophila dot chromosome. The tiny dot chromosome is of interest because it displays both euchromatic and heterochromatic properties that can vary between species. As a control for the dot chromosome, chromosome three was annotated in this project to serve as a euchromatic chromosome reference. Specifically, we annotated D. erecta Fosmid 55 and D. mojavensis DMAC 21 and DMAC 31. Within D. erecta Fosmid 55, we found six putative genes that corresponded to the following D. melanogaster orthologs: msn, dos, CG16984, RpL8, CG26985, and Pxn. In contrast, D. mojavensis DMAC 21 contained only one putative gene that was shown to be orthologous to D. melanogaster CG10566, whereas D. mojavensis DMAC 21 was found to not have any putative genes. The long-term goal of this project is to determine how DNA sequence information, including gene identity, gene density, and repeat density, influences higher order chromatin structure of the Drosophila dot chromosome. This research was performed in conjunction with a number of colleges and universities as part of the Genomics Education Partnership (GEP). The GEP provides undergraduate students the opportunity to participate in original comparative genomics projects, allowing students to contribute to public scientific databases and expand the understanding of how chromatin domains are assembled.

962C

The blog “Ciber-Genética” is a resource for teaching and learning. Rosario Rodriguez-Arnaiz, Lucero León Rangel, Isaac Reyes Martínez, Jovana Jasso Martínez, América Nitxin Castañeda Sortibrán. Dept Cellular Biol, Sci Fac, UNAM, Mexico, DF, D.F., Mexico.

Internet offers a wealth of opportunities to optimize the processes of teaching and learning. Blogs are resources with vast potential at the time of work in the classroom. Through a blog the students have greater interaction with the content, can access to information outside the classroom and also can leave comments that are answered by the teacher or other students. Likewise, the student can come into contact with news, articles and events that are conducted in the area. The use of the “Ciber-Genética" blog in the classroom represents a useful tool to transmit updated information that complement, reinforce and increase the information seen in the session. The contents are organized in chronological and in thematic order. External links (journals, books, and web-sites,among others) are also organized by topic, and help to create a network of related sites. A survey with 15 questions about the pertinence of the blog was done between students. 87.7% of the replies were between excellent and very good. The Google Analytics Service was used in order to obtain information about the blog: the blog has 944 posts; the tag “news” contains the highest number of posts (27.86%). From September 2009 till October 2012 the blog was visited 174,302 times and from September 21th until October 2oth the number of visits was 8,132, thus a mean of 270 visits/day were recorded. Visits were from 117 countries around the world but Mexico was the country from which more visits (44%) were received. The entry “Timeline of Genetics” was the one most visited.

963A

Mapping and cloning recessive wing mutations in an undergraduate course. Eric P. Spana, Samuel C. Arnold, S. Canon Brodar, Emily Chang, Karen Y. He, Andrew Hollis, Yanjun Anna Liu, David K. Lung, Sasha McEwan, Uchenna C. Osuji, Ann Prybylowski, Clara Starkweather, Diana L. Xie, Qingyun Li. Department of Biology, Duke University, Durham, NC.

Drosophila adult visible mutations are valuable for genetic mapping and studying a range of developmental processes. Despite their experimental utility, many wing mutations have not yet been cloned. By employing complementation tests against molecularly defined deficiencies, duplications, and insertion alleles, as well as RNAi knockdowns, we sought to identify genes responsible for four wing mutants: *fluted*(*fl*), *scooped* (*scp*), *folded* (*fo*), and *bloated* (*blo*). The *fluted* (*fl*) mutation, first identified by Helen Redfield in 1921, displays longitudinal creases adjacent to the 2nd through 5th wing veins. Deficiency crosses narrowed the map region to ~85kb within 90B1-90B2 (~5 genes), and further complementation crosses with insertion lines identified CG5873 as a candidate gene. Described by Muller in 1926, *scp*mutants display a subtly slight upward curve towards the lateral edges of the wing. We localized *scp* to two candidate regions spanning a total of ~450 kb through duplication and deficiency crosses (4F10-5C7 & 6B2-6C4). The wings of *folded* (*fo*), discovered by Grossman in 1932, remain unexpanded in a varying percentage of adults, with postscutellars bent forward. Using a reported genetic location of *fo*(1-63), we mapped the mutation to a ~450 kb interval residing in the 15F-16B cytogenetic region. We have also identified a wing pigmentation phenotype on *fo* flies with expanded wings; whether this wing pigmentation phenotype is associated with *fo* remains uncertain. The *blo* mutation, isolated in 1933 by Ives, gives rise to large blisters on the wings. We narrowed *blo* to a ~165 kb region (44E-44F) using deficiency complementation. We found that a knockdown of *sticks and stones* in wings phenocopied *blo*, suggesting that *blo* may be an adult viable allele of *sns*. Further molecular analysis will be performed to identify the lesions in the candidate genes.