Regulation of energy metabolism in *Drosophila*. Carl S. Thummel, William Barry, Daniel Bricker, Janelle Evans, Michael Horner, Geanette Lam, Jyoti Misra, Daniel Seay, Matthew Sieber, Rebecca Somer, Jason Tennessen, Wendou Yu. Dept of Human Genetics, Univ Utah School of Med., Salt Lake City, UT.

Energy metabolism is central to all aspects of animal life. Dietary nutrients are either set aside as stored reserves or burned to provide the energy needed for daily survival. Conversely, misregulation of metabolism is central to human disease - in particular the alarming rise in the incidence of diabetes and obesity. We are using *Drosophila* as a model system to define the molecular mechanisms of metabolic control and to better understand the causes of metabolic disorders. There are three projects currently underway in the lab. The first is focused on how nuclear receptors sense small metabolites and regulate the transcription of genes that play central roles in specific metabolic pathways. These studies currently address how dHNF4 maintains energy homeostasis under conditions of nutrient depletion, the role of dERR in regulating developmental growth, and the control of lipid metabolism by DHR96. The second project is a collaborative effort aimed at defining the functions of uncharacterized mitochondrial proteins that are conserved through evolution from yeast to humans. Current efforts are focused on two families of mitochondrial proteins, one of which appears to act as the long-sought pyruvate transporter that links cytoplasmic glycolysis with mitochondrial energy production, while the second protein family appears to regulate mitochondrial fatty acid oxidation. The third project is studying how temporary changes in parental diet can affect the metabolic state of their offspring. Hundreds of studies in rodents and humans have linked parental nutrition to metabolic dysfunction in subsequent generations. Although this transgenerational inheritance is thought to be epigenetically programmed, the published work in this area is only correlative. We have reproduced these effects in *Drosophila* and aim to define the molecular mechanisms involved.

Behavioral and anatomical analysis of the neural circuits that drive fly grooming. Julie H. Simpson, Stefanie Hampel, Primoz Ravbar, Andrew M. Seeds. HHMI, Janelia Farm Res Campus, Ashburn, VA.

We use Drosophila grooming behavior as a model to understand the neural circuit architecture that allows the brain to construct complex behavioral sequences from simpler reflexes and subroutines. Flies remove debris using an ordered progression of leg sweeps and rubs. The local subroutines needed to remove dust resemble the scratch reflex and can be discretely triggered or blocked. When the whole body is covered with dust, the fly grooms its anterior before proceeding to clean its posterior. Our detailed analysis of the way wild-type flies groom suggested both the modular organization of subroutines and the ordered progression between them. We conducted screens in which we activated or inhibited neural activity in different sets of neurons and determined the effect on spontaneous grooming and dust removal. The range of phenotypes we observed supports our hierarchical model of grooming, and the GAL4 lines targeting key neurons provide anatomical entry points to the circuitry that drives the behavior. We have identified sensory, motor, and interneurons that affect grooming. We are using a range of genetic tools to identify the minimal groups of neurons and circuits required for both the execution of behavioral subroutines and the coordinated progression of the grooming sequence.

News from the Niche. Stephen DiNardo[1,2], Tishina Okegbe[1,2], Lindsey Wingert[1,2], Qi Zheng[1,2], Judith Leatherman[3]. 1) Dept Cell & Developmental Biol, Perelman Sch Medicine; 2) Institute for Regenerative Medicine, Univ. Pennsylvania, Philadelphia, PA; 3) College of Natural and Health Sciences, University of Northern Colorado.

Niches regulate the behavior of many tissue-specific stem cells. However, in no case do we fully understand how a niche is specified and assembled in a tissue, and then how it executes control over stem cells. In the Drosophila testis, a small group of cells (hub cells) acts as part of the niche, leading to the activation of renewal pathways in adjacent cells. In this manner, nearby somatic cells adopt cyst stem cell fate (CySC), while nearby germline cells, intermingled with these CySCs, adopt germline stem cell fate (GSC). Work from us and others recently clarified how this niche operates, bringing together disparate observations into a cohesive framework. In particular we found that the CySCs are doubly intriguing. Aside from acting as stem cells, producing daughter cells that form an instructive epithelial ensheathment for differentiating germ cells, our work now shows that CySCs are also part of the niche: they act together with hub cells to renew the GSCs. We further showed that the zinc finger homeodomain protein Zfh1 governs both of these CySC properties, and is expressed highly in CySCs and dramatically downregulated both in the epithelial hub, and in their differentiating epithelial cyst progeny Our new focus on CySCs also led us to discover that hub cells and CySCs derive from a common pool of somatic gonadal precursor cells (SGPs) during gonadogenesis. Their common derivation provides some understanding for why hub cells and CySCs can each act as niche cells for the germline. But, more importantly, their lineal relationship drove us to investigate how somatic cells within the common SGP pool choose between hub fate and CySC fate, and how the hub forms during gonadogenesis. Our work, along with van Doren's, Tanentzapf's, Kobayashi's and Wawersik's, generates a model for the steps that occur before the hub can act as a niche. This presentation will summarize that progress and the outstanding questions that yet require resolution.

Lipoproteins in human and Drosophila Hedgehog signaling. Suzanne Eaton, Wilhelm Palm, Marta Swierczynska, Veena Kumari. The Max Planck Institute of Molecular Cell Biology and Genetics, Germany.

Hedgehog (Hh) family proteins play crucial roles in development and tissue homeostasis. Although they are covalently linked to both sterol and palmitate, they are secreted and can spread over long distances to receiving cells. In Drosophila, lipoproteins act as vehicles for the spread of Hh, but also repress the pathway in a ligand-independent manner. Here, we establish similar functions for lipoproteins in human Shh signaling. We further show that both human and Drosophila cells can secrete Shh/Hh in two distinct forms - as sterol-modified Hh proteins associated with lipoproteins, or, when lipoproteins are not available, as non-sterol-modified monomers or dimers (Shh-N*/Hh-N*). The association of Shh/Hh with lipoproteins alleviates their repressive effect on the pathway. Hh-N* and Shh-N* exert complementary effect to lipoprotein-associated forms, suggesting these distinct forms of Hh affect the pathway in different ways.

3D video tracking of Drosophila behavior and GFP expression and predictive biomarkers of aging. John Tower, Gary Landis, Reza Ardekani, Simon Tavaré. Molecular and Computational Biology Program, University of Southern California, Los Angeles, CA.

Evolutionary theory suggests that aging results from antagonistic pleiotropy of gene function between developmental stages and the sexes; uni-parental inheritance of the mitochondrial genome may make mitochondria-related genes particularly subject to sexual antagonistic pleiotropy (SAP), and contribute to mitochondrial malfunction, oxidative stress and proteotoxicity during aging. We find that *p53* and the mitochondrial redox-regulator *MnSOD* have sex-specific effects on life span consistent with SAP. Oxidative stress-response genes including *Hsp22*, *Hsp70*, and *Drosomycin* are up-regulated during normal aging, suggesting they are responding to age-related mitochondrial malfunction. Both MnSOD and Hsp22 can increase *Hsp22* expression during aging, suggesting an auto-regulatory pathway. Predictive biomarkers of aging would facilitate further analysis of aging mechanisms, but this requires non-invasive assays. Implementation of 3D tracking and GFP quantification has been hindered in the past by the complexity of operating systems. We report development of a user-friendly system (called Fluorescore) based on two synchronized cameras and recorded videos. Fluorescore quantifies fluorescent reporter proteins in groups of free moving flies, and provides 3D movement patterns with simultaneous fluorescence quantification for single flies. Longitudinal assay of fluorescent reporter constructs in young adult flies revealed that the oxidative-stress response genes *Hsp22*, *Hsp70*, and *Drosomycin* are predictive biomarkers of life span.

The Neurobiology of Monarch Butterfly Migration. Steven Reppert. UMass Medical School, Worcester, MA.

Studies of the migration of the monarch butterfly have revealed neural mechanisms behind its navigation using a time-compensated sun compass. Skylight cues, such as the sun itself and polarized light, are processed through both eyes and integrated in the brain's central complex, the presumed site of the sun compass. Time compensation is provided by circadian clocks that have a distinct molecular mechanism and that reside in the antennae. The monarch genome has revealed a full set of protein-coding genes, which yield insights into the long-distance migration. Gene targeting approaches are being developed to manipulate putative migration genes. The monarch butterfly is an outstanding system to study the molecular and neural basis of long-distance migration.

Oogenesis - where physiology and development meet. Denise J. Montell, Xiaobo Wang, Li He, Ho Lam Tang, Aeri Cho, Jessica Sawyer, Meg Waghray, Jennifer Jensen, Danfeng Cai, Jingchuan Luo, Fang Bu. Dept Biological Chemistry, Center for Cell Dynamics, Johns Hopkins Sch Med, Baltimore, MD.

The development of the Drosophila ovary serves as a useful model for general aspects of organogenesis. In contrast to embryonic development, which is highly stereotyped and insulated from environmental perturbations, oogenesis is responsive to changing physiological conditions. Starvation for example slows the rate of stem cell division and induces some egg chambers to undergo apoptosis while others arrest until conditions improve. The development of robust live imaging of most stages of oogenesis has opened the door to the use of sophisticated new tools, including photoactivatable proteins and FRET biosensors, to measure and manipulate molecules and physical forces in real time. I will describe recent genetic and live imaging studies, touching on the topics of somatic stem cell maintenance, mechanisms of cell fate diversification, cell motility, epithelial morphogenesis and the influence of physiological conditions on ovarian development.

Evolution and Phenotypic Effects of New Genes in Drosophila. Manyuan Long. Dept Ecology & Evolution, Univ Chicago, Chicago, IL.

New gene evolution is a generally important process. The young genes which originated recently, e.g. as short as 1~10 million years (mys) of divergence time between D. melanogaster and its eight close relatives, provided an excellent system to understand the origin of genes, because the signature of evolution in their early stage is detectable in their sequences before its disappearance in subsequent evolution. Several genetic mechanisms have been found responsible for the initial structures of new genes. New genes were fixed into a species with an appreciable rate, in which the lineage toward D. melanogaster generated ~1000 new genes since the Drosophila genus diverged 40-60 mys ago. These frequent origination events revealed a stable process of gene evolution in which the excess male genes, whose expression is male-biased or male-specific, frequently copied into autosomes; and the excess female genes into the X in a slower pace. This “gene traffic”, manifesting the interaction between the sex chromosome and autosomes that impacted genome evolution, is shaped by natural selection through various evolutionary genetic and mechanistic processes. Its consequence was evolutionarily and functionally to reorganize the genomes in the contents and types of genes between sex chromosomes and genomes. Furthermore, the functional analyses revealed an unexpected evolutionary role of new genes in phenotypic evolution: ~1/3 of young genes quickly evolved essential functions, terminating developmental process and blocking the formation of organs/tissues when silenced with knockdown or disruptive mutagenesis. These findings revealed that in Drosophila there are species-specific or lineage-specific components of the genetic system that controls development, defining a paradox to understand the divergence between closely related species with a potential solution that may lie in the evolution of gene interaction. The materials for presentation are based on the published (http://longlab.uchicago.edu/publication) and unpublished data in the Long lab at Chicago.

PIPs control cell morphogenesis in Drosophila. Julie A. Brill. Cell Biology Program, The Hospital for Sick Children, Toronto, ON.

Phosphatidylinositol (PI) phosphates (PIPs) regulate cell signaling, cytoskeletal organization and membrane trafficking in yeast and mammalian cells, yet surprisingly little is known about their effects on the development of multicellular organisms. We are investigating the roles of PI 4-phosphate (PI4P) and PI 4,5-bisphosphate (PIP_2) in cytokinesis, gametogenesis and organelle biogenesis using Drosophila as a model system. We discovered that the different PI 4-kinases, which synthesize PI4P, play distinct roles in these fundamental biological processes: Fwd (PI4KIIIβ) regulates Rab11 during spermatocyte cytokinesis; PI4KIIIα is essential for moesin activation and membrane trafficking during oogenesis; and PI4KII regulates glue granule biogenesis and acts with the PI 4-phosphatase Sac1 to promote pigment granule formation. We also identified roles for the PIP 5-kinase Sktl, which synthesizes PIP_2, in cytokinesis, cell polarity and nuclear shaping during sperm development. Our current understanding of the molecular mechanisms and targets of PIP regulation in these different processes confirms that PIPs serve as critical signaling molecules during Drosophila development.

Spindle orientation in neural stem cells. Chris Q. Doe. Inst Neuroscience, Univ Oregon, Eugene, OR.

I will discuss our work on spindle orientation in Drosphila neuroblasts and sense organ precursors. We have been using both in vivo genetics as well as an "induced cell polarity" culture system to dissect the mechanisms of spindle orientation. Neuroblasts and SOPs are polarized cell types, and the mitotic spindle aligns with cortical polarity cues to induce a molecularly asymmetric cell division in both cases. We show that loss of spindle orientation leads to failure of neuroblast homeostasis -- there is always an increase in neuroblast number and never a loss of neuroblast number. We use the induced cell polarity system to dissect the protein domains and amino acids in each domain that are required for proper spindle orientation, including structural studies of the relevant domains. We uncover a mechanism by which the mitotic kinase Aurora-A positively regulates the Pins-Mud-dynein spindle orientation pathway, and evidence for a second Pins-Dlg-Khc73 pathway. More recently we have characterized the role of the fly Afadiin ortholog Canoe in recruiting Mud to the cortex to promote spindle orientation. Lastly, I will discuss our progress in understanding the mechanisim and function of Dishevelled-mediated spindle orientation.

Deciphering the *cis*-regulatory code. Eileen E. Furlong. Genome Biology, EMBL, Heidelberg, Germany.

A central challenge in biology is to understand how the genome is utilized to generate diverse cell types. Embryonic development occurs through progressive restriction of cell fates, from pluripotent fields of cells to complex organs and tissues. This requires a directed progression through interlinked regulatory states, each defined by the total set of active transcription factors. At each development stage, the inputs of signaling and transcriptional networks regulate the expression of specific sets of genes that drive the transition to the next state. Hence, understanding how the underlying *cis*-regulatory networks produce spatial and temporal gene expression is a vital step towards deciphering metazoan development and many diseases. Recent approaches assaying transcription factor (TF) binding enable the location and combinatorial occupancy of enhancers to be experimentally measured at specific development stages genome-wide. A current challenge is to interpret these TF binding data in terms of their resulting spatio-temporal *cis*-regulatory activity. Our work uses mesoderm specification in *Drosophila* as a well-defined model system. Using machine learning, we demonstrated that transcription factor occupancy alone is sufficient to predict spatio-temporal enhancer activity. *In vivo* transgenic reporter assays demonstrated a high accuracy, with 80 percent of enhancers’ activity matching their predicted expression domains. We have now complemented this study by generating cell-type specific information on chromatin state within the context of a developing embryo, using a new method that we have developed. The data reveals heterogeneous combinations of chromatin marks linked to enhancers in an active state. Using a Bayesian network, we show that chromatin state is sufficient to predict not just the location, but activity state, of regulatory elements *de novo*. The model thereby enables the visualization of dynamic enhancer usage during development and uncovered a temporal link between RNA polymerase II enhancer occupancy and the precise timing of enhancer activity.

Full Abstracts – PLENARY SESSION II

A mechanism of morphogen protein dispersion mediated at points of direct contact. Thomas B. Kornberg. Cardiovascular Research Institute, University of California, San Francisco, CA.

Cytonemes, types of filopodia first identified in the Drosophila wing imaginal disc, were proposed to move signaling proteins between producing and target cells. We now have evidence for several types of cytonemes that are diverse in composition and exhibit both specificity for stimulating ligands and plasticity in orienting to novel sources of ligand. Receptors for ligands have punctal distributions specifically in responding cytonemes, and puncta containing both ligand and receptor can be found in cytonemes that make direct contact with ligand-expressing cells. These findings constitute experimental evidence for a mechanism of morphogen protein dispersion and support cytoneme-based movement of signaling proteins as a mechanism for cell-cell communication that transfers controlled amounts of signaling protein in a targeted manner to a specific recipient cell.

1

Evidence for monomeric α-catenin as a key regulator of adherens junctions in *Drosophila*. Ridhdhi Desai[1], Ritu Sarpal[1], Milena Pellikka[1], Noboru Ishiyama[2], Mitsuhiko Ikura[2], Ulrich Tepass[1]. 1) Cell & Systems Biol, Univ Toronto, Toronto, Canada; 2) Division of signaling biol, Ontario cancer Institute, Canada.

α-catenin is a component of the cadherin-catenin complex that binds to β-catenin/Armadillo (Arm) at adherens junctions (AJs). Our analysis of recently identified null mutations in *Drosophila* α-catenin are consistent with the conclusion that α-catenin is essential for cadherin function. To explore the molecular mechanism of α-catenin function in vivo, we carried out a structure-function analysis of α-catenin and tested α-catenin domains in the context of DE-cadherin::α-catenin fusion proteins. We found that the N-terminus of α-catenin, which has Arm-binding and dimerization activities, and the C-terminal region required for F-actin binding are essential for α-catenin function. In contrast, the central region of α-catenin is a strong positive modulator that likely undergoes secondary interactions to stabilize AJs.

As an alternative to the model that α-catenin physically links cadherin to actin, it was proposed that α-catenin acts in an allosteric manner: α-catenin is recruited to AJs through its binding to Arm, but then detaches and forms a dimer that binds and modulates actin. To test this model we examined three different constructs: (i) A construct which brings α-catenin dimers/oligomers to AJs without strong interactions with Arm failed to rescue a-catenin mutants arguing against the allosteric regulation model for α-catenin. (ii) A construct that has enhanced dimerization activity reduced rescue activity and enhanced cytoplasmic localization without any obvious enrichment on F-actin structures suggesting that α-catenin dimers are not obligatory actin binding proteins in vivo. (iii) Finally, we show that mammalian αN-catenin does not show any dimerization activity in vitro in contrast to mammalian αE-catenin, but nevertheless shows a strong rescue of *Drosophila* α-catenin mutants. Together, these data suggest that monomeric α-catenin acts as a physical linker between cadherin and the actin cytoskeleton.

2

Presenilin controls kinesin-1 and dynein activity in axonal transport. Shermali Gunawardena[1], Ge Yang[2], Lawrence S.B. Goldstein[3], Kunsang Dolma[1], Elizabeth Spina[1]. 1) Biological Sciences, SUNY at Buffalo, Buffalo, NY; 2) 2.Lane Center for Computational Biology & Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, 15213; 3) 3.Howard Hughes Medical Institute, Department of Cellular and Molecular Medicine and Neuroscience, University of California, San Diego, La Jolla, CA, 92093.

Neurons and other cells require intracellular transport of essential components for viability and function. Previous work suggested that the amyloid precursor protein (APP) can function as a kinesin-1 receptor during transport. However, how APP vesicle transport is regulated remains poorly understood. Here we show that reduction of presenilin (PS) suppresses axonal transport defects induced by excess human APP (hAPP). However, reduction of PS enhances axonal transport defects induced by loss of Drosophila APP (APPL). This PS-dependent enhancement is specific to APPL. No effect is seen with reduction of PS in the context of sunday driver (syd) mutants, which disrupt transport of a different class of vesicles. Further, increased anterograde and retrograde velocities are observed for APP vesicles in PS reduced axons, but not for synaptotagmin vesicles. These increased velocities require functional kinesin-1 and dynein motors. Our findings suggest that PS regulates APP intracellular transport by repressing kinesin-1 and dynein motor activity perhaps by influencing GSK3b. Thus our work has unraveled a novel function for PS in axonal transport.

3

Differential positioning of adherens junctions initiates epithelial folding during *Drosophila* gastrulation. Yu-Chiun Wang[1,4], Zia Khan[2,3], Matthias Kaschube[3], Eric Wieschaus[1,4]. 1) Department of Molecular Biology; 2) Department of Computer Science; 3) Lewis-Sigler Institute for Integrative Genomics; 4) Howard Hughes Medical Institute, Princeton University, Princeton, NJ.

During tissue morphogenesis, simple epithelial sheets undergo folding to form complex structures. The prevailing model underlying epithelial folding involves cell shape changes driven by Myosin-dependent apical constriction. Here we describe an alternative mechanism that requires differential positioning of adherens junctions controlled by modulation of epithelial apical-basal polarity. Using live embryo imaging, we show that prior to the initiation of dorsal folds during *Drosophila* gastrulation, adherens junctions shift basally in the initiating cells, but maintain their original subapical positioning in the neighboring cells. Junctional positioning in the dorsal epithelium depends on the polarity proteins Bazooka and Par-1. In particular, the basal shift that occurs in the initiating cells is associated with a progressive decrease in Par-1 levels. We show that uniform reduction of the activity of Bazooka or Par-1 results in uniform apical or lateral positioning of junctions and in each case dorsal fold initiation is abolished. In addition, an increase in the Bazooka/Par-1 ratio causes formation of ectopic dorsal folds. The basal shift of junctions not only alters the apical shape of the initiating cells, but also forces the lateral membrane of the adjacent cells to bend toward the initiating cells, thereby facilitating tissue deformation. We hypothesize that in epithelial tissues in which the levels of cortical Myosin are low and constant, junctional repositioning regulated by Par-1/Bazooka interactions may play a more prominent role to initiate folding than does differential activation of Myosin contractility. Dorsal fold formation represents an emergent model in which the insights into this alternative mode of epithelial folding could be further analyzed.

4

Talin: A Master Regulator of Cell-ECM Adhesion-Dependent Morphogenesis. Stephanie J. Ellis, Michael J. Fairchild, Stefan Czerniecki, Mary Pines, Guy Tanentzapf. Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, Canada.

Morphogenesis of a complex body plan requires coordinated regulation of cell adhesion molecules and the cytoskeleton to form distinct, organized tissues. Integrin adhesion receptors mediate ECM attachment and connect to the cytoskeleton through the adapter protein, talin. Talin interacts with many binding partners including integrin and F-actin. A delicate balance of these multiple interactions offers a means of fine-tuning integrin function and linkage to the cytoskeleton. Using targeted point mutations, we systematically investigate the role of different domains of talin during Drosophila embryogenesis. Our results suggest that morphogenetic events requiring short term, transient adhesions, such as germband retraction and dorsal closure, are highly sensitive to mutations in talin that compromise the ability to quickly disassemble adhesive contacts and linkage to the cytoskeleton. Conversely, in the embryonic and larval musculature, where myotendinous junctions form adhesive contacts that grow and persist over several days, talin interactions that strengthen attachment between integrins and the surrounding ECM are of greatest importance. Finally, using FRAP in the living embryo, we find that disruption of key domains in talin alters the dynamics of talin at adhesions, suggesting talin may be a master regulator of adhesion stability and cytoskeletal dynamics. Altogether, we demonstrate how the ability of talin to switch between multiple binding partners comprises an essential mechanism for modulating integrin function to elicit distinct developmental outcomes.

5

Septins are required for the establishment of adherens junction at the exit of mitosis of polarized epithelial cells. Nabila Founounou, Roland Le Borgne. CNRS UMR 6061-Institut de Génétique et Développeme, Rennes. France.

Septins are a conserved GTPases forming filament required at cytokinesis. Loss of Peanut, one of the four *Drosophila* septins, gives rise to binucleation in various cell contexts but its precise role remains poorly understood. Here, we have investigated the function of Septins during epidermal cell divisions and asymmetric divisions within the sensory organ (SOP) lineage. Two types of divisions are taking place within this lineage: SOP divide asymmetrically within the plane of the epithelium giving rise to two polarized epithelial cells, while one of the SOP daughter cells divide along the apical basal axis with one sibling that looses its epithelial characteristics. Sep2::GFP dynamics confirmed that Drosophila septins act as hetero-oligomer in vivo, and that loss of Pnut or tissue-specific silencing of one of *septins* is sufficient to cause cytokinesis defects. Time-lapse analyses revealed that in clones of cells homozygote mutant for pnut the early steps of SOP mitosis occur correctly but the abscission fails giving rise to a binucleated cell. Surprisingly, the binucleated cell undergoes an asymmetric division along the apical basal axis with a complete abscission indicating that Septins are dispensable for this type of asymmetric cell division. This is in striking contrast to the formin Diaphanous whose activity is required for the two types of asymmetric cell divisions. In both epidermal cells and SOP cells, time-lapse analyses indicate that cytokinesis occurs asymmetrically from basal to apical pole with Septins forming a horseshoe-shaped contractile apparatus. In a second step, an apical constriction takes place and precedes the establishment of adherens junctions as monitored using E-Cad::GFP knock-in line. In cells mutant for pnut, while basal to apical followed by apical constrictions occur properly, we observed a failure in the establishment of E-Cad junctions at mitosis exit. Our data reveal that septins are required in epithelial cell cytokinesis to allow adherens junction establishment, we are currently investigating the molecular link between Septins and E-Cad.

6

Actin turnover balances forces between cells during epithelial invagination. Adam C. Martin, Frank M. Mason, Mike Tworoger. Biology, Massachusetts Institute of Technology, Cambridge, MA.

Embryonic development requires that coordinated cell shape changes collectively deform tissues. Cell shape changes result from forces generated by actin networks that are coupled to adhesive complexes. During Drosophila gastrulation, a ratchet-like contraction of an apical actin and myosin II network coupled to adherens junctions drives apical constriction and generates epithelial tension, which are important for epithelial invagination. While the role of actin filament (F-actin) turnover is well established for membrane protrusion during cell migration, it is unclear how F-actin is remodeled to allow for myosin contraction and apical constriction during the coordinated movement of an epithelial sheet. Here, we combine live imaging, quantitative image analysis, and drug perturbation to define the role of F-actin turnover during apical constriction. We found that in contrast to Myosin-II levels, which steadily increase, total F-actin intensity decreases as cells constrict, suggesting actin network turnover. Pulses of Myosin-II accumulation are correlated with transient accumulations of F-actin, consistent with these events being contractions of the actin-myosin network that are subsequently remodeled. To determine the function of F-actin turnover, we titrated the rate of actin polymerization by injecting a wide concentration range of drugs that inhibit F-actin polymerization (cytochalasin D) or depolymerization (phalloidin). We observed a transition from a general disruption of contractility in all cells with high drug doses, to a mesoderm specific disruption in cell-cell connections at low doses. At low drug does neighboring actomyosin networks continually lost and reformed connections, resulting in an unbalanced ‘tug-of-war’ between mesoderm cells. During apical constriction, transient holes continually appear in the apical F-actin meshwork and these holes persist when F-actin turnover is inhibited. We propose that rapid F-actin turnover is required to fill holes generated during network contraction to balance forces across adhesive contacts.

7

Mechanisms of Epithelial Wound Repair. Jeffrey M. Verboon, Maria-Teresa Abreu-Blanco, James J. Watts, Raymond Liu, Susan M. Parkhurst. Division of Basic Sciences. Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA.

Upon injury, an epithelial tissue must have a rapid and robust repair mechanism to prevent invasion by microorganisms as well as to preserve tissue integrity. In other organisms, this repair response has been characterized to use either dynamic protrusions to crawl the wound edge forward or an actomyosin purse-string to cinch the wound closed. These mechanisms have been loosely correlated to large and smaller wounds respectively. We find that wound repair is achieved utilizing a combination of these mechanisms: an actomyosin cable circumferentially contracting and actin protrusions that knit the wound closed regardless of the wound size. Using 4D, high-resolution microscopy we set out to examine the relative contribution of the actin cable versus the protrusions using fluorescent expression constructs and genetic perturbations to components of the cytoskeleton in wounded fly embryos. Wounded embryos mutant for the core cytoskeletal proteins myosin and cadherin, as well as a transmembrane protein, Echinoid, have reduced actin cable in varying degrees. These mutants close using increased amounts of cellular protrusions, however, repair is delayed. In contrast, embryos mutant for Cdc42 decrease the quantity of protrusions during wound repair without affecting the cable. These wounded embryos are delayed and often unable to completely reseal the epithelial such that a small hole remains. Thus, protrusions play an indispensible role in completely fusing the final wound edges together. We are currently investigating additional components, which affect the actin cable and protrusions and their role in wound repair.

8

A Novel Mechanism for Actin Filament Disassembly Mediated by the Semaphorin/Plexin Axon Guidance Signaling Protein Mical. Ruei-Jiun Hung[1], Chi Pak[2], Jonathan Terman[1]. 1) Neuroscience; 2) Biochemistry, UT Southwestern Medical Center, Dallas, TX.

Semaphorins are one of the largest families of axon guidance cues and were characterized for their ability to rapidly disassemble actin filaments (F-actin) and “collapse” elongating neuronal growth cones. Mical, a multidomain cytosolic protein associated with cell-surface Semaphorin receptors (Plexins), is critical for Sema/Plexin-mediated neural connectivity and actin cytoskeletal rearrangements. Recently, we have found that Mical directly binds F-actin and provides a conduit between Semaphorin/Plexin and the actin cytoskeleton. Interestingly, Mical belongs to a class of flavoprotein monooxygenase/hydroxylase enzymes that associate with flavin adenine dinucleotide (FAD) and use the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH) in oxidation-reduction (Redox) reactions. Although MICALs have no known substrate/s, they employ their Redox region to bind F-actin and disassemble filaments in an NADPH-dependent manner. These observations suggest the possibility that MICALs are direct actin regulatory enzymes. To further address this hypothesis, we utilized in vitro biochemical assays and found that only very low, substochiometric levels of Mical were required for F-actin disassembly, lending additional support for a catalytic/post-translational mechanism underlying Mical-mediated F-actin disassembly. Moreover, this Mical-treated actin failed to re-polymerize even after removal of Mical/NADPH, indicating that Mical stably modifies actin to alter polymerization. Indeed, we found that actin was a specific substrate of Mical. Specifically, mass spectrometric analyses identified that F-actin subunits were directly modified by Mical on their conserved pointed-end that is critical for filament assembly. Mical post-translationally oxidized a conserved amino acid within the D-loop of actin, simultaneously severing filaments and decreasing polymerization. These results together with in vivo analyses using the Drosophila model bristle process present a specific oxidation-dependent mechanism that selectively regulates actin dynamics and cellular behavior.

9

Ras-oncogenic *Drosophila* hindgut cells use an "inflammatory-like cell program" to migrate to distant sites. Yiorgos Apidianakis[1,2], Erdem Bangi[3], Laurence Rahme[1], Ross Cagan[3]. 1) Department of Surgery, MGH, Harvard University, Boston, MA; 2) Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus; 3) Department of Developmental and Regenerative Biology, Mt Sinai School of Medicine, New York, NY.

Cell migration is the property of eukaryotic cells during tumor cell metastasis or inflammatory cell response to injury and infection. Here we find that adult Ras-oncogenic *Drosophila* hindgut cells migrate to distant sites when challenged by intestinal bacteria. While RasV12 ongogene suffices to induce migration, intestinal infection with the human pathogen *Pseudomonas aeruginosa* potentiates and induces an earlier onset of migration in adult flies. This cooperative migration progresses over time, is reversible upon bacterial clearance and can be inhibited by genetic and pharmacological means. We demonstrate that the Imd pathway converges with Ras signaling to induce JNK and Metalloproteinase 1 (MMP1), which in turn digests extra-cellular matrix (EMC) to promote hindgut cell migration. This property of genetically predisposed epithelial cells to produce MMP1, degrade EMC and migrate upon innate immunity stimulation resembles infiltrating macrophage activities , thus depicting innate immune response as a common trigger of inflammatory and metastatic tumor cell functions. Our previous work on the *Drosophila* midgut shows that virulent bacteria induce enterocyte compensatory proliferation that can be diverted in the presence of a tumorigenic genetic background to induce intestinal tumors. This mechanism is drastically different from the one we now propose for tumor cell migration in the fly hindgut. During tumor formation pathogenic bacteria use their virulence factors to cause cytotoxicity and enterocyte apoptosis, which in turn drives stem cell proliferation to promote tumor formation in genetically predisposed *Drosophila* midguts. While during tumor cell migration bacterial infection induces oncogenic cells to migrate via an innate immune response; independently of bacterial virulence factors that mediate damage to host cells.

10

Mediating a balance between tolerance and resistance. Moria C. Chambers, Karla L. Lightfield, David S. Schneider. MicroBiol & Immunology, Stanford Univ, Stanford, CA.

Immunity is a combination of both microbe clearance (resistance) and the host's ability to withstand the damage induced during infection (tolerance). Ets21c, a putative transcription factor, belongs to a newly characterized class of Drosophila mutants defective in tolerance to Listeria monocytogenes. Ets21c mutants don't affect all infections in this way, for example, we find that Et21c mutants more resistance to Streptococcus pneumoniae, as shown by increased survival and decreased bacterial loads. Microarray analysis of the Ets21c mutants after infection suggests that their phenotypes may be due to a combination of effects. Ets21c mutants show mis-regulation of melanization, metabolism and WntD, a negative regulator of the NFkB pathway. Melanization has been previously shown to impact both resistance and tolerance, and we can now show that that genetic modulation of metabolism and WntD independently affect both tolerance and resistance. These findings support a complex relationship between tolerance and resistance during infection and stress the importance of examining multiple infections when classifying genes as part of resistance or tolerance.

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Virus recognition by Toll-7 activates antiviral autophagy in *Drosophila*. Ryan H. Moy, Margaret Nakamoto, Jie Xu, Shelly Bambina, Ari Yasunaga, Spencer S. Shelly, Beth Gold, Sara Cherry. Department of Microbiology, Penn Genome Frontiers Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104.

The innate immune system relies on germline-encoded pattern recognition receptors (PRRs) for the detection and clearance of invading pathogens. The canonical PRRs are the mammalian Toll-like receptors (TLRs), which were originally identified through their homology to *Drosophila* Toll. Flies encode nine Toll receptors; however, whereas the immune functions for Toll are well-characterized, roles for the eight remaining Toll receptors have remained elusive. By using RNA interference screening techniques, we have identified a role for an additional Toll receptor, Toll-7, in conferring antiviral immunity to the negative-sense RNA virus Vesicular Stomatitis virus (VSV). Toll-7 knockdown in cells leads to increased VSV infection. Similarly, flies depleted of Toll-7 by RNAi or mutant for Toll-7 exhibit increased viral RNA replication and mortality upon VSV challenge. Like many mammalian antiviral PRRs, Toll-7 is transcriptionally induced by viral infection along with several other Toll receptors. Moreover, Toll-7 is present at the plasma membrane and binds VSV by co-immunoprecipitation, which is fundamentally distinct from the mode of recognition for Toll and more similar to that of mammalian TLRs. This interaction is required in both cells and flies to activate autophagy, an ancient pathway involved in the degradation of cytoplasmic components including pathogens. Taken together, these data unveil an evolutionarily conserved role for a second *Drosophila* Toll receptor that links virus recognition to autophagy and defense, suggesting that the mammalian TLRs and *Drosophila* Tolls are in fact more alike than previously appreciated.

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Study of Pallbearer, an E3-ubiquitin ligase that regulates phagocytosis of apoptotic cells in *Drosophila*. Hui Xiao, Nathalie Franc. The Department of Genetics, 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. In a quest to genetically dissect the molecular mechanisms of apoptotic cell clearance in *Drosophila*, we previously identified *pallbearer* (*pall*), a gene encoding a novel F-Box protein. F-box proteins form multi-molecular Skp/Cullin/F-Box (SCF) complexes, which act as E3 ligases that target modified proteins to degradation via the ubiquitin-proteasome pathway. In these complexes, the F-Box protein provides the substrate-binding function and specificity. Identifying the PALL substrate(s) is important to further our understanding of the molecular mechanisms of 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 macrophages, the opposite phenotype of *pall* mutants. We have also found that *RpS6* RNAi enhances apoptotic cell clearance in a genome-wide RNAi screen in S2 cells, and validated this finding using new *RpS6* specific siRNAs. Furthermore, we have found that overexpression of RpS6 in macrophages results in decreased phagocytosis *in vivo*. We are now testing whether *RpS6* and *pall* genetically interact *in vivo*. We propose that RpS6 is a substrate of PALL that negative regulates phagocytosis of apoptotic cells.

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Calcium signaling plays a role in Drosophila cellular immunity and is antagonized by parasitoid wasp venom. Nathan T. Mortimer, Todd A. Schlenke. Department of Biology, Emory University, Atlanta, GA.

Drosophila larvae are targeted by a wide range of parasitoid wasps, and mount a robust cellular immune response following parasitization. Parasitoids attempt to counteract this response by the co-injection of immunomodulatory venom proteins. Thus, identification of wasp venom proteins may allow for a better understanding of both parasite virulence and host response. We have focused on an uncharacterized parasitoid wasp species of the genus Ganaspis, caught in southern Florida (and referred to as GanFl). GanFl is a generalist of Drosophila species and has immunosuppressive venom. We used a combined transcriptomic/proteomic approach to identify GanFl venom proteins and discovered the presence of a highly abundant Serca-type ATPase pump. Endogenous Serca ATPases regulate ion homeostasis by pumping calcium from the cytosol into ER stores. The presence of such a calcium pump in GanFl venom led us to hypothesize that the fly cellular immune response may require cytosolic calcium signaling and that this may serve as a target of GanFl venom activity. We find that intracellular calcium levels are elevated in fly immune cells following parasitization and that this appears to be required to mount a successful immune response. Further, we find evidence that the virulence mechanism of GanFl is at least partially dependent on its ability to antagonize calcium signaling in fly immune cells.

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Beta-arrestin Kurtz regulates *Drosophila* Toll signaling and immune system homeostasis through an interaction with SUMO protease Ulp1. Wenjian Xu[1], Saima G. Anjum[1], Niusha Nikkholgh[1], Sukanya Basu[1], Mary Thomas[1], Tony Ip[2], Alexey Veraksa[1]. 1) Biology Department, University of Massachusetts Boston, Boston, MA; 2) Program in Molecular Medicine, UMass Medical School, Worcester, MA.

Our previous studies implicated the *Drosophila* β-arrestin Kurtz (Krz) in the regulation of Toll signaling in the early embryo. Here we extend these studies to probe the role of Krz in Toll regulation at the larval stages. *krz* mutants and RNAi knockdown animals show increased lamellocyte production, melanotic mass formation, as well as Dorsal and Dif nuclear localization, suggesting that loss of *krz* results in an upregulation of Toll signaling. Proteomic surveys and follow-up studies of Krz binding partners have identified Ulp1, a SUMO protease, as a direct Krz interactor. RNAi knockdown of *Ulp1* results in even stronger phenotypes than loss of *krz*. RT-PCR analysis demonstrated that *Drosomycin* expression is increased in both *krz* mutants and *Ulp1* knockdown animals. Furthermore, *krz* and *Ulp1* show dosage-sensitive synergistic genetic interactions, which suggests that these two proteins are involved in the same pathway. Because Ulp1 normally functions as a SUMO deconjugating enzyme, we tested whether Krz plays a role in protein sumoylation. Our studies of Dorsal sumoylation show that altering Krz levels can affect the efficiency of SUMO deconjugation mediated by Ulp1. Our data suggest that both Krz and Ulp1 are required for maintaining a precise level of Dorsal sumoylation, which can affect Dorsal activity in the Toll pathway and ultimately regulate larval immune system homeostasis.

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***Drosophila* immune responses to entomopathogenic nematodes and their mutualistic bacteria.** Julio César Castillo, Ioannis Eleftherianos. Department of Biological Sciences, The George Washington University, Washington DC 20052.

Drosophila melanogaster has been established as an excellent genetic and genomic model to investigate host-pathogen interactions and innate immune defense mechanisms. To date, most information on the innate immune response in Drosophila derives from studies that involves bacterial, fungal and viral pathogens. However, immune reactions to insect parasitic nematodes (entomopathogenic) are still not well understood. The nematode *Heterorhabditis bacteriophora* lives in symbiosis with the entomopathogenic bacteria *Photorhabdus luminescens* that together are able to invade and kill insects. Interestingly, these nematode parasites are viable in the absence of their mutualistic bacteria. Although the pathogenicity of *Heterorhabditis* nematodes has been attributed almost exclusively to the toxins and virulence factors produced by *Photorhabdus*, little is known about the contribution of the nematode vector to the pathology observed in insects. We have recently developed a method for infecting *Drosophila* adult flies with *Heterorhabditis* nematodes that contain (symbiotic worms) or lack (axenic worms) *Photorhabdus*. We have used this assay to show that axenic and symbiotic nematodes are equally pathogenic towards wild-type adult flies. We have further identified which particular immune pathways are activated in *Drosophila* adults following immune recognition of *Heterorhabditis* nematodes with or without mutualistic *Photorhabdus* and documented the signaling genes that participate in these processes. Our results generate for the first time fundamental information on the immune detection, interaction and transcriptional regulation of immune signaling pathways, and activation of effector mechanisms by which *Drosophila* responds to natural insect pathogens, such as the *Heterorhabditis-Photorhabdus* complex. Finally, our data suggest that the parasitic nematode *Heterorhabditis* can induce severe pathological effects on *Drosophila* adult flies, and provide important clues on how two pathogens can come together to exploit a common host.

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A model for intracellular parasitism in Drosophila. Dominique X. Ferrandon, Sebastian Niehus, Adrien Franchet, Marie-Céline Lafarge, David Giacomini. IBMC, CNRS UPR 9022, Université de Strasbourg, France.

While much is known about *Drosophila* host defense against extracellular bacterial and fungal pathogens, intracellular host defenses not mediated by autophagy remain a mystery. Also, no *Drosophila* model with a natural obligate intracellular eukaryotic parasite has been developed so far. Here, we report on the development of such a model using the microsporidium *Tubulinosema ratisbonensis*, which sometimes contaminates laboratory stocks, and which, if not kept in check, can eradicate Drosophila cultures. Microsporidia are highly derived intracellular fungi, which infect orally their fly host through spores. Spores infect host cells through a unique mechanism that involves the evagination of a polar tube through which the spore cytoplasm and nuclei are transferred directly into the host cells.

We have developed an adult infection model in which flies succumb to *T. ratisbonensis* proliferation in a temperature and dose-sensitive manner. Host defenses include the cellular and humoral systemic immune responses. We have also developed cell culture models. Some cell lines are resistant to the infection while others are permissive. We find that host cell invasion is an active process, which involves both the parasite and the host through phagocytosis. Strikingly, infected cells become multinuclear giant cells within three days and are reminiscent of xenomas described in other systems by classical authors about a century ago. New spores are produced within 9-12 days. We have performed transcriptomics and metabolomics analyses and, surprisingly, find that the parasite takes control of its host cell very early on during the infection.

This novel model will allow us to study host-parasite relationships at multiple levels and should yield an unprecedented insight into the molecular bases of parasitism. Finally, we have established methods for prophylaxis and protocols to cure microsporidia-infected stocks from the parasites.

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Genome-wide Association Mapping of Resistance to Oxidative Stress in *Drosophila* Identifies Genes Involved in Complex Disease. Allison L. Weber[1,2], George F. Khan[2,3], Michael M. Magwire[1], Crystal L. Tabor[1], Robert R. H. Anholt[1,2,3], Trudy F. C. Mackay[1,2]. 1) Department of Genetics, North Carolina State University, Raleigh, NC; 2) W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC; 3) Department of Biology, North Carolina State University, Raleigh, NC.

Oxidative stress has been implicated in ageing, the progression of cardiovascular and neurodegenerative diseases in humans and the disruption of cell signaling processes that control cell growth and death. Although several genes involved in cellular responses to oxidative stress have been identified there is limited knowledge concerning how natural genetic variation contributes to variation in resistance to oxidative stress. In order to identify these genes, we have recently completed genome-wide association mapping of acute oxidative stress resistance in a panel of 167 inbred wild-derived *Drosophila* lines. We assessed oxidative stress resistance for all lines by measuring survival times on exposure to 20mM paraquat and 75mM menadione sodium bisulfite. We found significant genetic variation, and genetic variation for sexual dimorphism under both treatment conditions. We detected 298 SNPs significantly associated with resistance to paraquat and 146 SNPs significantly associated with resistance to menadione sodium bisulfite. Among these associations, variation in only seven genes was significantly associated with resistance to both paraquat and menadione sodium bisulfite. Approximately half of the genes identified have human homologs and a quarter have previously been implicated in complex diseases. These results suggest that the genetic architecture of oxidative stress resistance is sex-specific and dependent on the method of induction. This study illustrates the potential of using the genetically tractable, easily manipulated *Drosophila* system to identify human candidate genes that may harbor risk alleles to oxidative stress dependent complex human diseases, including neurodegenerative disorders such as Parkinson's disease.

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ATM kinase inhibition in glial cells activates the innate immune response and causes neurodegeneration. Andrew Petersen, David Wassarman. Cellular and Regenerative Biology, University of Wisconsin-Madison, Madison, WI.

To investigate the mechanistic basis for central nervous system (CNS) neurodegeneration in the childhood disease Ataxia-telangiectasia (A-T), we analyzed flies mutant for the causative gene A-T mutated (ATM). ATM encodes a protein kinase that functions to monitor the genomic integrity of cells and control cell cycle and DNA repair programs. Mutation of the C-terminal amino acid in Drosophila ATM inhibited the kinase activity and caused neuronal and glial cell death in the adult fly brain and a significant reduction in longevity. These data suggest that loss of kinase activity is sufficient to cause neurodegeneration in A-T. ATM kinase mutant flies also exhibited prolonged upregulation of a transcription program in glial cells consistent with activation of the innate immune response. ATM knockdown specifically in glial cells was sufficient to cause neuronal and glial cell death, activation of the glial cell innate immune response, and a significant reduction in longevity, indicating that non-cell autonomous toxicity contributes to neurodegeneration in A-T. Taken together, these data suggest that early onset CNS neurodegeneration in A-T is similar to late onset CNS neurodegeneration in diseases such as Alzheimer's wherein uncontrolled inflammatory response mediated by glial cells drives progressive neurodegeneration. Current experiments are focused on elucidating the specific glial subtype initiating the immune response, as well as the signaling pathways responsible for immune gene expression.

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A Drosophila Model Linking Diet-induced Metabolic Disease and Cancer. Susumu Hirabayashi[1], Thomas Baranski[2], Ross Cagan[1]. 1) Mount Sinai School of Medicine, New York, NY; 2) Washington University School of Medicine, St. Louis, MO.

Epidemiological studies have demonstrated that increased cancer risk is associated with metabolic disease including obesity and diabetes, but the underlying mechanism remains poorly understood. To this end, we have developed whole animal Drosophila cancer models that permit us to explore tumor progression in 'diabetic' flies. Feeding Drosophila a diet high in carbohydrates was previously demonstrated to direct metabolic dysfunction including insulin-resistance, hyperglycemia, increased insulin levels, and accumulation of fat. We now show that high dietary sugar also transforms Ras/Src-activated tissue from localized growths to aggressive tumors with emergent metastases. Surprisingly, while most tissues displayed aspects of metabolic dysfunction including insulin resistance, Ras/Src-activated tumors retained insulin pathway sensitivity and exhibited an increased ability to import glucose as well as resistance to apoptotic elimination. We provide evidence that this reflects increased Insulin signaling, which in turn acts through Wingless/Wnt signaling to promote diet-mediated malignant phenotypes within Ras/Src-activated tumors. These fly models should provide useful paradigms to study the link between metabolic dysfunction and tumorigenesis in the context of a whole animal.

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Drosophila - a useful model for anti-amyloid drug development. Daniel Segal[1], Roni Scherzer-Attali[1], Ronit Shaltiel-Karyo[1], Sivan Peled[1], Moran Frenkel-Pinter[1], Dorit Farfara[2], Dan Frenkel[2], Ehud Gazit[1]. 1) Molecular Microbiol & Biotech, Tel Aviv Univ, Tel Aviv; 2) Neurobiology, Tel Aviv Univ, Tel Aviv.

Drosophila offers an attractive model for drug development which can reduce the use of vertebrates and cut costs. Our recent work on developing inhibitors of amyloid assembly exemplifies this strategy. We have previously identified a key role of aromatic residues in the molecular recognition and self-assembly leading to the formation of various amyloid assemblies. Aromatic interactions provide selectivity as well as stability to the interacting molecules. Our strategy is to use small aromatic molecules that would bind the aromatic residues of the beta-amyloid (Aβ) monomers in Alzheimer's disease (AD) thereby inhibit the early steps of the molecular recognition and structural transition of the monomers which lead to the formation of the toxic amyloid species. We have synthesized a series of N-linked tryptophan-modified quinones and screened them for anti-Aβ activity. Two compounds, NQTrp and Cl-NQTrp, were most effective. They inhibit Aβ oligomerization and fibrillization in vitro and reduce the cytotoxic effect of Aβ oligomers towards cultured cells. NMR spectroscopy and molecular dynamics simulations provide a mechanistic basis for the activity of these compounds. When fed to Drosophila expressing Aβ in their nervous system, these compounds alleviated their AD-related symptoms while having no effect on control flies. When injected intraperitonealy to 5xFAD transgenic acute AD mice they led to specific and significant improvement of their cognitive behavior, dramatic reduction in the level of both soluble and insoluble Aβ in their brain extracts and marked decrease in Aβ deposition in their brains. The compounds can cross the blood-brain-barrier and have no adverse effects. We have also shown that β-synuclein-derived peptidomimetics designed to inhibit the toxic amyloid assembly of α-synuclein in Parkinson disease (PD) have marked remedial effect in Drosophila expressing α-synuclein in their brain which serve as an established model for PD.

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Creating an epileptic fly by tipping the balance of *prickle* isoforms. Salleh Ehaideb[1], Atsushi Ueda[1], Alexander G Bassuk[1], 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.

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, *prickle* (*pk*) and *spiny legs* (*sple*). Strikingly, flies heterozygous for the pk^{sple1} mutation display pronounced seizures even though no planar cell polarity defects are visible, suggesting that the PCP and seizure phenotypes can be genetically separated. We now report that pk^{pk} mutations are actually protective against seizures, consistent with PCP data that *pk* and *sple* act antagonistically towards one another. Additionally, pk^{sple} mutant larvae have both anatomical and electrophysiological neuronal defects. Finally, targeted overexpression of the pk^{pk} isoform in motor neurons and muscles (which recapitulates the imbalance of *pk* vs *sple* isoforms seen in the pk^{sple} heterozygote) strongly induces fly seizures in an otherwise wild-type fly. These results likely pinpoint the tissues involved in human epilepsy in patients with Prickle mutations.

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microRNAs Orchestrate Muscular Dystrophy in Drosophila. April K. Marrone, Halyna R. Shcherbata. Laboratory of Gene Expression and Signaling, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.

In Drosophila, like in humans, Dystrophin Glycoprotein Complex (DGC) deficiencies cause a life span shortening disease, associated with muscle dysfunction and cognitive impairment. We have shown that in addition to mutations in DGC main components Dystrophin (Dys) or Dystroglycan (Dg), stress can induce muscle degeneration and accelerate age-dependant muscular dystrophy even in wild type animals. In an effort to explore mechanisms by which stress can cause dystrophy we have turned to microRNA biogenesis and have performed a microarray screen on whole adult flies to determine misregulation in genetic mutants for Dys and Dg and high temperature stressed animals. We have been able to define three categories of misexpressed microRNAs; stress derived, Dys and/or Dg dependent and those that are altered in stress and mutant situations. In this screen we were able to detect altered microRNA levels not only in dystrophic muscle as has been done previously in mammals, but in the brain and nervous system. Since abnormal functioning of Dg causes congenital muscular dystrophies that are associated with brain defects and the function of the DGC in the nervous system has not been fully defined, we have focused on misregulated microRNAs that have the potential to target Dg in this tissue. Interestingly, we found that Dg isoforms can have variant 3'UTRs that allow for specific regulation by certain microRNAs. The plethora of miRNAs implicated in the DMD pathology present a substantial and complex level of regulation that opens diverse avenues for future research and therapies.

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Cell-Specific MeCP2 expression changes Sleep Patterns and Aggressive Behavior in a Drosophila model of MeCP2 Spectrum Disorders. Sarah J. Certel, Tarun Gupta, David Hess-Homeier, Conor Jacobs, Brittany Felgate. Division of Biological Sciences, University of Montana, Missoula, MT.

Methyl-CpG-binding protein 2 (MeCP2) is expressed in nearly every cell in the human body and is one of the most dosage-sensitive genes involved in neuron function. Due to the widespread expression of MeCP2, the complexities of human disorders associated with mutations in the MeCP2 gene including Rett Syndrome and MeCP2 Duplication disorders are considerable. Key behavioral changes in patients can include increased aggression levels and significant sleep disturbances. To determine whether neuronal or glial changes in MeCP2 expression cause the neurological dysfunction and alterations in behavior, we expressed human MeCP2 in astrocytes and distinct subsets of amine neurons including dopamine and octopamine (OA) neurons. Our results indicate there are quantifiable behavioral differences between astrocytic MeCP2-expression and OA neuron-MeCP2 expression both in sleep patterns, sleep latency, and duration of sleep bouts. Specifically, expression of MeCP2 in astrocytes results in a significant reduction in the number of nighttime sleep bouts. A slight increase in latency to sleep is also observed indicating nighttime sleep is both delayed and reduced. In contrast, daytime sleep is significantly reduced in males expressing MeCP2 in OA neurons. Although activity levels are not reduced in MeCP2-expressing flies, male aggressive behavior is significantly decreased as compared to control males. Fights between males expressing MeCP2 in octopamine neurons or astrocytes exhibit a decrease in lunge number and an increase in latency to initiate aggression. Finally, we examined if the morphology of amine neurons is altered either non-cell-autonomously or cell-autonomously by astrocytic vs. neuronal MeCP2 expression. Given the complexities of MeCP2 function, our results provide insight into the distinct cell types, glial and neuronal, that mediate key behavioral changes tied to alterations in MeCP2 function. (NIH COBRE grant P20RR015583 to SJC).

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Organotypic models for kidney disease: *Drosophila* gets kidney stones, too. Julian A T Dow[1], Pablo Cabrero[1], Taku Hirata[2], Michael Romero[2]. 1) Institute of Molecular, Cell and Systems Biology, University of Glasgow, Glasgow, United Kingdom; 2) Physiology & Biomedical Engineering & O'Brien Urology Research Center, Mayo Clinic, Rochester, MN 55905 USA.

Although there are homologs for over 70% of human genes in *Drosophila*, the best disease models are organotypic - that is, when the fly homolog is expressed in the most appropriate tissue for the disease (1, 2). The Malpighian tubule provides an excellent model for renal function and disease (3). Nephrolithiasis represents a major health burden, costing over $5B/ yr in the US alone. Insect Malpighian tubules in fact express two major classes of stone (phosphate and urate) constitutively, and the rosy mutant exactly recapitulates the renal & metabolic phenotypes of human xanthinuria type I (4, 5). The most common stones in humans are of calcium oxalate, with a complex etiology including a dietary component, and here we show that dietary oxalate loading rapidly induces stones in *Drosophila* tubules (3, 7). Furthermore, stone formation in isolated tubules can be induced in minutes by addition of oxalate, allowing the nucleation event to be monitored in situ for the first time (3). The utility of the model would be increased if specific genes could be associated with stone formation. We have identified prestin, an SLC26A5/6 homolog with strong expression in the gut and tubules, as a key oxalate transporter that mediates stone formation, as dsRNA-mediated knockdown of prestin in just tubule principal cells is sufficient to reduce the rate of stone formation. Given the range of quantitative phenotypic readouts available in this tissue (7), it is clear that the Malpighian tubule offers an unusually good model for human function and disease. 1.J. A. T. Dow, S. A. Davies, J. Insect Physiol. 52, 365 (2006). 2.V. R. Chintapalli et al. Nat. Genet. 39, 715 (2007). 3.J. A. T. Dow, M. F. Romero, Am J Physiol Renal Physiol 299, F1237 (2010). 4.J. Wang et al., Genome Biol. 5, R69 (2004). 5.M. A. Kamleh et al. FEBS Lett. 582, 2916 (2008). 6.Y. H. Chen et al., Kidney Int. 80, 369 (2011). 7.J. A. T. Dow, S. A. Davies, Physiol. Reviews 83, 687 (2003).

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***Patched* together: sophisticated multi-modular *cis*-regulatory circuitry underlies a seemingly simple "constitutive" response to Hedgehog signaling.** Scott Barolo. Department of Cell & Developmental Biology, University of Michigan Medical School, Ann Arbor, MI.

The *Drosophila* gene *patched* and its mammalian ortholog *PTCH1* encode the receptor for the Hedgehog morphogen. Both *patched* and *PTCH1* are constitutive responders to Hedgehog signaling: they both contain binding sites for Hedgehog-regulated Gli transcription factors (Ci in flies), and both are activated in response to Hedgehog signaling in all tissues. In principle, this represents the simplest possible regulatory circuit: where Hedgehog signaling is active, Glis directly activate *patched*.

However, our dissection of the *patched* locus shows that this seemingly simple response depends on unexpectedly complex *cis*-regulatory circuitry, with many Hedgehog-responsive enhancers spread over tens of kilobases. These enhancers employ variable numbers of Gli/Ci binding sites of widely varying affinities: our previous work has shown that Gli binding affinity is a key patterning determinant in the tissue-specific Hedgehog target genes *dpp* and *wingless*. We have identified separate *patched* enhancer modules responsible for expression in the developing wing, eye, gut, segmental stripes, embryonic mesoderm, salivary gland, gonadal stem cells, etc., suggesting that *patched*'s "constitutive" Hedgehog response is in fact composed of many tissue-specific elements with diverse types of regulatory logic.

Interestingly, a large number of separate *cis*-elements contribute to *patched* expression in the wing, suggesting a "shadow enhancer"-like mechanism of transcriptional control. We will present the latest results of our investigation into (1) the role of Gli/Ci sites in this multi-modular Hedgehog response; (2) mechanisms by which tissue-specific responses are integrated into a constitutive response pattern; (3) the role of "shadow enhancers" in shaping the response to Hedgehog signaling in the wing; and (4) similar enhancer logic of the fly and mouse *patched* genes.

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The spatial and temporal activity of enhancers depends on combinatorial binding of transcription factors. Zhe Xu[1], Hongtao Chen[1], Paolo Struffi[2], Constance Mei[1], Darren Huang[1], Steve Small[1]. 1) Biology, New York University, New York, NY; 2) European Commission, Joint Research Center.

Although extensive research has been done on a few well-known regulatory elements, it is still poorly understood how they are activated or silenced in general. To better understand the activation/repression mechanisms used by enhancers that pattern the early embryo, we conducted a genome-wide search for Bcd-dependent enhancers by looking for clusters of predicted Bcd-binding sites or in vivo Bcd-binding, or both. More than 75 fragments were tested by reporter gene assays, and thirty-two new enhancers were identified in the search. However, sixteen of bio-informatically predicted enhancers showed no Bcd binding in vivo and did not direct early embryonic expression. By comparing sequences of positive and negative fragments, we found a strong correlation between enhancer activity and the number of predicted binding sites (TAGteam sites) for the ubiquitous maternal protein Zelda (Zld). Fragments activated by Bcd in the early embryo showed an enrichment of TAGteam sites compared to those that are not activated. Adding TAGteam sites to some negative fragments successfully converted them into Bcd-dependent enhancers in the early embryo. Adding more TAGteam sites increased Zld- and Bcd-binding to these enhancers, and resulted in earlier activation and stronger expression in regions of limiting Bcd concentration. Preliminary data shows change of histone modification with addition of Zld binding sites to negative enhancers, indicating that Zld possibly functions by modifying chromatin states. Our work underscores the importance of combinatorial binding in determining the spatial and temporal activity of enhancers, and provides data that will refine our ability to predict regulatory activity based on DNA sequence.

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Assessing the basic building blocks necessary to generate *cis*-regulatory modules (CRMs) that regulate spatio-temporal expression. Jelena Erceg, Charles Girardot, Eileen Furlong. Genome Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany.

cis-regulatory modules (CRMs) regulate the spatio-temporal activity of target genes through the recruitment of transcription factors (TFs) via a collection of TF binding sites (TFBSs). Understanding the rules of how different TFBSs are organized to drive specific activity is key to elucidate the logic of gene expression patterns. Are CRMs generally a composite of TFBSs as basic building blocks with very constrained positions or ‘grammar’ (enhanceosome) to which highly ordered TF complexes bind? Or is it a more relaxed and flexible composite, as suggested by the billboard model? Do novel spatio-temporal patterns arise through an increase in motif complexity? We addressed these questions using a synthetic biology approach focused on the key TFs essential for *Drosophila* mesoderm development. Synthetic CRMs with multimers of a single TFBS (homotypic) were assessed for their ability to drive expression in transgenic reporter assays. We found that homotypic synthetic CRMs were sufficient to recapitulate the endogenous expression patterns for some TFs, but not others. We further tested synthetic CRMs with a pair combination of TFBSs (heterotypic) where TFBSs were chosen for factors with evidence of protein-protein interaction (PPI). To assess the possible constraints at the sequence level due to impinging protein interactions, changes in both orientation and spacing were systematically explored for four pairs of TFBSs. Our data revealed emerging developmental patterns, which were not simply additives for the corresponding patterns obtained with homotypic synthetic CRMs. Furthermore, we found that minor variations in the spacing and orientation produce specific spatio-temporal patterns, suggesting constraints imposed by direct PPI. These findings provide valuable insights into the general toolkit required to generate CRMs, which drive restricted developmental patterns.

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Juxtaposed *cis*-regulatory elements contradict simple definitions of enhancer modularity. Tara L. Martin, Meghan Bragdon, Kelly Eckenrode, Zeba Wunderlich, Angela DePace. Department of Systems Biology, Harvard Medical School, Boston, MA.

Classically, enhancers are defined as modular *cis*-regulatory sequences which act independently of orientation or distance from the promoter to activate gene expression. However, a growing body of evidence suggests that this definition does not fully capture the behavior of all *cis*-regulatory elements. Multiple non-contiguous sequences can drive expression in the same domain (e.g., "redundant" enhancers), and *cis*-regulatory elements may interact with each other to produce the endogenous pattern, (e.g., *sloppy-paired-1*). To specifically test whether adjacent *cis*-regulatory elements would remain modular or adopt a combined function, we juxtaposed elements that are regulated by the same factors but respond to them with different sensitivities. We created transgenic lacZ reporter lines that combined the stripe 3+7 and stripe 4+6 *cis*-regulatory elements from the *even-skipped* locus. Using a quantitative cellular resolution imaging platform, we compared the average expression pattern from each line, and integrated it into an existing gene expression atlas for *D.melanogaster*. This method yields a dataset amenable to modeling where the expression patterns of all relevant regulators and their targets are present in the same cellular resolution framework. Current thermodynamic models predict that our constructs will produce intermediate expression patterns because the response to upstream regulators is an average of the sensitivities. However, in our experiments the expression domains did not blend. We find that the spacing, order and orientation of the *cis*-regulatory elements influence expression in both subtle and dramatic ways. One *cis*-regulatory element sometimes dominates, reducing the level of expression of it's neighbor without otherwise shifting the expression domain. We discuss models of gene regulation and enhancer structure that are consistent with our results and suggest methods to incorporate higher order interactions between *cis*-regulatory elements into expression pattern predictions.

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Temporal Coordination of Gene Networks by Zelda in the Early *Drosophila* Embryo. Hsiao-Lan Liang[1], Chung-Yi Nien[1], Stephen Butcher[2], Yujia Sun[1], Shengbo Fu[1], Tenzin Gocha[1], Nikoai Kirov[1], J. Robert Manak[2], Christine Rushlow[1]. 1) Department of Biology, Center for Developmental Genetics, New York University, New York, New York, USA; 2) Departments of Biology and Pediatrics, Roy J. Carver Center for Genomics, University of Iowa, Iowa City, Iowa, USA.

Our recent discovery of the transcriptional activator Zelda (Zld), which binds to CAGGTAG and related sequences present in the enhancers of many early-activated genes in *Drosophila*, suggested how a single molecule could collectively activate batteries of genes simultaneously. To explore further the function of Zld and to unravel the gene circuitry regulated by Zld, we used genome-wide binding and expression assays to identify Zld target genes in the blastoderm embryo. Our results showed that Zld binds to genes involved in early developmental processes such as cellularization, sex determination, neurogenesis, pattern formation, as well as early-expressed microRNAs. In the absence of Zld, many target genes failed to be activated, while others, particularly the patterning genes, exhibited delayed transcriptional activation, some of which also showed weak and/or sporadic expression. These effects disrupted the normal sequence of patterning-gene interactions and resulted in highly altered spatial expression patterns, demonstrating the significance of a timing mechanism in early development. In addition, we observed prevalent overlap between Zld-bound regions and genomic "hotspot" regions, which are bound by many developmental transcription factors, especially the patterning factors. This, along with the finding that the most over-represented motif in hotspots, CAGGTA, is the Zld binding site, implicates Zld in promoting hotspot formation. We propose that Zld promotes timely and robust transcriptional activation of early-gene networks so that developmental events are coordinated and cell fates are established properly in the cellular blastoderm embryo.

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The cis-regulatory code of Hox function in Drosophila. Maria Polychronidou[1], Sebastian Sorge[1], Nati Ha[1], Jana Friedrich[1], Daniela Bezdan[2], Petra Kaspar[1], Martin Schaefer[2,3], Stephan Ossowski[2,4], Stefan R. Henz[2], Juliane Mundorf[1], Jenny Raetzer[1], Fani Papagiannouli[1], Ingrid Lohmann[1]. 1) Centre for Organismal Studies, Heidelberg, Heidelberg, Germany; 2) Max Planck Institute for Developmental Biology, Tübingen, Germany; 3) Max Delbrück Center for Molecular Medicine, Berlin, Germany; 4) Center for Genomic Regulation, Barcelona, Spain.

Spatiotemporal control of gene expression is orchestrated by the combinatorial interplay of transcription factor (TF) complexes with cis-regulatory DNA elements. However, it remains mostly unclear how TFs, many of which are active in several cell types, acquire cell-type specific functions. An ideal model to study the mechanisms underlying TF tissue-specificity are the Homeobox (Hox) TFs, which despite their broad expression, activate or repress transcriptional programs in a highly context dependent manner. To address how a widely expressed transcriptional regulator is able to modulate downstream gene activity with high cellular specificity, we have quantitatively identified binding regions for the Hox TF Deformed (Dfd) in the Drosophila genome. By analyzing Dfd bound cis-regulatory modules (CRMs), we show that architectural features like motif-pair associations and motif distance preferences are essential for cell-type specific expression of associated target genes. CRM features indeed determine specificity, since they alone accurately predict target gene function and expression patterns. We also find that Dfd and Ultrabithorax (Ubx), another Hox TF specifying different morphological traits, interact exclusively with non-overlapping genomic regions in vivo, regardless of their similar DNA binding preferences. Despite their comparable basic design principles, Dfd and Ubx CRMs show distinct motif compositions and motif-pair associations, explaining the high functional specificity of the two Hox proteins. Our results uncover the regulatory code of Hox CRMs and elucidate the mechanisms underlying functional specificity of TFs in vivo.

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Unlocking specificity: Cofactor binding reveals latent differences in DNA binding specificity between Hox proteins. Matthew Slattery[1,2], Todd Riley[3,4], Peng Liu[4,5], Namiko Abe[2], Pilar Gomez-Alcala[3,6], Iris Dror[7], Tianyin Zhou[7], Remo Rohs[7], Barry Honig[4,5], Harmen Bussemaker[3,4], Richard Mann[2]. 1) University of Chicago, Chicago, IL; 2) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY; 3) Department of Biological Sciences, Columbia University, New York, NY; 4) Center for Computational Biology and Bioinformatics, Columbia University, New York, NY; 5) Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY; 6) Department of Electrical Engineering, Columbia University, New York, NY; 7) Molecular and Computational Biology Program, Department of Biological Sciences, University of Southern California, Los Angeles, CA.

Members of transcription factor families typically have similar DNA binding specificities yet execute unique functions in vivo. Transcription factors often bind DNA as multiprotein complexes, raising the possibility that complex formation might modify their DNA binding specificities. To test this hypothesis, an experimental platform was developed, termed SELEX-seq, that can be used to determine the relative affinities to any DNA sequence for any transcription factor complex. Applying this method to all eight Drosophila Hox proteins revealed that they obtain novel recognition properties when they bind DNA with the cofactor Extradenticle (Exd). Exd-Hox specificities group into three main classes that obey Hox gene collinearity rules and DNA structure predictions suggest that anterior and posterior Hox proteins prefer DNA sequences with distinct minor groove topographies. Together, these data suggest that emergent DNA recognition properties revealed by interactions with cofactors contribute to transcription factor specificities *in vivo*.

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Dopaminergic precursor fate is established by *gsb-n* and *slp* at the intersection of Wg and Hh signaling. Joseph D. Watson, Stephanie B. Stagg, Stephen T. Crews. Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill.

Our lab is focused on identifying the transcription factors that specify the dopaminergic H-cell from a pool of Midline Precursors (MPs). A screen of candidate genes expressed in the midline revealed that proper specification of dopaminergic fate required the paired homeobox gene *gooseberry-neuro* (*gsb-n*). Loss of *gsb-n* eliminated the gene that encodes tyrosine hydroxylase (*pale*) from the midline CNS, while ectopic expression of *gsb-n* generated additional dopaminergic neurons. In both cases, the specification of MP1 midline neurons was disrupted. Closer examination revealed that the loss of *gsb-n* generated additional MP1 neurons, while misexpression of *gsb-n* duplicated MP3 at the expense of the MP1 precursor. However, misexpression of *gsb-n* had only a mild effect on posterior MPs. These data suggest that transcription factors arrayed along the A-P axis spatially restrict dopaminergic specification to the anterior of each segment. We showed that the fork-head transcription factor *sloppy-paired* (*slp*) was expressed exclusively in MP1 and MP3. This expression required *wingless*, and mutations in *slp* resulted in additional posterior MPs and loss of MP1 and MP3. This suggests that *slp* segregates anterior MPs from posterior MPs. Thus, MP1 and MP3 initially have a common transcriptional program. What differentiates MP3 from MP1? We showed that ectopic expression of *hedgehog* throughout the midline led to the generation of two H-cells and the loss of MP1 neurons. Consistent with these results, *gsb-n* was ectopically activated in MP1s. We also showed that the transcription factors *lethal of scute*, *tup*, and *BarH1* act downstream of *gsb-n* to activate a dopaminergic program in H-cell. Finally, we observed similar cell fate changes in non-midline lateral CNS derived dopaminergic neurons in each of these mutant backgrounds. Thus, our data suggest that a common set of transcriptional programs specify dopaminergic precursor fate in the Drosophila CNS.

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Combinatorial input from two spatial axes generates neuronal diversity in the *Drosophila* medulla. Ted Erclik, Xin Li, Claire Bertet, June Ng, Claude Desplan. Department of Biology, New York University, New York, NY, USA.

The *Drosophila* medulla is the largest neuropil in the optic lobe and is responsible for the processing of both color and motion detection signals. While over 70 neuronal cell types have been identified in the medulla, little is known about how these neurons are specified. We have performed a transcription factor antibody screen in the medulla and have identified 35 genes that are expressed in subsets of medulla progenitors and neurons. By mapping these transcription factors onto the larval optic proliferation center, the structure from which the adult medulla is generated, we find that neuronal specification in the medulla is the product of the intersection of two spatial axes: (1) in the medio-lateral axis, neuroblasts switch between five distinct fates to generate neurons of different identities. As they age, neuroblasts first express Homothorax (Hth), then Eyeless, followed by Sloppy-paired 1, Dichaete and, finally, Tailless. We find that later neuroblast genes repress the expression of earlier ones; (2) in the dorsal-ventral axis, the neuroepithelial crescent from which the neuroblasts are generated is sub-divided into dorsal (Optix), central (Vsx1) and ventral (Optix+Hedgehog) compartments. While the sequential progression of neuroblasts is identical in each region, the types of neurons that are generated by a given neuroblast are region-specific. We have determined how these two axes intersect to generate diversity in the progeny of Hth neuroblasts. Hth neuroblasts generate Mi1 neurons throughout the larval crescent but Pm3 neurons specifically in the center. In these central neuroblasts, Vsx1 is co-expressed with Hth where it directs the formation of Pm3s; *vsx1* is required for the expression of the Pm3 markers Seven-up and Prospero but not the Mi1 marker Bsh. Thus, Pm3 specification is the product of combinatorial input from both the neuroblast (Hth) and dorsal-ventral (Vsx1) axes. We have extended this analysis to the other neuroblasts and have mapped 11 additional neuronal-types to distinct regions in the larval optic proliferation centers.

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Slit/Robo-mediated axon guidance in *Tribolium* and *Drosophila*: divergent genetic programs build insect nervous systems. Tim Evans, Greg Bashaw. Dept Neuroscience, Univ Pennsylvania Sch Med, Philadelphia, PA.

Roundabout (Robo) family receptors control diverse axon guidance decisions during nervous system development in bilaterian animals. In *Drosophila*, Robo and Robo2 mediate midline repulsion in response to Slit, while Robo2 and Robo3 specify the lateral position of longitudinal axon pathways. Alone among the fly Robos, Robo2 can also promote midline crossing. Notably, *Drosophila* robo2 and robo3 are products of a recent gene duplication, suggesting that their distinct roles in controlling midline crossing and promoting lateral and intermediate pathway formation may be recent evolutionary developments. To gain insight into the evolution of axon guidance receptor functions in insects, we have characterized Slit/Robo-mediated axon guidance in the flour beetle *Tribolium castaneum*, which unlike *Drosophila* has only two Robo receptors: Robo (TcRobo) and the ancestor of Robo2 and Robo3 (TcRobo2/3). Using RNAi, we show that *TcSlit* is required for midline repulsion of axons in the beetle embryonic CNS, and that both *Tribolium* Robos contribute to this activity. Longitudinal axon pathways in the *Tribolium* embryonic CNS form in distinct medial, intermediate, and lateral zones, despite the presence of only two Robos. Individual knockdowns demonstrate that beetle Robos have specialized axon guidance functions: *TcRobo* is dedicated to midline repulsion, while *TcRobo2/3* also regulates longitudinal pathway formation. *TcRobo2/3* knockdown mimics aspects of both *Drosophila* robo2 and robo3 mutants, suggesting that these two genes in flies share a role that is performed by a single ancestral gene in other insects. In addition, TcRobo2/3 is unable to promote midline crossing of *Drosophila* axons, suggesting that the pro-crossing role of fly Robo2 arose after its divergence from Robo3. Together, our results suggest that the functional diversification of Robo receptors during insect evolution has involved gene duplication and both loss and gain of axon guidance activities, and reveal that modern insects deploy divergent genetic programs to control equivalent axon guidance decisions during development.

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Axonal branching and synaptic connectivity of mechanosensory axons. Derya Ayaz, Dan Dascenco, Olivier Urwyler, Dietmar Schmucker. Vesalius Research Center, VIB, Leuven, BELGIUM.

During development neurons are precisely organized to connect with each other and give rise to the complex pattern of the nervous system. The wiring complexity is highly increased by the ability of a single neuron to connect with multiple target neurons by forming specific axonal and dendritic branches. In contrast to research on axon guidance, much less is known about basic mechanisms underlying spatially restricted axon branching. How **axon branching** is induced molecularly and how external stimuli influence this process **remains largely elusive**. We have established an ***in vivo* system** for studying axon branching using mechanosensory neurons that exhibit an invariant genetically hardwired branching morphology in the ventral nerve cord. We will present a time-lapse *in vivo* analysis of axon branching of afferent projections within the pupal CNS. Our analysis reconstructs the developmental sequence of sensory axon branching and document for the first time growth cone dynamics, spatially restricted axonal sprouting, and interstitial branch formation within the pupal CNS. Moreover we will present a systematic genetic analysis of key regulators of axon branching. Currently we are focusing on a novel kinase, distantly related to STE-20 like kinases, which is essential for sensory axon branching. Based on this we will discuss a new molecular pathway that selectively controls axonal branching of mechanosensory neurons.

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Defining microRNA function during synapse development using the Drosophila microRNA sponge. Elizabeth M. McNeill, Carlos Loya, Tudor Fulga, David Van Vactor. Cell Biology, Harvard Medical School, Boston, MA.

MicroRNAs (miRNAs) have emerged as key candidates to regulate the deployment of proteins that control synaptic morphogenesis and function. However, our understanding of the structural, physiological and cellular mechanisms by which miRNAs control synapse development remains relatively unexplored. We have generated a library of Drosophila miRNA sponge (miR-SP) transgenics, which we have screened to determine the effects of miRNA depletion to synapse structure. This screen has confirmed many of the previously known miRNA players at the synapse as well as begun to identify some unique miRNAs involved in synaptic morphogenesis. Using a miR-SP targeting the conserved miRNA, miR-8, we have discovered that although expressed on both sides of the synapse, miR-8 is an essential postsynaptic regulator of neuromuscular junction (NMJ) differentiation, subsynaptic reticulum elaboration, and physiology. A key to this postsynaptic-specific regulation of NMJ development is the miR-8 target gene Enabled (Ena), a well-established regulator of actin dynamics. Using a combination of microarray, in silico, genetic and biochemical tools, we find that muscle-specific repression of Ena accounts for most of miR-8's potent regulation of NMJ ultrastructure and morphogenesis. Moreover, structural mutant analysis of the conserved domains in mammalian Ena (Mena) suggest that the C-terminal Ena/VASP homology 2 (EVH2) domain, which is comprised of a G-actin binding, F-actin binding and coiled coil motif, is both necessary and sufficient for postsynaptic Ena localization and function. Consistent with the known antagonism between Ena/VASP proteins and actin capping proteins in F-actin dynamics at the molecular level, we see a partial rescue of Ena overexpression with capping protein beta gain-of-function at the synapse. Our study reveals a tissue-specific function for miR-8 in synapse formation through the control of the postsynaptic actin cytomatrix, and provides new insight into the regulatory repertoire of miRNA in nervous system development.

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Nucleotide sugar transporter Meigo regulates both dendrite and axon targeting of synaptic partners through Ephrin signaling in the olfactory system. Sayaka Sekine[1], Liang Liang[6], Miki Yamamoto-Hino[4], Satoshi Goto[4], Hideyuki Okano[4], Liqun Luo[5,6], Masayuki Miura[1,2], Takahiro Chihara[1,3]. 1) Genetics, Grad Sch Pharm Sci, Univ. Tokyo, Japan; 2) CREST; 3) PRESTO; 4) Dept. Physiol, Keio Univ, Tokyo, Japan; 5) HHMI; 6) Dept. Biol. Stanford Univ, CA.

The wiring of neural network results from accurate synaptic matching enabled by precise targeting of axons and dendrites. During the development of olfactory system, the axons of each first-order olfactory receptor neuron (ORN) and the dendrites of each second-order projection neuron (PN) target one of ~50 glomeruli in the antennal lobe (AL), resulting in one-to-one connections of proper synaptic partners. To elucidate the mechanism of dendrite targeting of PNs, we performed MARCM-based genetic screen and isolated a mutant, *meigo* (*medial glomeruli*). The dendrite of *meigo*$^{-/-}$ PN spilled over from destined glomeruli, and mistargeted to the medial side of the AL. Interestingly, axon targeting of *meigo*$^{-/-}$ ORNs was also medially shifted in the AL. The responsible gene (*meigo*) encodes a nucleotide sugar transporter that is located at ER. These results suggest that Meigo is cell-autonomously required in synaptic partners for neuronal targeting along the mediolateral axis in the AL, probably by regulating the glycosylation of cell surface proteins. To identify the cell surface proteins, we performed genetic modifier screen and found that overexpression of *ephrin* in *meigo*$^{-/-}$ PNs significantly suppresses the dendrite mistargeting phenotype. Downregulation of *ephrin* in PNs exhibited the dendrite spillover, which is similar to that in *meigo*$^{-/-}$ PNs. In contrast to medially shifted dendrites in *meigo*$^{-/-}$ PNs, overexpression of *ephrin* caused dendrite mistargeting to the lateral side of the AL. Moreover, biochemical and genetic analyses revealed that efficient *N*-glycosylation of Ephrin requires Meigo in S2 cells, and Ephrin *N*-glycosylation is essential for its full function in neuronal targeting *in vivo*. Thus, *meigo* regulates neuronal targeting of synaptic partners in the AL through facilitation of Ephrin signaling partly by *N*-glycosylation.

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Ly6 related proteins in Drosophila Blood Brain Barrier. Mubarak Hussain Syed[1], Alice Krudewig[2], Daniel Engelen[1], Tobias Stork[3], Christian Klambt[1]. 1) Institute for neurobiology, University of Muenster, Muenster, NRW, Germany; 2) Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland; 3) University of Massachusetts Medical School, Department of Neurobiology, 362 Plantation Street, LRB 740, Worcester, MA 01605.

The Blood Brain Barrier (BBB) is a highly specialised structure, which ensures the constant ionic microenvironment needed for reliable neuronal functions. The Drosophila BBB is formed by a thin layer of subperinurial glial cells, which cover the entire nervous system and posses laterally localised homotypic septate junctions. Disruption of these septate junctions leads to a defective BBB and fluorescent probes can easily penetrate into the CNS. In order to find new components of BBB, we performed dextran uptake screen and identified mutants in coiled and leaky having severe BBB defects. coiled encodes a GPI linked cell surface homophilic adhesion molecule, which is a member of the Ly6 super family, and is predicted to form three-finger folds. Coiled and Leaky are expressed in all septate junction forming cells, particularly in the BBB forming subperinurial glial cells. The kinetics of Dextran uptake in coiled mutants is similar to nrx IV mutants showing a severely affected BBB. Likewise leaky mutants result in a severely compromised BBB. In coiled and leaky mutants septate junction molecules like Neurexin IV, Discs large and Coracle are mislocalized basally. EM analysis of coiled mutants revealed the lack of septate junctions in the subperineurial glia. We could rescue septate junction assembly and dye penetration phenotype in a cell specific manner implying that Coiled functions cell autonomously in subperineurial glial cells. Clonal studies show that symmetric distribution of Coiled is required in both the cells participating in septate junction. Further analysis of coiled and leaky will be presented.

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Gene regulatory networks controlling hematopoietic progenitor niche cell production and differentiation in the *Drosophila* lymph gland. Tsuyoshi Tokusumi, Yumiko Tokusumi, Douglas A. Shoue, Robert A. Schulz. Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.

The lymph gland is a specialized organ for hematopoiesis, utilized during larval development in *Drosophila*. This tissue is composed of distinct cellular domains populated by blood cell progenitors (the medullary zone), niche cells that regulate the choice between progenitor quiescence and hemocyte differentiation [the posterior signaling center (PSC)], and mature blood cells of distinct lineages (the cortical zone). Cells of the PSC express the Hedgehog (Hh) signaling molecule, which instructs cells within the neighboring medullary zone to maintain a hematopoietic precursor state while preventing hemocyte differentiation. As a means to understand the regulatory mechanisms controlling Hh production, we characterized a PSC-active transcriptional enhancer that drives *hh* expression in niche cells. Our findings indicate that the GATA factor Serpent (Srp) is essential for *hh* activation in niche cells, whereas the Suppressor of Hairless and U-shaped transcriptional regulators prevent *hh* expression in hemocyte progenitors and differentiated hemocytes. Furthermore, Srp function is required for the proper differentiation of niche cells. With the *hh-GFP* transgene serving as a sensitive marker for the PSC, we initiated an *in vivo* RNAi screen to discover genes whose function was required for the correct production of niche cells. The Gal4 driver employed was the *P85col-Gal4* strain and it has been used thus far to express 820 RNAi sequences from VDRC. Thorough this screen, we have identified ~100 genes regulating PSC formation and function. Among them, we focused on two important mechanisms: Brahma complex and Akt1/Tor pathway. Brahma complex, which is an ATP-dependent chromatin remodeling complex, can genetically interact with *srp* to regulate *hh* gene expression and filopodia formation. On the other hand, Akt1/Tor pathway can regulate PSC cell growth depended on nutrient conditions. Our results demonstrate novel regulatory mechanisms at work within the stem-like niche.

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***hedgehog* regulates self-renewal and niche competition in the *Drosophila* testis.** Marc Amoyel[1], Michael Burel[1], Erika Bach[1,2]. 1) Pharmacology, New York University School of Medicine, New York, NY; 2) Kimmel Stem Cell Center.

Stem cells are critical for tissue regeneration during adulthood. The *Drosophila* testis is one of the best characterised stem cell-niche systems, as both the niche and stem cells are anatomically and molecularly well defined and some signals controlling self-renewal have been established. Somatic hub cells comprise the niche, which maintains two stem cell populations, germ-line stem cells (GSCs) and somatic cyst stem cells (CySCs). GSC self-renewal requires activation of Bone Morphogenetic Protein (BMP) receptors by BMP ligands produced by the hub and CySCs, while CySC self-renewal requires activation of the JAK/STAT pathway by Unpaired (Upd), produced only by hub cells. The requirement of additional signalling pathways in self renewal and niche occupancy of testis stem cells is not known. While it is known that Hh is expressed in niche cells, a role for Hh in testis stem cells has not been reported. We find that Hh signalling is detected in CySCs but not in GSCs. Consistent with this we find that Hh signalling is required cell-autonomously in CySCs but not in GSCs for self-renewal. To determine if Hh affects activity of Stat92E, we examined JAK/STAT pathway readouts in Hh mutant CySCs and vice versa. We confirmed that each pathway is not affected by disruption of the other. This suggests that Hh and JAK/STAT pathways act in parallel in CySC self-renewal, which we confirmed by clonal epistasis experiments. We also observed that CySCs with sustained Hh signalling compete with resident CySCs and GSCs and colonise the niche by displacing both wild-type populations. CySCs with increased Hh proliferate faster than wild-type CySCs. Experimentally accelerating the cell cycle in testis stem cells alters population dynamics at the niche, leading to preferential accumulation of the highly proliferative population at the expense of the wild-type. These data support a model in which Hh regulates cell cycle progression of CySCs, which is critical for regulating homeostasis at the niche.

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A histone demethylase dUTX regulates crosstalk among different cell types in the *Drosophila* testis stem cell niche. Xin Chen, Lama Tarayrah. Dept Biol, Johns Hopkins Univ, Baltimore, MD.

Adult stem cells reside in microenvironments, or niches, that provide signals from surrounding cells to the stem cells to prevent differentiation. The *Drosophila* male germline stem cell (GSC) niche is considered to be one of the best characterized niches in which GSCs associate with two types of somatic cells: hub cells and cyst stem cells (CySCs). It has been reported that somatic cells play important roles to maintain GSCs through the JAK-STAT signaling pathway. Here we show that dUTX contributes to the maintenance of male GSC niche by affecting all three cell types in the niche (i.e. GSCs, CySCs and hub cells). dUTX is the sole *Drosophila* homolog of mammalian UTX, which has been shown to act as a specific demethylase of trimethylation on lysine 27 of histone H3 (H3K27me3) *in vivo*. We found that GSCs from *dUTX* mutant testes have a significantly lower mitotic index and a higher rate of misoriented centrosomes, suggesting a decrease in GSC activity. In addition, the hub size and structure are abnormal in *dUTX* mutant testis. Interestingly, the hub phenotype could be recapitulated by knocking-down dUTX in germ cells, suggesting normal function of dUTX is required in germ cells to maintain the hub architecture. Surprisingly, not only the hub structure changes, a transcription factor zinc finger homeodomain-1(Zfh-1), which is normally turned on in CySCs and early cyst cells but not in hub cells, is ectopically expressed in *dUTX* mutant hub cells. Further analysis indicated that dUTX acts in CySCs to maintain the silenced state of Zfh-1 in hub cells. Finally, we found that dUTX may directly regulate the chromatin state of JAK-STAT signaling pathway components to coordinate behavior of different cell types in the testis niche. Together, our data demonstrate that a chromatin factor regulates the key signaling pathway and crosstalk among different cell types in the *Drosophila* testis stem cell niche: in addition to the known signaling from hub and CySCs to GSCs, both GSCs and CySCs can send feedback to hub cells to maintain the integrity and functionality of the niche.

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***robo2* is a JAK-STAT Target that Controls Stem Cell Maintenance in the *Drosophila* Testis Stem Cell Niche.** Rachel R. Stine, Erika L. Matunis. Johns Hopkins Sch Med, Baltimore, MD.

Adult stem cells are essential for tissue regeneration and must be carefully regulated by signals from their surrounding niche. The *Drosophila* testis contains a well-characterized stem cell niche consisting of a cluster of somatic hub cells surrounded by germline stem cells (GSCs) and somatic stem cells called cyst stem cells (CySCs). Although JAK-STAT and BMP signals are known to be secreted from the hub, few other hub signals have been identified. We find that *slit*, the only ligand for the *Drosophila* Robo/Slit pathway, is expressed specifically in hub cells. Slit signals via the Robo receptors to mediate axon guidance, cell migration and cell adhesion in *Drosophila* and mammals, but Robo/Slit signaling has not been previously studied in the testis. We show that the Slit receptor *robo2* is expressed in the somatic cells of the testis apex and that expression of *robo2* depends on JAK-STAT signaling. A luciferase-based JAK-STAT response assay further indicates that *robo2* is a direct target of Stat92E. Mosaic analysis indicates that *robo2* mutant CySCs are rapidly lost from the niche; this phenocopies *E-cadherin* mutant CySCs, suggesting that Robo2 may promote CySC adhesion to the niche. Multiple additional Robo/Slit pathway members are expressed in the testis apex including an additional receptor, *robo*, and several downstream effectors including *Abelson kinase*. As an important regulator of CySC maintenance, JAK-STAT is known to control genes important for self-renewal. We now show that JAK-STAT may also regulate CySC adhesion through its newly discovered integration with Robo/Slit signaling. Further investigation will reveal if these two pathways interact in other tissues where Robo/Slit signaling functions.

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A model for formation of the follicle stem cell niche in the Drosophila ovary. Stephanie Vlachos[1], Ryan Conder[2], Todd Nystul[3], Nicholas Harden[1]. 1) Molec Biol & Biochem, Simon Fraser Univerisity, Burnaby, BC, Canada; 2) Institute of Molecular Biotechnology, Dr. Bohr Gasse 3, 1030, Vienna, Austria; 3) Department of Anatomy, University of California, San Francisco, CA 41943.

The germarium in the Drosophila ovary is a popular structure for the study of stem cell niche formation. In the germarium, two or three germline stem cells (GSCs) located at the anterior tip in region 1 give rise to germline cysts that move posteriorly and encounter two follicle stem cells (FSCs) on opposite sides of the germarium at the region 2a/2b border, which encapsulate one cyst at a time in a monolayer of somatic follicle cells. The FSC niche is an example of a dynamic somatic stem cell niche in which the stem cell microenvironment demonstrates characteristics of both classic and flexible stem cell niches. Using females mutant for the p21-activated kinase Pak, we show that the FSC niche can encapsulate cysts when mispositioned in the germarium. Furthermore, we provide evidence that the number of FSC niches is increased in pak mutant germaria, thus enabling the encapsulation of two or even three cysts at a time. We propose a model for formation of the FSC niche in which the germline cyst determines the position of the niche.

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Piwi and the Polycomb Group Proteins interact to regulate Drosophila ovarian germline. Jamy C. Peng, Na Liu, Haifan Lin. Department of Cell Biology, Yale Stem Cell Center, Yale University School of Medicine, New Haven, CT.

We use *Drosophila* ovarian germline, a classic model system of tissue stem cells, to probe the molecular relationship between Piwi and Polycomb Group proteins. Piwi and its associated piRNAs epigenetically regulate the niche and the intrinsic mechanisms within stem cells to regulate germline stem cell division and differentiation. Despite detailed characterization of Piwi functions, its mechanistic action remains elusive. The Polycomb Group proteins are epigenetic modifiers that regulate key developmental genes to ensure proper stem cell differentiation. Using *Drosophila* genetic assays, we showed that Piwi and the Polycomb Group complexes PRC1 and PRC2 functionally interact to maintain germline stem cell division and germline development. Biochemical analyses revealed that Piwi physically associates with PRC2 core subunits, and that Piwi and PRC2 form a molecular complex in ovarian extract. Expression analysis determined that the Polycomb Group complexes participate in the Piwi-piRNA pathway to regulate retrotransposons, thereby stabilizing germline genome. Chromatin immunoprecipitation analysis indicated that Piwi binds selected Polycomb Group target sites to inhibit Polycomb Group functions; this promotes RNA Polymerase II localization at these sites. Comparative epigenomic analysis of wild type and *piwi* mutant ovarian germline indicated that Piwi specifically impacts Polycomb Group target genes that are regulators of transcription, developmental processes, and metabolism. These data led to our model that Piwi interacts with Polycomb Group proteins to regulate gene expression profiles required for proper germline stem cell maintenance and differentiation. Our study integrates Piwi and Polycomb Group proteins into a molecular pathway which regulates germline stem cell maintenance and differentiation. This work is funded by an NIH NICHD Pioneer Award to HL (DP1 OD006825) and an American Cancer Society Postdoctoral Fellowship to JP (115878-PF-08-160-01-DDC).

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Insulin levels control Delta-Notch signaling in the *Drosophila* female germline stem cell niche via the regulation of FOXO on *fringe*. Sheng-An Yang, Hwei-Jan Hsu. Institute of Cellular and Organismic Biology, Academia Sinica, Taipei City, Taiwan.

Stem cells reside in a specialized microenvironment, or the niche, which regulates stem cells for their participation in tissue maintenance, regeneration and repair. A functional niche can provide both physical contact and secreted factors to regulate stem cell retention and self-renewal, while the dysregulated niche impairs stem cell function. The stem cell niche also plays a role to integrate niche-local and systemic signals that mediate the balanced response of stem cells to the needs of organisms. How the niche itself is regulated and how these signals are merged in the niche, however, are largely unknown. We have previously shown that insulin nutrient-sensing signaling directly controls the competence of the *Drosophila* female germline stem cell (GSC) niche to respond to Notch ligands, and thereby the niche is maintained. Here, we further dissect the molecular and cellular mechanisms underlying these processes. We show that Notch activity in cap cells (a major component of the niche) is positively or negatively controlled by *fringe*. *fringe* is required for Notch activation, but excessive *fringe* also disturbs Notch activation. Further, *fringe* is up-regulated by FOXO in cap cells to suppress Notch activation when insulin signaling is inactivated, and resulting in loss of cap cells, and thus loss of GSCs. Additionally, *fringe* expression is also regulated by mouse *FOXO1*, suggesting that this regulation may be evolutionally conserved. Finally, we demonstrate that the Notch ligand, Delta, produced within the niche predominately activates Notch signaling in cap cells. Our results reveal that FOXO-Fringe regulation serves as a bridge to link insulin signaling and Notch signaling pathways in GSC niche in response to nutrition and highlight complex interactions between niche-local and systemic signals for proper stem cell niche function.

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Tissue specific analysis of chromatin marks identify temporal enhancer activity in development

. Robert P. Zinzen[1], Stefan Bonn[1], Charles Girardot[1], E. Hilary Gustafson[1], Alexis Perez Gonzalez[2], Nicolas Delhomme[1], Yad Ghavi-Helm[1], Bartek Wilczynski[1], Andy Riddell[2], Eileen E.M. Furlong[1]. 1) Genome Biology Unit, EMBL, Heidelberg, Germany; 2) Flow Cytometry Core Facility, EMBL, Heidelberg, Germany.

By using a new method to batch-isolate tissue-specific chromatin followed by immunoprecipitation (BiTS-ChIP), we uncovered mesoderm-specific signatures for histone modifications and Pol II positioning from developing embryos. We find that enhancers exhibit heterogeneous chromatin/Pol II states, and that specific states are highly correlated with spatio-temporal enhancer activity. While H3K4me1 enrichment provides no information on the activity state of regulatory regions, other features such as H3K27ac, H3K79me3 and in particular Pol II enrichment mark active enhancers with temporal precision. The uncovered enhancer signatures allowed for faithful *de novo* identification of new regulatory regions, which direct activity *in vivo* in the correct tissue at the predicted time. This cell type-specific data therefore identifies enhancers in active use during development, which will be instrumental in deciphering *cis*-regulatory networks.

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The transcriptional repressor Snail functions as activator during *Drosophila* mesoderm development. Martina Rembold[1], Lucia Ciglar[2], Jorge Omar Yáñez Cuna[3], Charles Girardot[2], Robert Zinzen[2], Martina Braun[2], Alexander Stark[3], Eileen Furlong[2], Maria Leptin[1]. 1) Institute of Genetics, University of Cologne, Cologne, Germany; 2) European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; 3) Research Institute of Molecular Pathology (IMP), Vienna, Austria.

Gastrulation and the patterning of the mesoderm anlage in *Drosophila* are controlled by the activities of the transcription factors Twist and Snail. Twist activates the expression of genes necessary for the morphogenetic changes leading to mesoderm internalisation and determination. Although Snail is known as dedicated repressor delimiting the expression of ectodermal genes, increasing evidence indicates that it might also act as activator of transcription. Whether Snail's activator function is direct or indirect is under debate.

We show that Snail directly activates the expression of mesodermal genes *in vitro* and *in vivo*. Chromatin immunoprecipitation and microarray (ChIP-on-Chip) analysis for Twist and Snail combined with expression analysis in *twist* and *snail* mutant embryos allowed us to identify direct target genes on a genome-wide basis. Snail shows extensive co-binding with Twist to active mesodermal enhancers for genes such as *Mef2*, *tinman* and *htl*, whose expression is lost in *snail* mutant embryos. In cell culture Snail acts as co-activator of Twist by augmenting Twist induced expression of luciferase by up to two-fold. In *snail* mutant embryos *lacZ* expression driven by these enhancers is lost. This effect depends on Snail direct binding to the enhancer, as mutation of two snail motifs in the *Mef2-I-D[L]* enhancer is sufficient to abrogate both the co-stimulatory effect in luciferase assays as well as *lacZ* expression *in vivo*. We are currently investigating the defining features of enhancers that are activated versus those that are repressed by Snail and have uncovered a new factor involved in this regulator switch.

Rembold, M and Ciglar, L contributed equally to this work.

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Yan binding at the eve locus confers robustness. Jemma L. Webber, Lauren Cote, Jie Zhang, Ilaria Rebay. Ben May Department for Cancer Research, University of Chicago, Chicago, IL.

The Ets transcriptional repressor Yan functions as part of a conserved network downstream of receptor tyrosine kinase (RTK) signaling. This network displays switch-like behavior, transitioning from a high-Yan to a low-Yan state following RTK activation. To ensure a reliable all-or-none response, such a system must also include mechanisms to buffer gene expression against developmental noise that might induce inappropriate oscillations between states. In our analysis of the genome-wide chromatin binding profile of Yan, we noticed that at developmentally important target genes, a significant fraction of Yan occupancy occurs at regions of high peak density that span multiple kilobases. Focusing on the Yan target gene even-skipped (eve), to which we mapped several high density Yan bound elements, including one that includes the previously identified muscle/heart enhancer (MHE), we used BAC recombineering of a genomic Eve-YFP construct to examine the contribution of individual Yan-bound regulatory regions to robustness of mesodermal Eve expression. Our results reveal a level of functional redundancy between the regions and suggest that robust regulation of mesodermal Eve expression requires Yan input at multiple elements across the locus, not just at the MHE. Mechanistically, we observe changes in Yan occupancy that are consistent with long-range interactions between elements spaced more than 10kb apart, suggesting that coordinated Yan occupancy at multiple sites across the eve locus may be critical to ensure robust expression. We are currently investigating whether Yan SAM-domain mediated self-association can facilitate such long-range interactions between enhancers. Extrapolating from our analysis at the eve locus, we speculate that the complex chromatin occupancy signatures observed with Yan may provide a filter against noise to ensure robust regulation of gene expression across development.

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Why do transcriptional repressors recruit more than one corepressor? Priyanka Upadhyai, Gerard Campbell. Biological Sciences, University of Pittsburgh, Pittsburgh, PA.

Transcriptional repressors function primarily by recruiting corepressors (CoRs), accessory proteins that antagonize transcription by modifying chromatin structure. Although a single CoR might be sufficient, many repressors, including the *Drosophila* protein Brinker, recruit multiple CoRs, with Brk recruiting the CoRs, CtBP and Groucho, in addition to possessing a third repression domain (3R). Possible reasons for this are: (a) Quantitative: multiple CoRs provide more activity; (b) Qualitative: different CoRs have distinct activities; (c) Availability: one CoRs may be inactive or not expressed in some cells, (d) Quality control: more than one protects against stochastic events. Previous studies indicated Gro is sufficient for Brk to repress targets in the wing, questioning why it should need to recruit CtBP, a 'short-range' CoR compared to Gro which can function over longer distances. To resolve this question we have generated a series of *brk* mutants in which the CtBP interaction motif (CiM), Gro interaction motif (GiM) and 3R are mutated individually or in combination. This was achieved by generating a *brk* knockout, replacing the coding region with an *attP* ΦC31 bacteriophage integration site. Modified/mutated forms of *brk* were then integrated into the *attP* site essentially replacing the endogenous gene with these forms. Analysis of these mutants reveals that recruitment of Gro alone is sufficient for Brk to make a morphologically wild-type fly, but in the absence of a CiM and 3R most animals die as embryos. Gro, however, is not sufficient for full fertility and CtBP or the 3R domain is required for normal oogenesis. Thus, Gro is not sufficient for Brk activity in specific situations outside of the wing. These results can be explained by recent studies showing that, although it is ubiquitously expressed, Gro activity is downregulated by Receptor Tyrosine Kinase activity which occurs in cells in which Brk functions during embryogenesis and oogenesis. Our results are consistent with Brk needing to recruit more than one CoR because its primary CoR, Gro, is not available in all cells.

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Transcriptional arithmetic during gene regulatory evolution. Albert J. Erives. Dept of Biology, University of Iowa, Iowa City, IA, USA.

Morphogen gradients allow cells to infer their positions in a multi-cellular body. These morphogenic systems require two components: (1) a concentration gradient of a substance over a field of cells; and (2) a mechanism whereby different genes can be induced when the concentration exceeds a gene-specific threshold level, which is encoded in *cis* at each responding locus. We previously documented an evolutionary molecular mechanism by which concentration threshold-specific responses to the Dorsal morphogen are encoded in Neurogenic Ectoderm Enhancers (NEEs) during *Drosophila* evolution. Here, I consider direct consequences of this model given known laws of additivity for transcriptional enhancers, which may act collectively at a locus. I enumerate all evolutionary paths by which an existing threshold response may be modified with a single mutational step, and find that these possible pathways depend on the directionality of threshold selection (higher versus lower concentration thresholds). This unexpected asymmetry suggests that partially-redundant enhancer activities will be frequently generated as an indirect byproduct of the evolutionary maintenance of threshold-sensitive regulatory DNAs. These results show that the arithmetic logic of transcriptional integration is sufficient to explain many apparent cases of redundant enhancer activities at a locus even when selection for robustness is minimal.

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Ancestral sequence reconstruction of the *even-skipped* stripe 2 enhancer in *Drosophila*. Carlos Martinez[1,2], Ah-Ram Kim[1,3], Joshua Rest[4], Kenneth Barr[5], Michael Ludwig[1,2], Kevin White[2], John Reinitz[1,2,6,7]. 1) Ecology & Evolution, UC, Chicago, IL; 2) Chicago Center for Systems Biology, UC, Chicago, IL; 3) Biochemistry & Cell Biology, SUNY, Stony Brook, NY; 4) Ecology & Evolution, SUNY, Stony Brook, NY; 5) Genetics, Genomics, & Systems Biology, UC, Chicago, IL; 6) Statistics, UC, Chicago, IL; 7) Molecular Genetics & Cell Biology, UC, Chicago, IL.

We have developed a novel computational approach for the custom design of complex *cis*-regulatory sequences capable of expressing in arbitrary patterns, as well as methods for predicting the evolution and putative ancestral sequences of extant enhancers in *Drosophila*. Our methodology involves the use of a feed-forward transcriptional model, capable of predicting gene expression patterns directly from enhancer sequence, as a tool for enhancer design and to provide a functional constraint for predicting enhancer evolution. In the former case, enhancer design was achieved through the use of simulated annealing in conjunction with a transcriptional model in order to efficiently search the sequence space for novel enhancers having the desired expression pattern. For the latter, Bayesian inference was used to generate a set of possible ancestral *eve* stripe 2 enhancer (S2E) sequences for a subset of the internal nodes of the *Drosophila* phylogenetic tree. Candidate ancestral sequences were selected for synthesis and experimental validation by checking the model predicted expression patterns of each sequence against a reference *eve* stripe 2 expression pattern from *D. melanogaster*. In addition, we synthesized and tested putative ancestral sequences predicted to lie along neutral evolutionary pathways between functionally conserved enhancer elements, such that at all points along the path the *eve* stripe 2 pattern is maintained.

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Evolution of Transcriptional Regulation in Early Embryos of the Drosophila Genus. Mathilde PARIS[1], Tommy KAPLAN[1], Susan LOTT[1], Xiao-Yong LI[2], Jacqueline VILLALTA[2], Michael EISEN[1,2]. 1) Molecular and Cellular Biology, QB3 Institute, BERKELEY, CA; 2) Howard Hughes Medical Institute, University of California Berkeley, Berkeley, CA.

To better characterize how variation in regulatory sequences drives divergence in gene expression, we undertook a systematic study of transcription factor binding and gene expression in four species that sample much of the diversity in the 60 million-year old genus Drosophila: D. melanogaster, D. yakuba, D. pseudoobscura and D. virilis. We used ChIP-Seq to examine the genome-wide binding of four transcription factors (BCD, HB, GT and KR) regulating segmentation along the anterior-posterior axis. As expected, genome-wide transcription factor binding divergence correlates with phylogenetic distance; regions more highly bound in D. melanogaster were more likely to be bound in other species, and to have similar overall levels of binding; and increases/decreases in binding were associated with gain/loss of transcription factor binding sites. To examine the consequences of these changes, we used single embryo mRNA-Seq to measure gene expression in sex individual blastoderm embryos of each species. Surprisingly, we found relatively few changes in gene expression, suggesting that differences in sequence and binding have limited effect on gene expression or act in a compensatory manner to maintain the overall expression levels of regulated genes. Using in situ hybridization, we also compared the expression pattern of genes showing variation in nearby regulatory TF binding among species. Finally, we used an evolutionary model of quantitative traits to link the evolution of gene expression with the evolution of regulatory TF binding. This analysis unravels the evolutionary links between various levels of transcriptional regulation, from DNA sequence to gene expression through protein binding.

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The Drosophila vesicular monoamine transporter mutation provides a sensitized system to identify drugs that regulate aminergic neurotransmission. Hakeem O. Lawal, Traci Biedermann, Filmon Mehanzel, David E. Krantz. Psychiatry & Biobehavioral Sci, Univ California, Los Angeles, Los Angeles, CA.

Most current treatments for neuropsychiatric disorders such as Parkinson's disease, depression, and attention deficit disorder target proteins expressed at aminergic synapses. However, methods to identify new molecular targets are limited. Using a mutation in the Drosophila vesicular monoamine transporter (dVMAT) as a sensitized genetic background, we screened for novel drugs that might potentiate aminergic signaling. In flies, as well as mammals, VMATs are required for packaging into synaptic vesicles all monoamine neurotransmitters, including dopamine, serotonin and in flies, octopamine. dVMAT mutants show several behavioral deficits including reduced larval locomotion. We screened a panel of 1039 drugs and identified 40 compounds that increase larval locomotion. In a secondary screen comparing the effects of dVMAT null and hypomorphic alleles, we showed that 7 drug are likely to act via increasing octopamine release at presynaptic sites. A second set of drugs acts post-synaptically to activate octopamine receptors. A third set of drugs is likely to act via cholinergic pathways, consistent with the proposed cholinergic input to glutamatergic motoneurons. Our screen represents an important new method to identify new drugs and targets for the treatment of neuropsychiatric disorders. In addition, it provides a new set of tools for dissecting the molecular mechanisms that control larval locomotion.

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A non-binary expression approach to generating brain-dopamine deficient Drosophila. Karol Cichewicz[1], Magali Iché-Torres[2], Serge Birman[2], Jay Hirsh[1]. 1) Biology, University of Virginia, Charlottesville, VA; 2) CNRS, ESPCI, Paris.

Drosophila tyrosine hydroxylase (DTH, *ple*), encoding the rate limiting enzyme in dopamine 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 (*ple*) null mutation, generating healthy flies with normal lifespan. These flies have undetectable brain dopamine, and show a number of behavioral defects that elucidate novel roles for neural dopamine (Hirsh et al., 2010; Riemensperger et al, 2010). These flies were constructed with the GAL4:UAS binary expression system, such that further genetic manipulations with GAL4:UAS are difficult. Here we present an approach, in which we switch off DTH expression in CNS without the use of binary expression tools, allowing for subsequent rescue using GAL4-UAS in dopamine neuron subsets. The DTH FS mutations were recombineered into a large genomic BAC clone, which was integrated site-specifically into a 3rd chromosome, and then recombined onto a ple mutant background. BAC plasmids containing the wild type 20kb DTH gene as well as DTH FS successfully rescued *ple* lethality, showing that all DTH cis-regulatory elements are contained within this segment. Our preliminary data shows dramatic reduction of activity and female infertility in flies rescued by DTH FS BAC the latter of which is rescued by L-DOPA feeding. The observed female sterility indicates that the dopamine deficiency is likely more severe than with the initial GAL-UAS approach. With this rescue background, dopamine neuron subset selective GAL4 drivers, in conjunction with UAS-DTH, will be used to elucidate the behavioral roles of dopamine in defined brain regions.

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Ryanodine receptor in neurons mediates volatile anesthetic sensitivity of Drosophila. Shuying Gao, David Sandstrom, Qun Gu, Robert Scott, Howard Nash. Laboratory of Molecular Biology, National Institute of Mental Health, NIH, Bethesda, MD.

Volatile anesthetics produce a profound and reversible alteration in the state of arousal in all motile metazoans that have been tested, yet few target molecules have been identified and validated behaviorally. Previous work in the lab has indirectly implicated the Drosophila ryanodine receptor (dRyr), an ion channel that governs Ca^{2+} release from the endoplasmic reticulum, as a prominent component of a gene network that controls the response to the volatile anesthetic halothane. Using an assay that evaluates the righting/climbing reflex, we found that heterozygous dRyr mutations conferred a strong and dominant resistance to halothane and weaker resistance to three other volatile anesthetics: isoflurane, enflurane, and sevoflurane. Addition of a transgenic copy of dRyr genomic DNA to a wild-type strain increased sensitivity to halothane. dRyr regulating halothane sensitivity in a gene dosage-dependent manner demonstrates that dRyr is a limiting factor for halothane sensitivity. Altering key residues of dRyr can increase halothane sensitivity. The function of dRyr in neurons, but not in muscle, is required for normal halothane sensitivity. RNAi knockdown dRyr in the nervous system induced halothane resistance. Conversely, expressing dRyr under the control of a panneuronal driver was sufficient to rescue normal halothane sensitivity in a mutant background. To determine whether anesthetics act directly on dRyr, anesthetic-induced Ca^{2+} flux was measured using Ca^{2+} -sensitive dyes and flow cytometry in Sf9 cells stably transfected with dRyr. Although untransfected cells were completely insensitive to anesthetics, dRyr-expressing cells responded in a concentration-dependent way. Halothane also induces $^{Ca2+}$ transients and hyperpolarization in larval RP2 motoneurons. This rise in Ca^{2+} correlated with hyperpolarization was blunted by expression of dRyr RNAi. Thus, Ryr function in neurons is critical for anesthetic responsiveness, and its expression in heterologous cells confers anesthetic sensitivity, suggesting that Ryr is a bona fide target of volatile anesthetic action.

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***stallone* and *balboa* are DEG/ENaC genes required for mechanical nociception.** Stephanie Mauthner[1], Richard Hwang[2], Jason Caldwell[3], W. Daniel Tracey[1,2,3]. 1) Univ Prog in Genetics and Genomics; 2) Dept of Neurobiology; 3) Dept of Anesthesiology, Duke University, Durham, NC.

Drosophila larvae respond to potentially tissue-damaging stimuli with nocifensive escape locomotion (NEL). NEL is a stereotyped withdrawal behavior in which the larva rotates in a "corkscrew" pattern distinct from normal locomotion and is triggered by noxious heat (>39oC) or noxious mechanical (>30mN) stimuli. The class IV multidendritic (md) neurons are the polymodal nociceptors responsible for triggering NEL. Recent evidence suggests that the *pickpocket* (*ppk*) gene, a degenerin/epithelial sodium channel (Deg/ENaC) subunit, is involved in the mechanotransduction of these neurons. *ppk* mutants show reduced NEL responses to noxious mechanical stimuli without showing defects in thermal or optogenetic NEL responses. While removal of this ion channel diminishes the larval response to noxious mechanical force, it does not completely abolish it leading us to hypothesize that additional mechanonociceptive channels have yet to be identified. The objective of this study was to 1) identify novel channels and 2) functionally characterize their role in mechanical nociception. We carried out an *in vivo* forward genetic screen by utilizing the VDRC RNAi collection to reduce expression of all known and predicted ion channels specifically in the class IV md nociceptors. Taking this approach, two novel genes showing a reduced mechanical response were identified. We named these genes *stallone* and *balboa*. Remarkably, both genes are predicted to encode Deg/ENaC subunits suggesting an important role for this ion channel family in sensory perception of noxious stimuli. In addition, neither gene is required for optogenetic NEL responses indicating that both *stallone* and *balboa* function at or near the transduction step, perhaps forming a multimeric channel with ppk. We used a hybrid element insertion (HEI) approach to delete the stallone locus; this genetic mutant phenocopies mechanical nociception defects observed in RNAi mutants.

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Analysis of escape and avoidance behavior in *Drosophila* larvae. Tomoko Ohyama, James Truman, Rex Kerr, Marta Zlatic. Janelia Farm Research Campus/HHMI, Ashburn, VA.

Nervous systems, which allow organisms to respond flexibly to their environments, must transform the sensory inputs they receive into appropriate behavioral outputs. To study this we use the somatosensory system of *Drosophila* larvae. All somatosensory neurons have been anatomically identified and are known to project to the ventral nerve cord (VNC). The VNC contains a relatively small number of neuronal classes, and the Truman lab has identified a GAL4 line for each of these (generated by the Rubin lab). These Gal4 lines allow us to test the function of each class of interneuron downstream of somatosensory circuits. We have established a high-throughput behavioral analysis system to elucidate the function of each neuron class. We use a tracking software developed by Kerr Lab for *C. elegans*, to monitor the reactions of larvae. This system allows us to analyze the dynamic parameters (speed, curvature, hunching, change of direction, etc.) of populations of animals as well as of individual animals. First, we analyzed the reactions to vibration and pain. We found that larvae show stereotypical reaction sequences during continuous vibration—stop (startle) > head retraction > turning > forward crawling (avoidance) > off response—and pain stimulation—roll > rapid forward crawling. By inactivating sensory neurons, we also confirmed that chordotonal (ch) neurons are necessary for sensing vibration and that multi-dendritic type IV (MD IV) neurons are necessary for sensing pain-inducing stimuli. Similar but different motor patterns (avoidance and escape; fast forward crawling) elicited by vibration and pain-inducing stimuli pose an interesting question as to how the circuitries downstream of ch and MD IV neurons elicit similar but non-identical reaction sequences. We performed inactivating/activating each interneuron class in the VNC to assess the correspondence between sensorimotor processing and neural connectivity in the circuits associated with ch and MD IV neurons. We found several interneuron classes that involed in vibration and pain stimulations.

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Decision-making neurons for feeding behavior revealed by genetic activation in *Drosophila*. Motojiro Yoshihara[1], Thomas Flood[1], Michael Gorczyca[1], Shinya Iguchi[1], Benjamin White[2], Kei Ito[3]. 1) Neurobiology, UMass Medical School, Worcester, MA; 2) Laboratory of Molecular Biology, NIMH, Bethesda, MD; 3) Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan.

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. We randomly activated subsets of the *Drosophila* brain in 835 Gal 4 lines established by the NP consortium[(1)] through activation of a cold-activated channel, TRPM8[(2)], or a heat-activated channel, TrpA1[(3)] in Gal 4 expressing cells. In the unbiased screening, we identified a Gal 4 line showing feeding behavior. By restricting the Gal 4 expression through the "flip-out Gal80" technique, we identified a critical pair of neurons, Fdg (feeding) neurons, in the brain that induced the entire feeding sequence when activated. The large dendritic arbor of the Fdg-neurons suggests a role in integrating multiple information types. Consistent with this, functional calcium imaging revealed that Fdg-neurons responded to food presentation only in the starved state. The study of Fdg-neuron function may help elucidate how feeding decisions are made generally. 1) Yoshihara and Ito (2000) Dros. Inf. Ser. 83:199. 2) Peabody et al. (2009) J Neurosci 29, 3343-3353. 3) Hamada et al. (2008) Nature 454, 217-220.

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A long-term memory circuit from mushroom bodies to central complex in the Drosophila brain. Tsung-Pin Pai, Ann-Shyn Chiang. Institute of Biotechnology/Brain Research Center, National Tsing Hua University, Hsinchu 30013, Taiwan.

A functional memory circuit must (i) register (acquire) an experience via a persistent neural activity, (ii) consolidate (store) a lasting memory via (protein synthesis-dependent) structural/functional changes somewhere in that circuit and (iii) retrieve a long-term memory via reactivation of (some or all) of the circuit. Neural activity in the mushroom body (MB) neurons contributes to acquisition, consolidation and retrieval of olfactory associative long-term memory (LTM). We found that outputs of speicific MB effernt neurons giving dendrites in the verticle lobes are required for LTM retrieval. These neurons were intrinsically responsive to a diversity of odors and exhibited enhanced GCaMP activity to the conditioned odors. Using GFP Reconstitution Across Synaptic Partners (GRASP) labeling, we identified a group of neurons interconnected between MB effernt neurons and the fan-shape body (FB), the premotor center controlling Drosophila locomotion. A behavioral screen for neurons in which their neurotransmission outputs are required for LTM retrieval is still ongoing. We will present a comprehansive neural circuit in which neurons are structurally and functionally interconnected for LTM retrieval.

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How Do Endocycling Cells Block Apoptosis? Bingqing Zhang, Christiane Hassel, Suozhi Qi, Brian R. Calvi. Department of Biology, Indiana University, Bloomington, IN.

Eukaryotic cells employ multiple checkpoints to preserve the integrity of the genome. Excessive DNA damage, however, can trigger a programmed cell death called apoptosis, which is a major barrier to genome instability and cancer. An important remaining question is how cell cycle programs and checkpoints differ among cells in development. We have been using *Drosophila melanogaster* as a model system to address this question. We have found that cells that enter an endocycle, which is a variant cell cycle that consists of only 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 p53 target genes at the H99 locus. This suggests that apoptosis is repressed because p53 cannot induce transcription of its targets, a hypothesis that we have confirmed using reporters and qPCR. Chromatin immunoprecipitation (ChIP) from larval brain-disc (mitotic) and salivary gland (endocycle) using antibodies against modified histones suggests that the H99 locus is epigenetically silenced in endocycling cells. ChIP against Myc tagged p53 suggests that this epigenetic silencing may partially block binding of p53 to H99 promoters, but also prevent it from activating the promoter once bound. To further explore mechanism and identify regulators of apoptosis in endocycling cells, we are performing a novel genetic screen using GFP-labeled salivary glands. Initial results indicate that knockdown of several genes that encode epigenetic silencing proteins sensitize salivary gland endocycling cells to p53 over-expression. We will also present data that remodeling of the cell cycle is sufficient to repress apoptosis in some cell types of Drosophila, and our efforts to determine if the repression of apoptosis is conserved in human polyploid cancer cells. This study will provide general insights into the developmental regulation of the cellular response to stress and the decision to activate the apoptotic pathway.

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Lack of E2F activity protects cells from irradiation-induced cell death. Aaron M. Ambrus[1], Abul B.M.M.K. Islam[2], Mary Truscott[1], Núria López-Bigas[2], Maxim V. Frolov[1]. 1) Department of Biochemistry & Molecular Genetics, University Illinois at Chicago, Chicago, IL; 2) Research Unit on Biomedical Informatics, Department of Experimental Health and Sciences, PRBB, Universitat Pompeu Fabra, Barcelona, Spain.

Both overexpression of dE2f1, and the deregulation of endogenous dE2f1 activity have been shown to promote cell death. Moreover, dE2f1 induces a pro-apoptotic gene expression program. Here we utilized the *Drosophila* eye imaginal disc to examine irradiation-induced apoptosis in cells lacking dE2f activity (*dDP* mutants). dDP is the dimerization partner for both dE2f proteins and is needed for them to bind DNA and regulate gene expression. It has been previously reported that following irradiation, cells in *dDP* mutant eye discs fail to undergo apoptosis, despite having full activation of *hid* and *reaper*, two genes essential for irradiation-induced apoptosis (Moon et al. 2008. PLoS Gen.). By microarray analysis, we confirmed that the normal transcription program induced in response to irradiation in wild-type animals was also induced in *dDP* mutant animals. Furthermore, irradiated *dDP* mutants showed a strong enrichment for down-regulated oxidative metabolism related genes. Interestingly, a general block in energy metabolism was sufficient to protect eye disc cells from irradiation-induced death. Consistently, *dDP* mutant animals have an energy deficiency compared to wild-type animals. Moreover, the promoters of these metabolic genes were directly bound by dDP. Thus, the absence of irradiation-induced apoptosis in *dDP* mutants is not a consequence of the failure to induce the normal apoptotic response, but rather the result of a separate gene expression program modulating genes involved in oxidative metabolism, which protects against irradiation-induced cell death. Therefore, we identified a previously unappreciated set of dE2F-regulated metabolic genes. This new role for E2f in regulating oxidative metabolism extends our understanding of the function of E2f as a switch between regulating cell cycle progression, and promoting cell death.

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JNK and Draper regulate the engulfment of nurse cells by follicle cells during starvation induced mid-oogenesis cell death in the Drosophila ovary. Jon Iker Etchegaray[1], Allison Timmons[1], Adam Klein[1], Tracy Pritchett[2], Elaine Welch[1], Kim McCall[1]. 1) Boston University, Boston, MA; 2) Boston University Medical School, Boston, MA.

Programmed cell death and the subsequent removal of cell corpses is an important process in animal development and tissue homeostasis. Failure to engulf cell corpses can lead to leakage of cellular contents, secondary necrosis, and ultimately disease caused by inflammation. The Drosophila ovary provides an excellent system to study engulfment because the germline can be induced to die by starvation and their remnants are subsequently engulfed by the surrounding epithelial follicle cells. To address what are the components of engulfment in this system, we have conducted genetic analysis on two genes: drpr (drpr) and JNK (basket). We have found that drpr, a known effector of engulfment, is required in the follicle cells for proper phagocytosis of the dying germline. drpr mutants show impaired cell clearance, which is evident by lingering nurse cells remnants as well as reduced enlargement and premature death of the follicle cells. Moreover, drpr expression is upregulated in the follicle cells during the engulfment process. Interestingly, our lab has also found that JNK activity is induced in engulfing follicle cells. As with drpr, disruption of JNK expression in the follicle cells disrupts engulfment. By conducting epistasis analysis we found that drpr is required for JNK activity and JNK activity upregulates drpr expression. Furthermore, we found that expressing a constitutively active form of Hemipterous (HepCA), a kinase that specifically activates JNK, suppresses the engulfment defects seen in drpr null egg chambers. We also observed that over-expression of drpr or (HepCA) induces the germline to undergo cell death even when the flies are not starved, suggesting that JNK and drpr, contribute to the death process. Our overall model is that drpr activation by the dying germline leads to JNK activation in the follicle cells. Once activated, JNK upregulates drpr through a feed-forward mechanism, as well as other genes involved in engulfment.

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A role for the Drosophila histone variant H2Av in mitotic chromosome segregation. Giovanni Cenci[1,2], Fiammetta Verni[3]. 1) Dip. Biologia di Base ed Applicata, Università dell'Aquila, Via Vetoio, 67100 L'Aquila, Italy; 2) Sbarro Institute for Cancer Research and Molecular Medicine, Dept. of Biology, Temple University, PA 19122, USA; 3) Dip. di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma, Roma 00185, Italy.

We found that mutations in the Drosophila *H2Av* gene, which encodes both H2A.X and H2A.Z variants, impair compaction of pericentric chromosome regions and lead to irregular chromosome segregation during larval mitotic divisions. Analysis of spindle assembly revealed that ~23% of H2Av-depleted mutant cells displayed apparent anaphase-looking bipolar spindles with chromosomes not connected to the spindle poles by bundles of kinetochore microtubules (MTs). In addition, we found that *H2Av* mutant chromosomes showed defective sister chromatid separation and were not able to congress to the equator of the cell. Moreover, ~85% of irregular mitotic figures exhibited high levels of Cyclin B with respect to control anaphases (~3%), suggesting that the anaphase-like figures are indeed in a metaphase status. Consistently, the checkpoint proteins ZW10 and BubR1 remained strongly localized at centromeres of mutant chromosomes. Interestingly, ZW10 failed to stream towards the poles suggesting that loss of H2Av caused defective MT attachment to the kinetochore. Indeed, MT regrowth experiments after cold exposure revealed that loss of H2Av impaired MT capture by kinetochores. All phenotypes were rescued by a *H2Av* transgene with a C-terminal truncation that lacks of H2A.X function, suggesting that it is the loss of H2A.Z activity that affected chromosome behavior. Our Co-Ip experiments showed that H2Av interacts with Hp1. Furthermore, Hp1 immunostaining revealed that ~50% of mutant neuroblasts displayed reduced pericentric Hp1 localization on mitotic chromosomes, although Hp1 levels in interphase cells remained normal. Altogether, our results suggest that H2Av is required for the regulation of mitotic chromosome segregation in *D. melanogaster* highlighting an unanticipated role of this histone variant for the Hp1 localization on mitotic chromosomes.

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The Tumor Suppressor APC2 and the Chk2 DNA Damage Checkpoint Promote Genomic Stability in the Early Embryo. John Poulton, Frank Mu, Mark Peifer. Dept of Biology, Linberger Comprehensive Cancer Center, Univ North Carolina, Chapel Hill, NC.

APC proteins regulate Wnt signaling, cytoskeletal processes, and genomic stability, though their precise roles in these processes are controversial. One of APC's best characterized cytoskeletal roles is in the early fly embryo, where it helps maintain nuclei at the embryonic cortex. Loss of APC2 leads to detachment of nuclei from the cortex (nuclear fallout), but its mechanism of action is unclear. To better understand APC2's cytoskeletal function we sought to identify mechanisms underlying nuclear fallout in *APC2*$^{-}$ embryos. From live imaging, we observed that nuclear fallout is frequently preceded by defects in chromosome segregation. Previous work showed that DNA damage activates the Chk2 pathway, leading to centrosome inactivation and nuclear fallout. We hypothesized that nuclear fallout in *APC2*$^{-}$ embryos is due to Chk2 activation. Consistent with this, we found that nuclei undergoing fallout in *APC2*$^{-}$ embryos accumulated high levels of the DNA damage marker γH2Av, and underwent centrosome inactivation prior to fallout. Importantly, embryos double mutant for *chk2* and *APC2* displayed almost no nuclear fallout, indicating fallout in *APC2*$^{-}$ embryos requires Chk2. Surprisingly however, these double mutant embryos had many more mitotic defects than either single mutant, suggesting Chk2 can correct some defects caused by loss of APC2. We also elucidated the upstream defects that lead to increased DNA damage in *APC2*$^{-}$ embryos. *APC2*$^{-}$ embryos had a high frequency defect in centrosome separation; this appears to lead to ectopic pseudocleavage furrows which disrupted mitotic spindles. We propose a model where initial errors in centrosome separation lead to cytoskeletal misregulation, including formation of ectopic pseudocleavage furrows. These cytoskeletal defects disrupt some mitotic nuclei, causing chromosome segregation defects that activate the Chk2 DNA damage pathway. This can either promote damage correction or nuclear fallout. Our findings provide in vivo evidence that the cytoskeletal function of an APC protein serves an important role in promoting mitosis and genomic stability.

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A FISH-based RNAi screen identifies genes involved in somatic homolog pairing of heterochromatic regions. Eric Joyce, Ting Wu. Genetics, Harvard Medical School, Boston, MA.

Pairing of homologous chromosomes is generally considered to be a special property of the meiotic cell. However, reports have also implicated pairing during the somatic cell cycle, with evidence for its impact in both double-strand break (DSB) repair and gene regulation. In Drosophila, homologous chromosomes are intimately paired in virtually all cell types throughout development; however, our understanding of the underlying mechanism remains unclear and only a few genes have been implicated in this process. Here, we introduce a novel high-throughput FISH (fluorescent in-situ hybridization) technique that enabled us to rapidly screen for factors involved in this robust level of somatic pairing. Using a genome-wide RNAi library, we identified both candidate 'pairing promoting genes,' as well as candidate 'anti-pairing genes,' providing evidence that pairing may be a dynamic process that can be both enhanced and antagonized. Many of the genes found to be important for pairing are especially enriched for functions associated with mitotic cell division, providing a genetic framework to understand a long-standing link between chromosome dynamics during mitosis and homologous pairing in interphase. In contrast to the pairing promoting genes, several of the candidate anti-pairing genes have known interphase functions associated with S-phase progression, replication, and chromatin compaction, including several components of the condensin II complex. These findings complement studies conducted over several decades regarding pairing mechanics from many labs including that of Henikoff, Sedat, Hawley, Bosco and others. These results, in combination with a variety of secondary assays, has led to new insights into the mechanism and dynamics of somatic pairing that have implications for gene regulation, DSB repair, and cell cycle progression. This work is supported by grants from the National Institutes of Health to E.F.J. (F32CA157188) and T.W. (RO1GM085169).

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Identification of factors that cooperate with rbf1 mutations. Nam-Sung Moon, Kate Krivy, Mary-Rose Bradley-Gill. Department of Biology, Developmental Biology Research Initiative, McGill University, Montreal, Quebec H3A 1B1, Canada.

Mutations of rbf1, the Drosophila homolog of the Rb tumor suppressor gene, generate defects in cell death and cell cycle control during development. In the Drosophila eye, RBF1 protects cells entering the Morphogenetic Furrow (MF) from Hid-dependent cell death and cooperates with Dacapo (DAP) to promote cell cycle exit of differentiating photoreceptors. Interestingly, the EGFR/Ras pathway regulates both Hid and DAP, suggesting that extensive crosstalk exists between RBF1 and the EGFR/Ras pathway. In an effort to identify downstream effectors of the EGFR/Ras pathway that participate in this crosstalk, we discovered that Capicua (Cic) regulates both survival and proliferation of rbf1 mutant cells. Cic is a transcription factor whose activity is post-transcriptionally regulated by the EGFR/Ras pathway, and has been shown to function in restricting proliferation. Immunostaining of imaginal discs revealed that cic rbf1 double mutant cells display a decreased level of cell death and bypass developmentally controlled cell cycle arrest. We will discuss our effort to investigate the molecular mechanism underlying this genetic interaction.

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Role of oenocytes in metabolic response to starvation. Debamita Chatterjee, Heinrich Jasper. Department of Biology, University of Rochester, Rochester, NY.

Organisms need to maintain metabolic homeostasis in order to live a healthy lifespan. In mammals, the liver plays a critical role in metabolic homeostasis under different environmental perturbations. Surprisingly, there is very little evidence of a corresponding tissue in Drosophila. Gutierrez and coworkers (2007) demonstrated a novel hepatocyte-like role for a previously mysterious class of specialized cells in Drosophila larva called oenocytes. The oenocytes are arranged in ribbon-like clusters along the inner cuticle of each abdominal segment. They specifically showed that oenocytes regulate the uptake of lipids during starvation much like the mammalian hepatocytes. In addition, they also express a specific set of genes homologous to mammalian liver. We wanted to characterize oenocytes in adult flies especially with respect to their role in regulating metabolic homeostasis under starvation conditions.We performed a transcriptome analysis of the genes expressed in oenocytes under starvation. We observed an upregulation of several key metabolic genes like neoglucogenic enzyme pepck, triacylglycerol lipases and adipokinetic hormone receptors GRHRI and II. In order to test the oenocyte-specific expression of several transgenes, we cloned a oenocyte-specific mifepristone-inducible driver. Using this driver, it was found that there is increased starvation sensitivity of flies in which oenocytes were ablated and of flies in which there was a oenocyte-specific blocking of Insulin signaling pathway (IIS) pathway. In addition, we observed a loss of upregulation of pepck and GRHR1 transcript levels under starvation conditions when IIS pathway was blocked in the oenocytes as compared to the wild type flies. Preliminary studies of the metabolic profile indicated that there was less glycogen and trehalose in the oenocyte-ablated flies compared to wild-type. All these results suggest a oenocyte-dependent regulation of metabolic homeostasis through the novel interaction of IIS and adipokinetic-hormone pathways. Future experiments are directed to corroborate the aforementioned hypothesis.

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Juvenile hormone regulation of lipid metabolism through insulin signaling. Hua Bai, Ping Kang, Marc Tatar. Ecology & Evolutionary Biol, Brown University, Providence, RI.

Juvenile hormones (JH) produced in corpora allata (CA) are involved in a variety of biological processes. Beside the role on female vitellogenesis, the effects of JH and its analog on lipid metabolism have also been studied in several insects. However, the underlying mechanism is poorly understood. Here we investigated the regulation of JH on lipid metabolism in female Drosophila. We found that short period application of JH analog (methoprene) increases the level of triglyceride (TAG), while CA ablated (CAKO) flies and putative JH receptor (Met) mutants have reduced TAG. Interestingly, we identified an insulin-like peptide (dilp6) whose expression can be directly induced by methoprene application in fat body culture in vitro. This induction requires Met and Kr-h1, two key players involved in JH signaling network. Overexpression of either Kr-h1 or dilp6 using a fat body driver promotes the accumulation of TAG in fat body. Furthermore, the expression of Kr-h1 and dilp6 are altered in responding to different nutrient conditions. These results suggest that JH may regulate lipid metabolism through a fat body expressed insulin-like peptide.

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An Interleukin-6 like cytokine regulates systemic insulin signaling by conveying the 'fed' state from the fat body to the brain. Akhila Rajan[1], Norbert Perrimon[1,2]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute, 77 Ave Louis Pasteur, Boston, MA.

Organisms constantly adapt their energy needs in accordance to their nutritional status. This process, referred to as 'energy homeostasis', involves the regulation of sugar and lipid stores depending on nutritional availability. Genetic studies of physiological responses in *Drosophila* have revealed that many parallels exist between invertebrate and mammalian homeostasis. In particular, many of the organs that control nutrient uptake, storage and catabolism in vertebrates, have analogs in the fly.

The fat body (FB) in the fly functions as the 'nutrient sensor' and sends remote signals to the islet-like cells in the brain to release insulin and regulate energy balance. Though the TOR pathway has been shown to play a crucial role in nutrient sensing in the FB, the identity of the humoral signal(s) involved in communication between the FB and the brain is unknown.

We have identified a *Drosophila* Interleukin (IL)-6 like gene, which, when perturbed specifically in the FB, results in a systemic reduction in cell size. In addition, these flies have reduced triacylglycerol levels and increased circulating sugars indicative of metabolic defects. They feed normally but accumulate lipids in the oenocytes even under 'fed' conditions, implying that they physiologically phenocopy starvation. Altering the levels of this gene in the FB affects insulin accumulation in the brain. However, a knock-down of this gene in other tissues such as the muscle has no effect on systemic growth and metabolism. Additional data suggest that this IL-6 family member conveys the ‘fed’ state in flies and affects systemic growth and metabolism by remotely controlling insulin secretion in the brain.

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MASOP coordinates imaginal disc growth and maturation with developmental timing. Julien Colombani, Ditte Andersen, Pierre Leopold. Institute of Developmental Biology and Cancer, Nice, France.

Little is understood about how developmental timing is coordinated with maturation/patterning of an animal during development. Earlier wing disc transplantation/regeneration experiments have demonstrated an important role for imaginal tissues in this coupling. Wounding of discs delays pupal molt allowing the wounded tissue to regenerate before entering metamorphosis. The nature of the molecular signal released from the discs to control developmental timing has yet to be identified. We have carried out an RNAi-based genome wide screen to identify molecules produced in the disc that are responsible for the developmental delay observed in animals with either neoplastic or minute-like imaginal discs. We screened 10.100 RNAi lines of the VDRC phiC31 collection and isolated 121 transgenic lines able to rescue developmental delay induced in the neoplastic growth conditions. Among them, we identified MASter Of Pupae (MASOP), a gene whose silencing rescued the delay in both of our testerlines. MASOP encodes a putative secreted peptide and is regulated at the transcription level as we found MASOP levels highly up regulated in a microarray experiment for developmentally delayed neoplastic discs. Overexpression of MASOP in imaginal tissues with a 2-day development delay without any visible effect on disc shape or patterning. We propose that MASOP is an inhibitory signal produced and potentially secreted from injured/slow growing mitotic tissues to delay pupariation, permitting regeneration/completion of growth to occur.

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Drosophila Lipoprotein Metabolism. Wilhelm Palm, Julio Sampaio, Suzanne Eaton. Max Planck Institute of Molecular Cell Biology , Dresden, Germany.

Lipoproteins transport dietary and endogenously synthesized lipids between different organs, and their dysfunction is associated with many pathological conditions. However, it is not well understood how lipoproteins influence tissue lipid composition. Here, we identify the major Drosophila lipoproteins and characterize their assembly and lipidation. To probe for lipoprotein function, we genetically perturb interorgan lipid transport, and use lipid mass spectrometry to quantitatively describe the resultant changes in the lipidomes of individual organs. The apoB family lipoprotein lipophorin (Lpp) is the major lipid carrier in the larval hemolymph. Lpp is produced in the fat body in a process that depends on Microsomal Triglyceride Transfer Protein (MTP). Subsequently, Lpp is recruited to the intestine, where it is further loaded with lipids in a process that depends on Lipid Transfer Particle (LTP), a second apoB family lipoprotein originating from the fat body. The fat body exports phospholipids bearing long-chain fatty acid residues to Lpp. The intestine loads Lpp with sterols, and with diacylglycerols bearing medium-chain fatty acid residues that are derived partly from dietary sources and partly from endogenous synthesis in enterocytes. Lpp delivers lipids from fat body and intestine to imaginal discs, which utilize them to build their fat stores. In addition, Lpp provides a significant fraction of membrane phospholipids to imaginal disc cells. In contrast, fat storage in the brain relies only on Lpp lipids derived directly from the fat body, and Lpp is not a major source of brain phospholipids. These studies define the major routes of interorgan lipid transport in Drosophila, and uncover surprising tissue-specific differences in lipoprotein lipid utilization.

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MPC1 plays an essential role in pyruvate metabolism in yeast, *Drosophila* and human disease. Daniel K. Bricker[1], Thomas Orsak[2], John Schell[2], Yu-Chan Chen[2], Eric Taylor[2], Michele Brivet[3], Audrey Boutron[3], Jared Rutter[2], Carl S. Thummel[1]. 1) Dept of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT; 2) Dept of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT; 3) Laboratoire de Biochimie, AP-HP Hôpital de Bicêtre, Le Kremlin Bicêtre, France.

ATP production through oxidative metabolism is critical to cellular function and survival. The ability of pyruvate to enter mitochondria is central to this process, as pyruvate connects glycolysis in the cytosol to the mitochondrial TCA cycle. Despite extensive biochemical characterization of mitochondrial pyruvate uptake, the genetic identity of the transporter responsible is unknown. Through our work in fly and yeast systems, we have identified a candidate for the mitochondrial pyruvate transporter and linked this function to human disease. Our current effort is focused on the Mitochondrial Pyruvate Carrier (MPC) proteins MPC1 and MPC2, which associate with each other at the inner mitochondrial membrane. Deletion mutants of *Drosophila MPC1 (dMPC1)* are viable, but die rapidly on a diet of only sugar due to an inability to generate ATP. Metabolomic profiling revealed that *dMPC1* mutants accumulate pyruvate and are depleted of TCA cycle intermediates, consistent with a defect in pyruvate metabolism. Studies in yeast have demonstrated that MPC1 is required for pyruvate transport across the mitochondrial membrane. A human genetic analysis has identified three families with children displaying lactic acidosis and hyperpyruvatemia. Biochemical characterization of cells isolated from affected individuals revealed no defects in pyruvate dehydrogenase, but an inability of mitochondria to take up pyruvate for oxidative metabolism. A causal locus was identified through linkage analysis to mutations that change conserved amino acids in MPC1. Taken together, our data supports the model that the MPC proteins act as a pyruvate transporter, providing an essential link between glycolysis and mitochondrial oxidative metabolism.

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Mitochondrial genotype alters the nuclear transcriptional response to varied levels of hypoxia in Drosophila. David M. Rand, Patrick A. Flight, Nicholas Jourjine, Lei Zhu. Ecology & Evolutionary Biol, Brown Univ, Providence, RI.

Exposure to reduced oxygen tension, or hypoxia, is a common occurrence in a wide array of environmental conditions ranging from cancer to stressed microhabitats in nature. One response to hypoxia is to reduce cellular demand for oxygen by down-regulating mitochondrial functions. Despite the central role mitochondria play in oxygen consumption, the effect of alternative mitochondrial genotypes on the hypoxic response has not been examined in flies. 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. Replicate cultures of adult males of each genotype were exposed to four different oxygen tensions (normoxia, 6%;, 3%;, and 1.5%) for 2 hours, and flash frozen. Expression profiles were determined using Affymetrix 2.0 arrays. The mtDNA genotype design allows for partitioning effects to alternative mtDNAs within a species, or fixed differences between Dmel and Dsim mtDNAs. MtDNA has subtle effects on gene expression under normoxia (~10 genes altered) and strong hypoxia (1.5%; ~25 genes altered), but had pronounced effects at 3% (>200 genes altered) and 6% (>500 genes altered). These results provide strong evidence for mitochondrial retrograde signaling in the nuclear transcriptional response to different levels of hypoxia. Gene ontology analyses reveal that alternative mtDNAs within species alter genes associated with ‘oxidation-reduction’ and ‘mitochondrion’, while the effects of Dmel vs. Dsim mtDNAs alter very different classes of genes (‘ribonuclear protein’; ‘ribosome biogenesis’). These studies offer the first evidence that genes in mtDNA play a critical role in modulating the nuclear transcriptional response to hypoxia, and do so with varying degree under different levels of hypoxic stress.

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Gustatory Regulation of Aging in *Drosophila melanogaster*. Michael J. Waterson[1], Zachary M. Harvanek[2], Ivan Ostojic[3], Joy Alcedo[3], Scott D. Pletcher[1,4]. 1) Cellular and Molecular Biology Graduate Program, University of Michigan; 2) Medical Scientist Training Program, University of Michigan; 3) Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland; 4) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI.

The process of aging is known to be regulated by the integration of environmental information within the central nervous system [CNS], yet the circuitry of this regulation from the sensory system to target tissues via the CNS remains poorly understood. Gustatory signals represent one such set of information, which are capable of modulating behaviors such as feeding rate, reproductive output, and mating choice, all known to influence organismal aging. The precise mechanisms through which specific gustatory components regulate behavior and physiology, including aging, however, remain to be determined.

External gustatory perception in *D. melanogaster*, a rapid and genetically tractable model system, is mediated by gustatory receptors [GRs] expressed in specialized GR neurons [GRNs]. To determine which specific gustatory inputs regulate certain aging phenotypes, we have utilized multiple genetic techniques to systematically manipulate the expression of single GRs or GRNs and have measured the effect on lifespan, as well as several measures of overall organismal health.

Our analyses have yielded insights into the effects of gustatory inputs on *Drosophila* aging. The functional manipulation of individual GRs or GRNs that represent a range of taste modalities was sufficient to mediate both lifespan extension and decline, as compared to background controls. Additionally, several measures of overall health were significantly altered due to these manipulations. This work provides evidence that specific components of the gustatory system are critical in the regulation of organismal aging. Given the similarities between insect and vertebrate taste perception, these results and our future work may have crucial implications for how chemosensory systems regulate human aging.

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Dynamic and Tissue Specific Regulation of Gene Activation and Silencing by Noncoding PRE/TRE Transcription. Leonie Ringrose[1], Adelheid Lempradl[1,2], Frank Ruge[1], Helena Okulski[1], Christina Altmutter[1], Gerald Schmaus[1], Karin Aumayr[1,3], Hasene Basak Senergin[1], Andrew Dimond[1,4]. 1) IMBA (Institute of Molecular Biotechnology), Vienna, Austria; 2) MPI (Max Planck Institute of Immunology and Epigenetics), Freiburg, Germany; 3) IMP (Institute of Molecular Pathology), Vienna, Austria; 4) Nuclear Dynamics ISP, Babraham Institute, Cambridge, UK.

Many Drosophila Polycomb/Trithorax Response Elements (PRE/TREs) give rise to noncoding transcripts, but whether these transcripts are involved in gene activation or silencing is currently unclear. Here we show that the Drosophila vestigial PRE/TRE undergoes a developmental switch, in which embryonic transcription of the PRE/TRE reverse strand is linked to vestigial gene activation, whereas larval transcription of the PRE/TRE forward strand is involved in vestigial gene repression. Transgenic reporter constructs identify a 100bp region of the PRE/TRE as a key switching point for this developmental transition. High ectopic transcription of a transgenic PRE/TRE leads to tissue specific misregulation of the vestigial gene, demonstrating a transcription dependent and strand specific interaction between the transgenic and the endogenous loci. By showing that PRE/TRE transcription is involved in both activation and silencing, and that this is dependent on developmental context, these findings have broad implications for understanding the dynamic dual nature of PRE/TRE elements.

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An RNA Memory Mechanism to Inherit Epigenetic Marks. Maria Cristina Onorati, Walter Arancio, Davide F.V. Corona. STEMBIO, Telethon Dulbecco Institute c/o University of Palermo, Italy.

A central question in epigenetics is to understand how, terminally differentiated daughter cells can inherit complex patterns of chromatin modifications from their mother cell. Even if several mechanisms have been hypothized to explain the establishment and maintenance of cell identity, it is still unclear how during mitosis covalent and ATP-dependent chromatin modifications are transmitted after DNA replication. Indeed, a simple way for daughter cells to restore the transcriptional profile of mother cells is to directly ‘sense’ the transcriptome of their mother cells. In order to unveil the molecular nature of somatic cell epigenetic memory, we used classic Position Effect Variegation assays to check if non functional alleles of the white gene could modify the eye color variegation caused by an heterochromatin inversion of the white gene called white-mottled 4 (wm4h). Our data show that several white alleles suppress the variegation of the wm4h line. Unexpectedly, the presence of white alleles causes an increase in the white gene transcript as well as an opening in the chromatin structure at the wm4h locus. Remarkably, this effect is inheritable, a phenomenon highly reminiscent of RNA mediated paramutation. The changes in the levels of expression of the wmh4 gene, induced in trans by several white alleles, indicate that the presence of a non functional gene that does not produce a coding transcript but potentially only ncRNA, could influence in trans the expression of a functional copy of the same gene silenced by heterochromatin. Our data indirectly indicates that cells can 'sense' the presence of non coding RNA’s inherited from their mother cells and can use them to epigenetically reset their transcriptional program after DNA replication.

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Insulators bring active genes into transcription factories in Drosophila. Hua-Bing Li, Vincenzo Pirrotta. Molecular Biology & Biochemistry, Rutgers University, Piscataway, NJ.

Transcription factories are nuclear sites where active genes undergo transcription but it is still not clear how genes are targeted to a given transcription factory. Insulators might play a key role in the formation of this kind of nuclear body. We previously have used transgenes containing the Mcp or Fab-7 (insulator+PRE) elements to show that two remote transgene copies interact and co-localize in around 7% of the nuclei, as shown by live-imaging and by 3C assays. This interaction is not caused by the binding of Polycomb complexes but depends on the presence in both copies of the insulator element. We observed a much higher frequency of co-localization in eye imaginal discs (in 50-90% of the nuclei) but not in wing imaginal discs when both transgenes contained the eye enhancer, indicating that interaction is much stronger when the transgenes are transcriptionally active. This high-level interaction requires also the insulator and the binding of Trithorax but not Polycomb to the PRE. The high-level interactions require CTCF but are modulated by CP190 and by RNAi genes, particularly by Ago2. The two transgenes do not co-localize with each other when one copy is active and the other is silenced. The transgenes also interact with endogenous homeotic genes, co-localizing with them when both transgene and endogenous gene are active but not when one is active and the other repressed. From a combination of genetics, live imaging, 3C, ChIP, and transcriptional data, we conclude that the insulators mediate the contacts but target the genes to different nuclear structures, depending on the state of activity. When both genes are repressed, they co-localize in Polycomb bodies. When both genes are active, they are targeted to transcription factories in a Trithorax-dependent fashion that is not directly related to strength of transcriptional activity.

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A genetic screen for recessive Polycomb group mutants. James A. Kennison, Mark A. Mortin, Monica T. Cooper. Program in Genomics of Differentiation, NIH, Bethesda, MD.

The Polycomb group genes were first identified because mutations in them fail to maintain HOX gene silencing in Drosophila. In an F1 screen for new Polycomb group genes, we are generating clones of homozygous mutant cells in the eye using the yeast FLP/FRT system. The mutagenesis is done in a genetic background in which Polycomb group Response elements (PREs) in transgenes silence the mini-white reporter gene by pairing-sensitive silencing. New silencing mutations are identified by derepression of the mini-white reporter in the homozygous mutant clones. On the second chromosome, we have recovered 32 mutations in seven previously-identified Polycomb group genes and 39 mutations in at least 16 new sileincing genes. The transcription units for three of the new silencing genes have been identified.

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Mapping of chromosomal proteins of the bithorax complex in single parasegments. Welcome Bender[1], Heber Domingues[1], Sarah Bowman[2], Robert Kingston[2]. 1) BCMP Dept, Harvard Medical Sch, Boston, MA; 2) Dept. of Molecular Biology, Massachusetts General Hospital, Boton, MA 02114.

The Drosophila bithorax complex (BX-C) is thought to have a different chromosome structure in each of the parasegments that it regulates. Thus, chromatin immunoprecipitation experiments done on whole embryos or cultured cells are of limited value for the study of the homeotic complexes. We have created embryos that produce GFP in PS4, PS5, PS6, or PS7. These are the parasegments most important for the expression of *Ultrabithorax* and *abdominal-A*. Enhancer trap P elements were swapped into the BX-C and the Antennapedia complex; these produce GAL4 or GAL80 with anterior expression limits at specific parasegmental borders. P elements with embryonic enhancers derived from the BX-C have also been constructed, which give similarly restricted expression patterns. Appropriate combinations of GAL4 and GAL80 producers restrict GAL4 activity to single parasegments. We have used FACS to isolat nuclei making GFP under GAL4 control, and ChIP/Seq experiments are in progress to map histone modifications and chromosomal proteins.

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Spliceosomal Dynamics is Required for Nurse-Cell Chromatin Dispersal In the *Drosophila* Germ Line. Stephen M. Klusza, Shirelle Figueroa, Amanda Novak, Billy Palmer, Wu-Min Deng. Dept Biological Sci, Florida State Univ, Tallahassee, FL.

In *Drosophila* oogenesis, nurse-cell nuclei display a significant progressive change in their chromatin morphology, from a highly-compact polytenic configuration to a diffuse, dispersed state largely devoid of any visible structures. Mutations in splicing proteins such as Hrb27C, Squid, and PUf68 have been found to affect nurse-cell chromatin dispersal (NCCD) through persistence of the intermediate '5-blob' phenotype in later stages of oogenesis, suggesting that splicing is an integral part of chromatin dynamics in polytene-polyploid nurse cells. In an effort to identify cellular processes that may be significantly affected by NCCD failure, we identified mutations in *peanuts* (*pea*), the *Drosophila* homolog of the yeast Prp22p DEAH-box RNA helicase, as an enhancer of the condensed '5-blob' morphology phenotype of *ovarian tumor* (*otu*) heterozygotes. Prp22p has been shown to be involved in RNA exon ligation and recycling of spliceosomal components through unwinding of the spliceosome from spliced RNA. FLP-FRT-induced whole germline clones of a null allele of *pea* invariably arrest at stages 2-3 of oogenesis, while half-germline clones display NCCD failure in *pea*-null nurse-cell nuclei at stages 6-7 of oogenesis. By using a Prp38 antibody that recognizes activated spliceosomes, we also discover that enrichment of spliceosomes in the inter-chromatin spaces, as well as a subset of chromatin, occur during the transiently-condensed phase of NCCD; in *pea*-null nurse-cell nuclei, the spliceosomes remain enriched in these areas, suggesting a defect in the re-distribution and/or recycling of spliceosomal fractions as a result of NCCD failure. Previous research in yeast spliceosomal mutants have identified splicing aberrations in many ribosomal proteins, and cursory examination of ribosomal-protein loss-of function (LOF) in *Drosophila* ovaries also retain similar NCCD defects. Therefore, we are currently examining ribosomal function through spliceosomal studies as a potential general mechanism for NCCD that may implicate chromatin dynamics as a regulator of translation in the cell.

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Phylogenomic analysis of the Heterochromatin Protein 1 gene family defines new germline-restricted functions. Mia Levine[1], Connor McCoy[1], Danielle Vermaak[1], Mary Alice Hiatt[1], Frederick Matsen[1], Harmit Malik[1,2]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute.

Heterochromatin is the gene-poor, satellite-rich eukaryotic genome compartment that supports many essential cellular processes, from chromosome segregation to genome defense. Extensive tracts of repetitive DNA in heterochromatin, however, challenge traditional methods of sequence assembly and experimental manipulation. Fortunately, the functional diversity of proteins that bind and often epigenetically define heterochromatic DNA sequence mirrors the diverse functions supported by this enigmatic genome compartment. To identify new such surrogates for dissecting heterochromatin function and evolution, we conducted a comprehensive phylogenomic analysis of the Heterochromatin Protein 1 gene family over 40 million years of Drosophila evolution. Our study expands this gene family from a modest 5 genes to at least 27 genes, including several uncharacterized HP1 genes in *D. melanogaster*. The 22 newly defined HP1s introduce unprecedented structural diversity, lineage-restriction, and germline-biased expression patterns into the HP1 family. We find that dynamic evolution occurs via prolific gene gains and losses while HP1 gene number per lineage remains remarkably constant, consistent with a 'revolving door' model. The high resolution dating of one such loss event—that of HP1E in the obscura group, introduces the notion that heterochromatin content and/or distribution across Drosophila evolution may drive at least some of these gain/loss dynamics. We have begun to test this hypothesis by functionally and cytologically characterizing the testis-restricted HP1E, which is dispensable over evolutionary time scales but essential for male fertility, at least in *D. melanogaster*. Our preliminary analyses are consistent with HP1E acting to epigenetically transfer information via the male germline to the egg. These data support the utility of this expanded compendium of ovary and testis-restricted HP1 genes guiding functional analyses of germline chromatin dynamics.

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The Unusual Features of Active Genes on Drosophila melanogaster Chromosome Four: Reinterpreting the Roles of Chromatin Modifications. Sarah CR Elgin[1], Nicole C Riddle[1], Tingting Gu[1], Youngsook L Jung[2], Monica Sentmanat[1], modENCODE Drosophila Chromatin Consortium. 1) Washington Univ, St Louis, MO; 2) Harvard Medical School, Boston, MA.

The small fourth (dot) chromosome of Drosophila melanogaster exhibits overall characteristics of a heterochromatic domain, including high levels of H3K9me2/3 and HP1a, but contains ~80 genes in its 1.2 Mb banded portion. Mapping of histone modifications and chromosomal proteins in S2 and BG3 cells shows that these genes have unique characteristics, distinctive from active genes in either euchromatin or pericentric heterochromatin. H3K9me2/3 and HP1a are depleted at the TSSs (transcription start sites) of active fourth chromosome genes; the TSSs, as expected, are occupied by H3K4me2/3 and RNA pol II. However, POF (painting of fourth), HP1a, and H3K9me3 are found at high levels across the gene body. There is no evidence of euchromatin "islands" supporting expression. Rather reporter sites permissive for expression appear to be those regulated by the Polycomb system in at least some cell types. Fourth chromosome genes exhibit the same range of expression levels seen in other domains, but there are few if any associated with a paused polymerase. The continued presence of HP1a and H3K9me3 at active genes results in a shift in association patterns, with H3K9me2 now being unique in its stronger correlation with inactive genes. Depletion of POF results in loss of HP1a from fourth chromosome gene bodies, but HP1a continues to be present in the pericentric heterochromatin and at repeat clusters on the fourth, indicating a dual targeting mechanism for HP1a association with the fourth chromosome. These findings challenge our prior interpretations of the roles of some histone modifications in gene regulation and focus attention on the modifications at the TSS. Supported by NIH grants U01HG0004258 to GH Karpen and R01 GM068388 to SCRE.

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The Tbx-20 transcription factor Midline functions as a localized negative regulator of epidermal growth factor receptor signaling. Mariana Fregoso Lomas[1], Fiona Hails[1], Jean-François Boisclair Lachance[1,2], Laura Nilson[1]. 1) Biology, McGill University, Montreal, QC, Canada; 2) Biological Sciences, University of Chicago, Chicago, IL, USA.

We use the ovarian follicular epithelium as a model to study signaling pathways and patterning during development. This epithelium is responsible for the secretion of the eggshell, including the two dorsal appendages derived from two dorsal anterior primordia. These primordia are recognized through the high expression of the Broad-Complex (Broad) transcription factor, and are separated by a region of cells at the dorsal midline that do not express Broad. The remaining follicle cells express basal levels of Broad. Dorsally-localized EGF signaling coming from the underlying oocyte initiates this spatial pattern along the dorsal-ventral axis, but the signals that pattern the anterior-posterior (AP) axis are less well understood. To better understand AP patterning, we characterized a new locus called F27 required for this process. In F27 mutant epithelia, the posterior limit of the high Broad domain is shifted toward the posterior, suggesting that dorsal anterior fates have been determined in posterior follicle cells. Consistent with this change in cell fate, the resulting eggshells exhibit extra appendage material posterior to the normal appendage position. The altered fate in this region can be detected in earlier stages because the mutant cells express the transcription factor *mirror*, which we have shown can induce dorsal-anterior fates. When we ectopically activate the EGFR signalling cascade in the F27 background, the follicular epithelium can respond inducing dorsal fates accordingly. We mapped the F27 mutation to the *midline* gene, which encodes a Tbox transcription factor. Midline is expressed in the posterior follicular epithelium and, moreover, when we ectopically express it in the anterior it represses dorsal fates. We show that the *midline* paralog *H15* participates in this process as well. Our data show that these factors are regulating posterior fate in the follicular epithelium, and are acting as novel negative regulators of EGFR signaling output.

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Evolutionary variation in the Dorsal gradient distribution. J. Sebastian Chahda[1], Claudia M. Mizutani[1,2]. 1) Case Western Reserve University, Cleveland, OH. Deparment of Biology; 2) Case Western Reserve University, Cleveland, OH. Deparment of Genetics.

The specification of primary germ layers along the dorso-ventral (DV) axis by morphogenetic gradients is highly conserved among bilaterians. Currently, little is known about how those gradients respond to modifications in embryonic size and how scaling affects DV cell fates. We recently found that related *Drosophila* species that vary in embryo size exhibit unequal scaling across their germ layers. While the neuroectodermal domain remains unchanged, there are striking variations in the mesodermal domain of Drosophilids. Here we sought to investigate the underlying causes for this evolutionary variation in the mesoderm size. Among possible factors affecting the mesodermal domain are alterations in nuclear size and densities, divergence in cis-regulatory sequence of mesodermal genes, and variation in the range of Toll receptor activation, which modulates the nuclear intake of Dorsal, a key morphogen responsible for mesoderm specification. Comparative quantifications of Dl reveal that Dl concentration levels have changed to an either broader or sharpened distribution across the DV axis in different *Drosophila* species. To rule out the possibility that the nuclear intake of Dl was changed due to divergence in the Toll signaling pathway, we generated *D. melanogaster* embryos with varying nuclear size and density packing and unmodified Toll signaling. Our data show that alterations in the Dl gradient distribution similar to other species can be solely generated by physical changes in nuclear size and packing. Finally, we also show that the activation of the mesodermal gene *snail* from sibling *melanogaster* species occurs at same threshold levels of Dl. Together, our results provide an unexplored route to modifying primary germ layers without the alteration of cis-regulatory sequences of Dl target genes.

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Computer-aided estimation of the motor neuron morphology patterns. Xiao Chang[1], Ashutosh Kale[1], Lauren Dodge[1], Jennifer Brazill[2], Michael D. Kim[2], Gavriil Tsechpenakis[1]. 1) Computer and Information Science, Indiana University-Purdue University Indianapolis, Indianapolis, IN; 2) Department of Molecular and Cellular Pharmacology, University of Miami School of Medicine, Miami, FL.

Our goal is to exploit the single-neuron resolution achieved by MARCM to create a comprehensive morphological categorization of motor neuron subtypes in the Central Nervous System (CNS) of the Drosophila larva. Such morphology-based classification of motor neurons allows for their automated, unbiased tracing and identification in complex single-cell resolution imagery, an essential step towards the bottom-up reconstruction of a fully annotated brain. The morphological features that we use to classify motor neuron subtypes involve the topological relationship between compartments and the reference ventral nerve cord (VNC) midline: relative positions between soma, axon, dendrites and reference; direction and extent of the axon; the extent and approximate shape of dendrites. The major challenge in our task is the high variability between the morphology of neurons of the same subtype, and the morphological similarities between motor neurons of different subtypes. The problem is translated computationally as clustering neuron morphologies into distinct classes, given the relatively limited number of manually annotated neurons that can be used for computer training. Our solution consists of a Machine Learning-based classification framework, namely a hidden Conditional Random Field, which incorporates the above features while avoiding the accurate estimation of shapes and locations in three dimensions with image processing. Our study has revealed so far nine distinct morphological classes that span across 30 motor neuron subtypes, independently from the target muscle and the terminal synaptic bouton morphology.

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Physical mechanisms shaping the *Drosophila* dorsoventral compartment boundary. Christian Dahmann[1,2], Maryam Aliee[3], Jens-Christian Röper[1], Katharina Landsberg[1], Constanze Pentzold[1], Thomas Widmann[1], Frank Jülicher[3]. 1) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 2) Institute of Genetics, Dresden University of Technology, Dresden, Germany; 3) Max Planck Institute for the Physics of Complex Systems, Dresden, Germany.

Separating cells with distinct identities and fates by straight and sharp compartment boundaries is important for growth and pattern formation during animal development. The physical mechanisms shaping compartment boundaries, however, are not fully understood. We combine theory and quantitative experiments to investigate the roles of different mechanisms to shape compartment boundaries. Our theoretical work shows that cell elongation created by anisotropic stress, cell area pressure, cell proliferation rate, orientation of cell division, and cell bond tension all have distinct effects on the morphology of compartment boundaries during tissue growth. Our experiments using the developing *Drosophila* wing reveal that the roughness of the dorsoventral compartment boundary is dynamic and that it decreases during development. By measuring tissue relaxation in response to laser ablation of cell bonds at different developmental times, we demonstrate that decreased boundary roughness correlates with increased cell bond tension along the compartment boundary. Finally, by using experimentally determined values for cell bond tension, cell elongation and bias in orientation of cell division in simulations of tissue growth, we can reproduce the main features of the time-evolution of the dorsoventral boundary shape. We conclude that a local increase of cell bond tension along the interface as well as global anisotropies in the tissue contribute to shaping interfaces in cell networks. We propose a simple scenario that combines time-dependent cell bond tension at the boundary, oriented cell division, and cell elongation in the tissue that can account for the main features of the dynamics of the shape of the dorsoventral compartment boundary.

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Inverse Regulation of Target Genes Distant from the Dpp Morphogen Source. 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; 2) Department of Molecular Biology, Princeton University.

Morphogenetic capacity of Dpp is contingent upon how an extracellular gradient is established and read across the A-P axis in the wing disc. It can thus pattern the cellular field by modulating gene expression in a concentration-dependent manner. Furthermore, the profile of the transcriptional response to the graded activity of Dpp relies upon two counter active gradients of pMad and Brk. However the conclusions, thus far, have relied heavily on a region proximal to the source in the wing pouch,. Earlier studies have assumed that the target gene expression in the regions distant from the source is independent of the signaling influence. We demonstrate that this supposition is clearly incorrect i.e. Dpp signaling is active all across the patterning field including at the periphery of the wing disc. We show that the current patterning model is inadequate to explain the expression pattern of Dpp targets, such as vestigial and spalt (sal), in lateral regions of the wing disc far from the Dpp source where Brk levels peak. Most importantly, in a classic 'role reversal' mode, the expression of the same targets is positively regulated by Brk and negatively by Dpp signaling. How is this opposite regulation achieved? By studying sal expression in the different regions of the wing disc, we identify a new mechanism where a classic Dpp target is regulated through an enhancer that contains neither Brk nor Mad-Med-Shn complex binding sites. We provide evidence that Brk induces expression of sal at the periphery of the wing disc indirectly through repression of a negative regulator (NRS). On one hand, NRS represses sal 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.

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Association Mapping to Identify New Leg Development Genes. Megan Leach, Nathaniel Grubbs, Xin Su, Tiffany Petrisko, Catherine Longo, James Mahaffey. Genetics Department, North Carolina State University, Raleigh, NC.

In order to further understand how genes, pathways, and networks establish and differentiate appendage systems, we used a genome wide association study to identify previously unknown genes that are involved in establishment and differentiation of Drosophila legs. Femur, tibia, and tarsal segments of the first (T1) and second (T2) thoracic leg were measured from a total of 12 males and 12 females of 117 inbred lines from the Drosophila Genetic Reference Panel (DGRP). Natural variation in proportionality of leg segments was used to conduct association mapping of the variance in segment proportionality between DGRP lines. We examined proportionality of segments instead of total leg length to 1) avoid only looking at larger vs. smaller flies, and 2) the proportionality variation studied can help to better explain the effect of altering Wingless or Decapentaplegic signal strength in the current bullseye model of Drosophila leg development. A change in the signal strength can alter the proportion of Drosophila imaginal discs expressing the leg determinant genes homothorax, dachshund, or Distal-less. Single Nucleotide Polymorphisms (SNPs) with the lowest p-values across male and female T1 and T2 were chosen for further study. Genes near SNPs of interest were identified independently for each leg segment analyzed. After candidate genes were identified, we used in situ hybridization to determine gene expression in Drosophila imaginal discs. RNAi lines, obtained from Bloomington and Vienna Stock Centers, were crossed with several GAL4 drivers for loss-of-function analysis. We identified genes that, when reduced, resulted in phenocopies with deleted leg segments, reduction of segment leg lengths and altered leg segment morphology, as well as, some that altered antenna segments and caused deformation of wing. Our findings include several newly discovered genes, implicating new genetic pathways with specific effects on leg development.

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Domain specific E3 ubiquitin ligase mediated Wingless degradation promotes Dorso-Ventral lineage in the developing *Drosophila* eye. Meghana Tare[1], Madhuri Kango-Singh[1,2,3], Amit Singh[1,2,3]. 1) Dept of Biology, Uninversity Dayton, 300 College Park Drive, Dayton OH; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton OH; 3) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton,300 College Park Drive, Dayton OH.

During early eye development, axial patterning transforms a single sheet of organ primordium cells to a three-dimensional organ by generating Dorso-ventral (DV), antero-posterior (AP), and proximo-distal (PD) axes. Drosophila eye anlagen initiates with a ventral ground state on which the dorsal eye fate is established, which requires a large number of eye specific proteins. Members of the Notch signaling pathway, Lobe (*L*; PRAS40 in vertebrates) and Serrate (*Ser*; Jagged-1 in vertebrates), play an important role in ventral eye growth and development. Loss of function of *L*/*Ser* results in loss of ventral half of the eye. In a genetic modifier screen, *cullin-4* (*cul-4*) was identified as a modifier of *L* mutant phenotype in the ventral eye. *cul-4* encodes an E3 ubiquitin ligase - an enzyme that ligates ubiquitin molecules to the proteins targeted for degradation. However, the pathway through which *cul-4* exerts its effects on L is not known. Using *Drosophila* eye as a model system, we characterized the functions of *cul4*, and its interactions with *L* using loss and gain of function approaches. Our studies suggest that *cul-4* acts downstream of *L*, and promotes cell survival in the ventral region of the developing eye by targeting Wingless (Wg) signaling components for degradation. Here we present a novel mechanism of DV specific ubiquitin mediated protein degradation that promotes and maintains dorsal ventral (DV) lineage in the developing early eye field.

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Fat and Mitochondrial Complex I component NdufV2 interact to regulate planar cell polarity. Anson Sing[1], Maïlis Bietenhader[2], Lacramioara Fabian[3], Robyn Rosenfeld[1], Julie Brill[3], G Angus McQuibban[2], Helen McNeill[1]. 1) Molecular Genetics, University of Toronto, Toronto, ON, Canada; 2) Department of Biochemistry, University of Toronto, ON, Canada; 3) Program in Developmental & Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada.

Planar cell polarity (PCP) is a form of tissue organization that is important for the proper development and function of organs. The cell adhesion molecule Fat has been previously identified as a mediator of PCP signaling; however the mechanisms and functions by which fat effect PCP signaling remain unclear. By yeast-2-hybrid and genetic screens we uncover NdufV2, a core component of Mitochondrial Complex I, as a Fat interactor. Depletion of NdufV2 in the Drosophila eye and wing causes PCP defects highly reminiscent of fat hypomorphic phenotypes. We also find that NdufV2 regulates the expression of four-jointed, the only known target of Fat signaling in PCP. Furthermore, loss of Fat or NdufV2 leads to an increase in Reactive Oxygen Species, which are implicated as signaling molecules in diverse pathways. We also detect fragments of Fat localized in the mitochondria. These data together suggest a model whereby cleavage and mitochondrial import of the Fat cytoplasmic domain leads to ROS-dependent signals essential for PCP.

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***oskar* translational activation: a 5' activating region directs translational activation throughout the oocyte.** Matt Kanke, Goheun Kim, Young Hee Ryu, Paul Macdonald. Molecular Cell and Developmental Biology, University of Texas at Austin, Austin, TX.

Embryonic patterning is dependent on proper regulation of *osk* RNA expression. *osk* is transcribed in the nurse cells, transported to the oocyte, and subsequently localized to the posterior of the oocyte. A program of translational repression and activation restricts Osk protein accumulation to the posterior of the oocyte. Repression is mediated by Bruno, which binds to sites in the *osk* mRNA 3' UTR. Activation relies on multiple types of regulatory elements, positioned in a 5' region and in the 3' UTR. The element(s) in the 5' region, which is defined by large deletion and inversion mutations, is thought to act only at the posterior of the oocyte (Gunkel et al., 1998, Localization-dependent translation requires a functional interaction between the 5' and 3' ends of *oskar* mRNA. *Genes Dev.* 12, 1652-1664). To better understand the role of the 5' region we tested a series of GFP reporter transgenes, with or without the *osk* 5' region. Surprisingly, inclusion of the *osk* 5' region causes enrichment of GFP throughout the oocyte, not just at the posterior pole. Oocyte enrichment is abolished by the 62 nt inversion mutation that defines the 5' regulatory element. The inversion mutation has a much lesser effect on GFP levels in the nurse cells. Thus, the *osk* 5' region provides one or more elements that specifically activate translation in, and throughout, the oocyte. We suggest that expression of *osk* specifically at the posterior relies on at least two different forms of activation. One makes the mRNA translationally competent throughout the oocyte; this form of activation is obscured in the *osk* mRNA by concomitant Bruno-mediated repression. A second form of activation involves the release from Bruno repression specifically at the posterior of the oocyte, allowing for accumulation of Osk protein.

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Local trafficking sorts germ plasm RNPs into distinct cortical domains. Jack J. Lee, Kristina S. Sinsimer, Shawn C. Little, Stephan Y. Thiberge, Eric F. Wieschaus, Elizabeth R. Gavis. Princeton University, Princeton, NJ.

RNA localization is a conserved mechanism for generating cell and developmental polarity. Although different mRNAs may have overlapping patterns of localization, it is not known whether transcripts with similar destinations are co-transported. In the process of germ plasm assembly that occurs during late stages of *Drosophila* oogenesis, *oskar (osk)* and *nanos (nos)* mRNAs accumulate concurrently at the posterior oocyte cortex using a common localization machinery, suggesting that they might co-habit the same transport particles. Using a combination of live imaging and high sensitivity fluorescence in situ hybridization to visualize *osk* and *nos* at high resolution, we find that the two mRNAs form distinct RNP particles within the oocyte cytoplasm. Upon localization at the posterior cortex, *nos*, but not *osk*, associates with Vasa (Vas) protein and the two types of RNPs occupy non-overlapping domains. To explore the basis for these unique distributions, we visualized fluorescently labeled *osk*, *nos*, and Vas in live oocytes using 4D two-photon microscopy. While previous studies have shown that posterior localization of germ plasm RNPs is maintained by the actin cytoskeleton, this high-resolution analysis revealed surprisingly that localized *osk* and *nos* RNPs undergo directed movements. Mutational and pharmacological analyses showed that this motility is microtubule and dynein-dependent. When dynein function is disrupted, *osk* and *nos* RNPs overlap, indicating that the observed motility is necessary for the segregation of *osk* and *nos* RNPs. This dynein-dependent organization of *osk* and *nos* RNPs in distinct domains is maintained into embryogenesis. During embryogenesis, *nos* is incorporated into germ cell progenitors, where it functions in germline development. In contrast, *osk* is not enriched in the germ cells, consistent with its prior role in nucleating germ plasm in the oocyte. We propose that local trafficking at the posterior cortex of the late oocyte sorts germ plasm RNPs into distinct domains that facilitate their functions in germline development.

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The Drosophila pan gu kinase complex regulates RNP stability via ubiquitin-dependent proteolysis. Risa Broyer[1], Brian Sato[2], Jim Wilhelm[1]. 1) Section on Cell and Developmental Biology, UC San Diego, La Jolla, CA; 2) Dept of Molecular Biology and Biochemistry, UC Irvine, Irvine, CA.

While mRNA is considered to be quite labile, maternal mRNA is highly stable with a half-life greater than two weeks in the oocytes of some organisms. This stability is thought to be due to the protective effects of the protein subunits of maternal RNA-protein (RNP) complexes. While an asset when the oocyte is stockpiling transcripts to drive early embryogenesis, this mRNA stability poses a problem for the maternal to zygotic transition when many maternal messages are degraded to shift to zygotic transcriptional control. Previous studies have focused on defining the pathway for maternal mRNA degradation: activation of the protein kinase, Pan gu, triggers translation of the RNA binding protein, Smaug, which in turn recruits the CCR4/Twin deadenylase causing message destruction. However, these studies also demonstrated that there is an additional pan gu dependent branch in the pathway that is required for mRNA degradation. We have found that in addition to regulating mRNA stability, pan gu is required for ubiquitin-mediated degradation of three subunits of the maternal RNP complex - the translational repressor, Cup, the RNA helicase, Me31B, and the LSm domain protein, Tral. In order to identify components of this pan gu dependent, smaug-independent pathway, we screened for mRNA degradation mutants where Smaug protein was expressed normally. This screen identified numerous regulators and components of the meiotic anaphase promoting complex, an E3 ubiquitin ligase. All of these mutants were defective in ubiquitin-mediated degradation of RNP subunits consistent with the meiotic APC being an important regulator of RNP stability. Furthermore, this effect is unlikely to be secondary to cell cycle arrest since meiosis is completed in one of our APC regulators. These results suggest that mRNA stability in the early embryo is regulated by both activating mRNA degradation factors, such as Smaug, and removing RNA stabilizing factors via ubiquitin mediated proteolysis.

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Messenger RNA nuclear retention as a novel facet of the DNA damage response. Eric Lecuyer[1,2,3], Mélanie Douziech[1], Carole Iampietro[1], Neal Cody[1], Xiaofeng Wang[1], Moineau-Vallée Karine[1,2], Henry Krause[4]. 1) Systems Biology Research Axis, IRCM, Montréal, QC, Canada; 2) Département de Biochimie, Université de Montréal, Montréal, QC, Canada; 3) Division of Experimental Medicine, McGill University, Montréal, QC, Canada; 4) Donnelly CCBR, University of Toronto, Toronto, ON, Canada.

The subcellular trafficking of mRNAs to specific destinations in the cell is emerging as an important and prevalent layer of gene regulation. While mRNA transport is often coupled to localized translation, which enriches the encoded proteins in the corresponding region of the cell, regulated mRNA localization may also serve as a mechanism to prevent translation of specific messages. In a previous global study of mRNA localization in Drosophila embryos, we identified a group of zygotically transcribed mRNAs that accumulate in nuclei that are eliminated from the forming embryonic epithelium into the underlying yolk by a process of nuclear fall-out. This serves as a quality control mechanism to eliminate damaged cells from the somatic precursor pool during development. The mRNA nuclear accumulation phenotype is strongly induced in embryos treated with genotoxins and is dependent on the function of the DNA-damage response regulator Checkpoint kinase-2 (Chk2). Nuclear retention blocks the translation of this group of mRNAs, which includes transcripts encoding core histones and developmental regulatory proteins. In the case of histone mRNAs, our results suggest that nuclear targeting stems from an interference with the function of specialized regulatory factors involved in histone transcript maturation and nuclear export. We conclude that mRNA nuclear retention, via the inactivation of specific mRNA export pathways, represents a new layer of regulation within the DNA damage surveillance system that is crucial for preserving genome integrity in eukaryotes.

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Identification of chemical compounds that inhibit Ago2-mediated small RNA silencing in *Drosophila*. Christophe Antoniewski[1], Caroline Jacquier[1], Anne-Laure Bougé[1], Fabrice de Chaumont[2], Jean-Christophe Olivo-Marin[2], Hélène Munier-Lehman[2], Clément Carré[1], Hélène Thomassin[1]. 1) Developmental Biology Laboratory, CNRS UMR 7622 - University Pierre & Marie Curie, 75252 Paris CDX 05, France; 2) Institut Pasteur, 25 rue du Docteur Roux 75724 Paris CDX 15, France.

MicroRNAs fulfill essential regulatory functions in metazoan development and homeostasis. Defects in miRNA silencing machinery or in miRNA expression have been associated to development abnormalities, genetic diseases and cancers. In *Drosophila*, miRNAs are predominantly loaded in Argonaute-1-containing RNA induced silencing complexes (RISCs), which they guide for translational inhibition or destabilization of complementary messenger RNAs. In addition, the miRNA silencing pathway is partially overlapping with the RNAi pathways in this organism, as miRNAs may also associate in part with Argonaute-2, the mediator of RNA interference. We set up a miRNA-repressed reporter system in which a single inducible promoter directs the expression of the GFP fluorescent protein and of two artificial miRNAs perfectly matching the GFP coding sequences. We showed that strong self-silencing of the resulting automiG reporter requires Drosha and Dicer-1 functions and involves exclusively the Argonaute-2 RISC complex loaded with the anti-GFP miRNAs. Hence, automiG provides a powerful system that reports in vivo for both miRNA biogenesis and Ago-2 mediated silencing. We used the automiG reporter as a biosensor to screen a chemical library of 15’104 compounds and identified 29 small molecules that strongly inhibit miRNA silencing, out of which 4 also inhibit RNA interference triggered by long double-stranded RNA. These molecules may be used to further dissect the overlapping small RNA silencing pathways as well as to develop therapeutics of diseases linked to miRNA overexpression.

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Forward genetic screens for genes affecting nonsense mediated mRNA decay reveal Smg6 is not an essential decay factor. Kimberly A. Frizzell, Shawn Rynearson, Mark M. Metzstein. Dept Human Gen, Univ Utah, Salt Lake City, UT.

Nonsense mediated mRNA decay (NMD) is a cellular surveillance mechanism that targets specific mRNAs for rapid degradation. Targets of NMD consist primarily of mRNAs containing premature termination codons (PTCs), typically resulting from genomic mutation, frameshifts, and alternative splicing. Evolutionarily conserved trans-acting factors involved in NMD were identified in suppressor screens in yeast and *C. elegans*. Of the six NMD genes conserved in *Drosophila*, null mutations in *Upf1* and *Upf2* are lethal, while loss of *Upf3* or *Smg1* are viable. Alleles of *Smg5* and *Smg6* have yet to be characterized.

To identify alleles of known NMD genes and also new genes involved in NMD, we have performed forward genetic screens of the X and right arm of the 3rd chromosome. To detect mutations affecting NMD, we use an NMD-sensitive fluorescent reporter whose expression increases in NMD mutants. We combine this reporter with a mosaic approach, allowing us to both bypass developmental defects associated with loss of NMD, and to identify mutations in the F1 generation, greatly increasing screen throughput. We have isolated 31 alleles on the X-chromosome which affect NMD. We have found these are all alleles of known NMD genes *Upf1*, *Upf2*, and *Smg1*. On 3R we have isolated 13 lines. Six lines carry alleles of *Smg6*, the only known NMD factor on 3R, and are the first alleles of *Smg6* in *Drosophila*. *Smg6* is an endonuclease that cleaves target mRNAs near the PTC, and we find all our mutations disrupt the conserved PIN-endonuclease domain. We have found that null alleles of *Smg6* are viable and fertile, and that loss of *Smg6* has only a moderate affect on NMD function, suggesting that Smg6 has an auxiliary role in NMD. This alters the current NMD model, which proposes Smg6 is the sole endonucleolytic factor required for degradation, and suggests other degradation pathways may be involved in NMD *in vivo*. Additionally, we have found the other seven lines we have identified on 3R complement *Smg6*, indicating we have isolated a new gene or genes involved in NMD.

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Zfrp8 and piRNA pathway components in Drosophila hematopoiesis. Svetlana Minakhina, Ruth Steward. Waksman Inst, Rutgers Univ, Piscataway, NJ.

Zfrp8/PDCD2 is a conserved factor with an essential function in several types of stem cells. In Drosophila, *Zfrp8* mutants were identified by severe abnormalities of the hematopoietic organ, the lymph gland, where it is required for HSC maintenance. We have also established that *Zfrp8* is essential in somatic and germ line stem cells in the ovary. Despite the conserved structure of Zfrp8/PDCD2 across eukaryotes, the molecular function of the protein is still unknown. We found that *Zfrp8* genetically interacts with the Argounaute family member, *piwi*. To test if *piwi* and piRNA pathway may regulate hematopoiesis we determined the mutant and knock-down phenotypes of several pathway components, including *piwi, AGO3, aub, zuc and spn-E*. Lack of each factor causes distinct abnormalities in hemocyte differentiation suggesting that the piRNA pathway is essential not only in germ cell differentiation, but also in hematopoiesis.

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Essential and equivalent roles for ligands signaling through the EGFR in Drosophila development. Christina L. Austin, Amanda A. Simcox. Department of Molecular Genetics, The Ohio State University, Columbus, OH.

Drosophila Epidermal Growth Factor Receptor (dEGFR), the sole fly homolog of the vertebrate EGFR/ErbB receptor family, is a receptor tyrosine kinase that signals through the canonical Ras/MAPK pathway, mediating diverse developmental processes by communicating both proliferative and differentiation cues. Dysfunction of the pathway is commonly implicated in human cancers, including amplification of the orphan receptor ErbB2/Neu. dEGFR shares a structural configuration with ErbB2/Neu, which differs from that of other ErbBs, and has been described as 'poised' to dimerize in the absence of ligand binding. Here we provide definitive evidence that dEgfr is absolutely dependent on its ligands for signaling: quadruple mutant embryos that lack all EGF-like ligands are phenotypically indistinguishable from receptor null embryos, ruling out the possibility that an unliganded fly receptor is active. This comparison reveals a previously undescribed role for the ligand *keren (krn)* in early development, and further investigation reveals a cryptic role for krn in wing development. Yet, while essential, the ligands do not appear to have unique roles. Surprisingly, we found that mutants of the neuregulin-like ligand *vein (vn)* can be rescued by expression of any one of the three TGFα-like ligands, if expressed under the control of the native *vn* promoter. Optimal rescue was achieved with the higher-affinity TGFα-like ligands by reducing the dose. This functional equivalence suggests, therefore, that unique outcomes attributed to each ligand are more dependent on expression pattern (and cellular context) than qualitatively distinct properties of individual ligands and favors the idea that different signaling outcomes are purely quantitative effects.

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Trafficking of the EGFR ligand Spitz to distinct membrane domains regulates signaling capacity in polarized tissues. Josefa Steinhauer[1], Jessica Treisman[2]. 1) Department of Biology, Yeshiva College, New York, NY; 2) Skirball Institute of Biomolecular Medicine, NYU Langone Medical Center, New York, NY.

EGFR ligands undergo complex processing during their maturation to active signaling proteins. Like the mammalian ligands, the predominant *Drosophila* EGFR ligand Spitz (Spi) is produced as a transmembrane precursor. In the secretory pathway, Spi is proteolyzed within its transmembrane domain, and the soluble active ligand is released into the secretory lumen. The soluble ligand has been shown to be palmitoylated in a late secretory compartment, and palmitoylation mediates membrane association at the cell surface following secretion. We have found that the precursor can reach the cell surface in the absence of proteolysis but is unable to signal, even when it is not palmitoylated. It is unclear why the transmembrane precursor is inactive, while membrane association of the processed ligand via the palmitate group promotes activity. We generated a panel of chimeric Spi constructs containing the Spi extracellular region and exogenous transmembrane domains. All the chimeras can activate the EGFR, despite varying orientation and distance of the Spi domain from the membrane. Thus, the presence of a transmembrane domain does not inhibit signaling, and orientation of the Spi domain with respect to the membrane is not determinative. Despite the fact that the Spi transmembrane precursor is unable to activate the EGFR in polarized imaginal tissues, we have found that it can signal in a tissue culture assay. Thus, tissue polarity may be a determinant for signaling ability in vivo. In support of this hypothesis, we have found that our chimeric Spi constructs localize to the basolateral membrane, whereas unprocessed Spi precursor localizes apically. We currently are investigating whether active processed Spi and the EGFR also localize basolaterally, and whether the intracellular domain of the precursor contains apical targeting sequences. Together, our data support the model that productive signaling is limited to the basolateral compartment.

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Mechanisms of Evi/Wls mediated Wnt-secretion - novel pathways beyond bulk secretion. Julia C. Gross, Varun Chaudhary, Michael Boutros. Division Signaling and Functional Genomics, German Cancer Research Center (DKFZ), Heidelberg, Germany.

During development, cells need to communicate to transform an undifferentiated sheet of cells into a functional, heterogenous type of tissue. This is achieved by spatially restricted secretion of morphogens, such as proteins of the Wnt family. These secreted glycoproteins are required for a variety of developmental processes, which are highly conserved from fly to human. How hydrophobic Wnt proteins spread in the extracellular space has been a long-standing question. Different hypotheses have been proposed to explain how Wnt could travel in the extracellular space, such as coating of lipoprotein particles and/or as part of secreted vesicles. Nevertheless, the mechanism of Wnt secretion and gradient formation remains poorly understood. Furthermore, a complete set of proteins involved in various aspects of Wnt trafficking still remains to be identified. We have previously identified the transmembrane protein Eveness interrupted (Evi/Wls) as a core component of the Wnt secretion process. Evi shuttles Wnt proteins from the Golgi to the plasma membrane and is then recycled by the retromer complex. Newer data suggest that Evi/Wnt complexes are not just dissociating at the plasma membrane and that Evi, as a multipass membrane protein can transfer from one cell to another. Our current research focuses on understanding how and when Evi releases Wnt on the cellular level. We are interested in finding additional factors required for further processing of Wnt proteins and trafficking of Evi/Wnt complexes for the secretion of functional signaling entities. Here we present our data on this novel functional role of Evi in Wnt secretion and our RNAi screening approaches along with candidates that are under investigation.

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Wingless is secreted on exosome-like vesicles in *Drosophila* S2 cells. Karen Beckett[1], Solange Monier[2], Hannah Green[1], Roland LeBorgne[2], Jean-Paul Vincent[1]. 1) Developmental Biology, National Institute for Medical Research, London, United Kingdom; 2) CNRS UMR 6061, Université de Rennes 1, 35043 Rennes Cedex, France.

Cell communication is essential for embryonic development and normal physiology of multicellular organisms. Wingless (Wg), the main *Drosophila* Wnt, controls several developmental processes including growth, differentiation and survival. Wg is lipid-modified and thus tightly associated with cellular membranes. However, Wg can be released to act at a distance from its source of production. Therefore an outstanding question is how Wg is packaged for release from producing cells. To answer this question we have revisited the argosome model of Wg secretion and tested whether Wg is secreted on exosome-like vesicles. Exosomes are 40-100nm microvesicles that are produced in multivesicular bodies (MVBs) and released by fusion of MVBs with the plasma membrane. We have found that Wg is secreted on vesicles resembling exosomes by *Drosophila* S2 cells. These vesicles have the density, size and morphology of exosomes and can activate downstream signalling. Proteomic analysis of Wg-containing vesicles has identified known mammalian exosome proteins such as those involved in membrane trafficking, signalling and metabolism. We found that membrane trafficking proteins such as Hrs, Vps28 and Rab35 are present in Wg-containing exosomes. However, so far they appear dispensable for exosome production. Wg secretion requires a multipass transmembrane protein called Evi that is thought to transport Wg from the Golgi to the plasma membrane. Retromer-mediated recycling of Evi is subsequently required to replenish Evi levels in the Golgi. We found that Evi is secreted on exosome-like vesicles in S2 cells independently of Wg. We propose a model whereby Evi is continually trafficked to MVBs and released on exosomes. We suggest that, in Wg producing cells, following transport from the golgi to the plasma membrane, Evi and Wg would be co-endocytosed, taken to MVBs and packaged into exosomes for release. We are currently developing tools to test this model in vivo.

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Polarized biosynthesis and secretion of Collagen IV during organ morphogenesis. Sally Horne-Badovinac, David Lerner, Darcy McCoy, Gary Gerlach II. Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL.

The elongation of tissues and organs along a particular body axis is a common theme in the development of multicellular organisms. Though initially spherical, Drosophila egg chambers lengthen along their anterior-posterior (A-P) axes to create the elongated shape of the mature egg. Egg chambers are composed of an internal germ cell cluster surrounded by an epithelial layer of follicle cells. At the onset of egg chamber elongation, the entire organ undergoes an unusual rotation perpendicular to its A-P axis. It has been proposed that this rotation leads to the remodeling of the Col IV matrix on the outside of the egg chamber, which in turn promotes elongation morphogenesis. However, the molecular and cellular mechanisms that underlie Col IV matrix remodeling remain obscure. Starting from a mutation that specifically blocks the secretion of Col IV, we have found that Col IV biosynthesis occurs in a specialized sub-region of the endoplasmic reticulum (ER) near the basal follicle cell surface. Interestingly, these basal ER cisternae are also polarized within the tissue plane, such that they are enriched at the back of each migrating follicle cell during rotation. We have further identified a specialized golgi population associated with the basal ER, and have shown that disrupting proteins associated with the basal golgi leads to the mistrafficking of Col IV to the apical surface. Together these data demonstrate a striking compartmentalization of the secretory machinery that controls polarized secretion of Col IV to the basal surface. Moreover, the polarization of this machinery within the tissue plane may lend insight into the mechanisms controlling Col IV matrix remodeling during egg chamber elongation.

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A Novel Role for UDP-GlcNAC in Dpp Signal Antagonism. Gregory B. Humphreys, Kate Monroe, Molly Jud, Anthea Letsou. Human Gen, Univ 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* 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. Considering that Jun is required for LE *dpp* transcription, and as Jun activity and localization are unaffected in *mmy* mutants, we utilized a *mmy Jra* double mutant to specifically test if Jun-initiated LE dpp expression is required to ectopically express *dpp* in a *mmy* mutant background. *mmy Jra* mutant embryos lack both LE and ectopic *dpp* expression, indicating a requirement for an initiating round of LE Dpp to enact ectopic expression. These data suggest that Mmy antagonizes paracrine Dpp in the epidermis, consistent with earlier evidence identifying Dpp-dependent *dpp* transcription in the LE epidermis. To test this model, we assayed embryonic Dpp signaling activity by probing Mad phosphorylation. P-Mad is found broadly in early embryonic epidermis, but undergoes a Mmy-dependent restriction to LE cells prior to dorsal closure. P-Mad remains broad in the dorsolateral epidermis in *mmy* mutant embryos, suggesting that Dpp undergoes a Mmy-dependent transition from paracrine to autocrine signaling in the embryonic epidermis. To identify downstream effectors of Dpp signal restriction, we screened the 25 Drosophila glycosyltransferases potentially utilizing UDP-GlcNAc downstream of Mmy. In embryos depleted of one of these transferases, *dpp* expanded ectopically beyond the LE epidermis, identifying this transferase as a *dpp* antagonist and providing new insights into regulation of Dpp signaling via glycosylation.

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The classic fibrodysplasia ossificans progressiva mutation reveals the latent kinase activity of the *Drosophila* BMP type I receptor Saxophone. Viet Le, Kristi Wharton. MCB Dept, Brown University, Providence, RI.

Bone Morphogenetic Protein (BMP) signaling is important for processes such as cell proliferation, apoptosis, patterning and specification during development. Dysregulation of BMP signaling is implicated in many disease states. In the developing Drosophila wing the BMP type I receptor, Saxophone (Sax) has been shown to exhibit a dual behavior where it can inhibit as well as facilitate BMP signaling. Here, we demonstrate that Sax is unable to phosphorylate Mad, the transducer of the pathway, in response to the BMP ligand Gbb. Two mutations downstream of the GS activation domain of Sax uncover its latent kinase activity in a ligand-dependent fashion. Curiously, both mutations result in ligand-independent type I receptor hyperactivity in the context of other type I receptors. One mutation, K262H, corresponds to the mutation in ALK2 (R206H), the human Sax ortholog that is responsible for the classic form of the bone disease fibrodysplasia ossificans progressiva (FOP). The other mutation, Q263D, corresponds to the standard mutation made in all BMP/TGF-β type I receptors to generate constitutively active receptors. These results suggest that the GS domain is an important region that masks the kinase activity of Sax. We are investigating the possibility that the activity of the Sax kinase is only stimulated when Sax is in a complex with the other BMP type I receptor, Tkv. We are also testing the possibility that Sax acts as a silent co-receptor in conjunction with Tkv, acting to facilitate the binding of heterodimeric ligands (i.e. Dpp:Gbb) in particular. Lastly, we consider how differences in the effect of the classic FOP mutation on Sax and ALK2 kinase activity impact our understanding of FOP.

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The impact of mutational biases and positive selection on the distribution of Copy Number Variants among five worldwide populations of *Drosophila melanogaster*. Margarida Cardoso-Moreira, Jennifer K. Grenier, Andrew G. Clark. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Copy Number Variants (i.e. duplications, deletions and insertions of DNA segments) can create novel genes, alter gene structures, modify gene regulation and dosage, and abolish gene function. They are widespread in genomes and underlie a wide range of phenotypes ranging from lethal to adaptive. In order to understand the impact of mutation and selection in shaping patterns of CNVs, we set out to identify CNVs in the genomes of 92 *D. melanogaster* lines originating from five geographical locations (United States, Netherlands, Australia, China and Zimbabwe). The genomes of these 92 lines were fully sequenced using Illumina paired-end technology and CNVs larger than 25 bp were identified using a bioinformatics pipeline that combined read-pair and split-read methods to identify the exact breakpoints of the CNVs, an essential requirement to understand the functional impact of these variants. We identified ~150,000 variants, mostly small insertions/duplications and deletions, but also complete gene duplications and deletions, retroposed genes and novel gene structures. We carried out extensive validation of these variants by PCR and Sanger sequencing. The distribution of CNVs throughout the genomes, between individuals and between populations, was strongly shaped by both mutational biases and natural selection. Regarding mutation, we found the distribution of duplications and deletions to be highly non-random, with duplication and deletion hotspots identified throughout the genome. We investigated the causes underlying the existence of hotspots by associating their presence with: 1) DNA replication timing; 2) chromatin accessibility; and 3) presence of non-B DNA structures. Regarding selection, we found purifying selection to be pervasive, eliminating most variants associated with functional regions. However, by leveraging haplotype information with levels of population differentiation we also found evidence for positive selection acting on a subset of the CNVs.

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Variation in Genome Structure in *Drosophila yakuba*. Rebekah L. Rogers, Kevin R. Thornton. Ecology and Evolutionary Biology, Thornton Lab, Irvine, CA.

Chromosomal rearrangements, which shuffle the locations of genes within the genome, can cause changes in where and when neighboring genes are expressed. If these rearrangements do not respect gene boundaries, they may also split genes into pieces and combine them with other genetic material, thereby modifying the proteins that the genome produces. We have used paired-end Illumina sequencing reads to identify chromosomal rearrangements in 20 inbred strains recently derived from natural populations of *D. yakuba*. Chromosomal rearrangements can be identified through paired end reads that map in parallel rather than properly paired orientation or which map to distant sections of the genome. Using these criteria we have identified over 200 putative chromosomal rearrangements segregating within the population. Some 15% of these events contain breakpoints that fall within genes, suggesting that the species houses a vast amount of diversity in gene content as well as exceptional variation in genome structure.

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Evolution of New Genes with Essential Functions in Drosophila Development and Reproduction. Sidi Chen, Manyuan Long. Dept Ecology & Evolution, The University of Chicago, Chicago, IL 60637.

Essential genes are often portrayed as conserved and ancient, while younger lineage-specific genes have been considered to be more dispensable and to perform relatively minor organismal functions. It is unclear how essential genes arise and how new genes accumulate essential functions. To investigate the origin and evolution of essential genes, we used newly evolved genes as a model. Because new genes arise continuously through time, and, when first arose, they were expected to be non-essential since their immediate ancestral species survived without them, thus they must subsequently evolve essentiality. However, little is known about the phenotypes and degrees of essentiality for new genes. We identified over five hundred new gene origination events in the last 35 million years. We phenotyped 195 newly arisen genes with RNAi knocking down and found that 30% of lethal. Interestingly, the proportion of lethal genes is similar in every evolutionary age group that we examined. Lethality was highly enriched in the pupal stage, and also found in the larval stages. Lethality was attributed to diverse cellular and developmental defects, such as organ formation and patterning defects. Our data suggested that new genes frequently and rapidly evolve essential functions. In addition, we assayed adult stage phenotypes of the genes that are not essential for pre-adult stage development. We found a large proportion of them played essential roles in reproduction in both males and females. The mechanism for the evolution of essentiality would change with the types of new genes. A de novo gene has to evolve essentiality through neofunctionalization. A duplicated gene, generated from a parental copy, could become essential from the lost of parents, the switch of essentiality from paralogs, subfunctionalization, or neofunctionalization. Evolutionary analyses revealed strong Darwinian selection and structure renovation for these genes, as well as their independent essentiality from parental genes, support the neofunctionalization origin as a primary mechanism.

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A genome wide association study reveals genetic evidence of the mutation accumulation theory of aging in age-specific fecundity in *Drosophila melanogaster*. Mary F. Durham[1], Michael 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.

Senescence is a phenomenon experienced by virtually all organisms and despite decades of research on a myriad of species and age-related diseases, mechanisms of aging remain poorly understood, especially at the genetic level. Evolutionary theories of aging are centered on lifespan and reproduction since organisms must survive long enough to reproduce in order to contribute alleles to subsequent generations and thereby maintain alleles that influence senescence in a population. Additionally, senescence is often believed to be due to trade-offs between lifespan and fecundity or early-age vs. late-age fecundity. Although it is clear that lifespan and age-specific fecundity are genetically controlled, the specific genes influencing natural variation in these traits and their relationship with each other, as well as their influences on aging are largely unknown. The goal of this work was to identify genes influencing mated lifespan and age-specific fecundity in *Drosophila melanogaster*. To do this we completed a genome wide association study (GWAS) using the *Drosophila* Genome Reference Panel (DGRP) to reveal candidate genes affecting these traits. We quantified lifespan as days lived and estimated age-specific fecundity as a two-day egg total every other week for each female until death. Our results indicate that there is extensive natural genetic variation in lifespan and age-specific reproduction. We identified over 2000 candidate single nucleotide polymorphisms (SNPs) involved in lifespan and age-specific fecundity. Our data also provide solid support for the mutation accumulation theory of aging as we see a significant increase in the number of genes contributing to variation in fecundity with increasing age. These results shed light on our understanding of the genetic mechanisms that drive lifespan, age-specific fecundity and senescence.

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Strong Purifying Selection at Synonymous Sites in *D. melanogaster*. David S. Lawrie[1], Philipp W. Messer[2], Ruth Hershberg[3], Dmitri A. Petrov[2]. 1) Dept. of Genetics, Stanford University, Stanford, CA; 2) Dept. of Biology, Stanford University, Stanford, CA; 3) The Ruth and Burce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel.

Synonymous sites are generally assumed to be subject to weak selective constraint. For this reason, they are often neglected as a possible source of important functional variation. We use site frequency spectra from deep population sequencing data to show that contrary to this expectation a substantial proportion of synonymous sites in *D. melanogaster* evolve under very strong selective constraint while few, if any, synonymous sites appear to be under weak constraint. Linking polymorphism with divergence data, we further find that the proportion of synonymous sites exposed to strong purifying selection is higher for those positions that show slower evolution on the Drosophila phylogeny. However, fewer synonymous sites are conserved across the entire tree than expected given the estimated percentage of strongly constrained sites. Together, these results suggest that the strong constraint at synonymous sites is episodic, such that any particular position may spend part of its evolutionary history evolving under tight constraint and part evolving neutrally or almost neutrally. This model of episodic strong selection appears to explain the rates of evolution of synonymous sites better than the alternative model of consistent weak selection.

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Small-scale Hitchhiking Effects in *Drosophila* . Grace Y C Lee, David J Begun, Charles H Langley. Ctr Population Biol, Univ California, Davis, Davis, CA.

Accumulated evidence suggests that beneficial substitutions are common in *Drosophila*. Selective fixation of initially rare alleles leads to a transient reduction of nearby neutral genetic variation, a phenomenon known as “genetic hitchhiking” or “selective sweep”. The physical extent and magnitude of reduced variation depends on the intensity of selection and the time since fixation of the favored variants. Previous estimates of selection coefficient for weakly beneficial mutations suggested that the extent of selective sweep of these prevalent variants is expected to be within hundreds of base pairs, which may play an important role in the overall genome-wide pattern of polymorphism. Here, we used the population genomic data of *D. melanogaster* and *D. simulans*, two species that have similar geographic distribution and demographic histories yet significantly different patterns of genetic variation, to examine the extent of small-scale (several hundreds to a few thousands of base pairs) genetic hitchhiking around substitutions in regions with high recombination. Our preliminary analysis found variants fixed from ancestral polymorphism show a weaker reduction in nearby neutral polymorphism than those that are not. Surprisingly, amino acid fixations at sites that substituted multiple times over the phylogeny showed stronger hitchhiking effects than those at sites that are highly conserved. We also observed substitutions leading to changes in amino acid polarities or charges have different levels of trough in neighboring diversity. Although selection for codon bias is generally viewed as a weak selective force, especially in *D. melanogaster*, its effect on variation reduction is readily detectable. We will also present similar analyses from noncoding regions.

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Molecular Evolutionary Analysis of Flightin Reveals a Novel Protein Motif unique to Pancrustacea. Jim O. Vigoreaux[1], Pedro Alvarez-Ortiz[1], Felipe Soto[1,2]. 1) Department of Biology, University of Vermont, Burlington, VT; 2) Illinois Natural History Survey, Champaign, IL.

Flightin is a thick filament protein that in Drosophila melanogaster is uniquely expressed in the indirect flight muscles (IFM). Flightin imparts rigidity and structural stability to the thick filament and its expression is essential for flight. Given the importance of flight acquisition in the evolutionary history of insects, here we study the distribution and phylogeny of flightin. Flightin has been identified in all hexapods (Collembola, Protura, Diplura and insect Orders Thysanura, Dictyoptera, Orthoptera, Pthiraptera, Hemiptera, Coleoptera, Hymenoptera, Lepidoptera, Diptera) and crustaceans (Orders Anostraca, Cladocera, Isopoda, Amphipoda, Decapoda) examined to date. The presence of flightin in Entognatha, the basal crustacean Daphnia, and derived insect and crustacean orders suggest flightin is widespread in Pancrustacea. It is not present in chelicerates, myriapods, or any phyla outside Arthropoda suggesting flightin dates back to the origin of extant Pancrustacea, ~600 MYA. Flightin is characterized by a well conserved sequence that is 52 amino acids long in hexapods and 48 to 56 amino acids in crustaceans. At least six sites are invariant, including an N-terminal tryptophan (W), two closely spaced tyrosines (Y), and a C-terminal arginine (R). Our data suggest this sequence represents a novel motif, herein referred to as WYR, that is unique to flightin and paraflightin, a putative flightin paralog detected only in decapods. Mosquitoes, sandflies and the amphipod (Gammarus) express two kinds of ESTs but only in the amphipod are the isoform differences within WYR. Phylogenetic analysis suggests that paraflightin originated before the divergence of amphipods, isopods and decapods. In summary, flightin evolved well before the appearance of flight in insects suggesting flightin was secondarily adapted for its flight muscle-specific function in higher dipterans.

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The YAN Network is robust against YAN protein variation in the developing eye. Nicolás Peláez[1,2,3], Hiba Eltahir[1,3], Alec Victorsen[3], Kevin White[3,4], Ilaria Rebay[3,4], Luís Amaral[1,2,3], Richard Carthew[1,3]. 1) Molecular Biosciences, Northwestern University, Evanston, IL; 2) Howard Hughes Medical Institute; 3) Chicago Center for Systems Biology, IL; 4) Universitry of Chicago, Dept. Human Genetics / Dept. for Cancer Research, IL.

A central problem in systems biology is how molecular networks accurately regulate the transition of cells from a multipotent to a differentiated state. The YAN molecular network controls this transition in the developing eye imaginal disc. YAN is an ETS-domain transcription factor that represses expression of key genes such as Dpax2, which are necessary for differentiation. YAN switches from high to low activity as cells transition into photoreceptor or cone differentiation. Modulation of Notch and EGFR signaling alters a variety of YAN regulators as part of this network during the multipotent to differentiated state transition. To quantitatively study the YAN network more directly we used a combination of genetics, confocal microscopy, image processing and computational modeling. We recombineered monomeric YFP to the C-terminus of YAN within a 33 kb genomic transgene. This YAN:YFP transgene fully rescued loss of endogenous YAN. We measured YFP fluorescence within eye disc cells as a proxy for YAN protein abundance. From this we have made several surprising discoveries. First, multipotent cells show remarkably large variation in YAN abundance, even between neighboring cells. Second, differentiated cone cells also show high levels of variation. Moreover, the level of YAN:YFP in cone cells is highly comparable to levels in multipotent cells. Third, changing the transgene copy number results in large changes in YAN:YFP abundance, but has little to no effect on the robustness of cell differentiation. We provide potential mechanisms to explain these observations.

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Quantitative insights into enhancer architecture of dorsal-ventral patterning in Drosophila. Rupinder Sayal[1], Jacqueline Dresch[2], Irina Pushel[1], David Arnosti[1]. 1) Biochem & Molec Biol, Michigan State Univ, East Lansing, MI; 2) Mathematics, Michigan State Univ, East Lansing, MI.

Enhancers facilitate gene regulation by binding sequence-specific transcription factors to generate precise temporal and spatial control of transcription. We are interested in understanding the enhancer function by utilizing thermodynamic models, which compute gene expression as a probability of binding of transcription factors (TFs). We used the enhancer of rhomboid gene for our studies, which is activated by Dorsal and Twist proteins, and repressed by Snail protein. We mutated each activator-binding site singly and measured its effect on gene expression, which showed little attenuation. Subsequently, we mutated two activator binding sites in all possible combinations, which revealed a hitherto unknown pattern of soft spots and idiosyncrasies in this enhancer. We used a global parameter estimation strategy (CMAES) to derive parameters for transcription factor scaling factors and cooperativities, as well as quenching of activators by repressors. To investigate the distance-dependent functions of cooperativity and quenching, various types of mathematical functions were applied. Since the in vivo binding affinity of sites is still unknown, we also tested the model using different thresholds for affinities of binding sites. This allowed us to observe the effects of including or disregarding weaker binding sites. In all, approximately 100 variants of the model were tested. To test the predictive power of parameters, we compared our model predictions with experimental data on 6 other evolutionary variants of this enhancer from sequenced fly species. The parameters were also tested on enhancers of other genes regulated by the same set of transcription factors. To our knowledge, this is the first systematic study based on a rigorous mutational analysis of a single enhancer for thermodynamic mathematical modeling. The results illustrate the power of applying quantitative mathematical models to shed insight on biochemical mechanisms underlying enhancer function.

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Increasing discriminative power in computational evaluation of the BMP activity distribution in wing disks. Alexi Brooks[1,2], Tara Brosnan[1,2], Mohit Bahel[2,3], David Umulis[4], Laurel Raftery[1,2]. 1) School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV; 2) CBRC, MGH/Harvard Medical School, Charlestown, MA; 3) New York University, New York, NY; 4) Department of Agriculture and Biological Engineering, Purdue University, Lafayette, IL.

Bone morphogenetic proteins (BMPs) are morphogens in many tissues of vertebrates and invertebrates. Extracellular BMP activates intracellular R-Smads, via receptor-kinase phosphorylation of R-Smad C-termini. For fly BMPs, Mad is the critical R-Smad. Nuclear levels of receptor-phosphorylated Mad (C-phospho-Mad) are proportional to the level of BMP activity and resultant target gene expression. In the larval wing primordium, the distribution of C-phospho-Mad is a gradient ranging from two high level peaks straddling the anterior/posterior compartment boundary to low levels near the edges of the wing pouch. The characteristics of this gradient, through various downstream targets, define the locations of the veins of the adult wing. Recent computational analyses have demonstrated that a linear representation of the posterior gradient may be characterized simply by exponential decay, but the anterior gradient remains an open question. We have developed additional computational tools to quantitatively characterize the C-phospho-Mad gradient in two dimensions, for a population of wing disks of the same genotype. To test for subtle alterations in BMP activity gradients, we express a potential modulator from the Ras-MAP kinase pathway in the dorsal compartment, using Apterous-Gal4. Even in the presence of natural cell-to-cell and field-to-field variability, we can now use reliable metrics of gradient length, amplitude, and shape throughout the wing pouch. Availability of such metrics for both compartments allow us to discriminate between models for pathway cross-talk in BMP signaling.

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Reverse-engineering the evolutionary and developmental dynamics of the gap gene system. Johannes Jaeger, Karl Wotton, Anton Crombach, Damjan Cicin-Sain. EMBL/CRG Research Unit in Systems Biology CRG - Centre de Regulació Genòmica Barcelona, Spain.

To gain a mechanistic and quantitative understanding of the genotype-phenotype map is one of the big challenges in biology today. Tackling this challenge requires a quantitative systems-level understanding of the gene networks underlying development across multiple levels, from the molecular to the organismic. This is difficult due to the large number of factors involved. We depend on computational models for this task. I present a reverse-engineering approach, where gene regulatory interactions are inferred from quantitative expression data, using data-driven mathematical models (called gene circuits). We have established that the gap gene network can be consistently reconstructed in this way using both protein or mRNA expression data. Gap gene circuit models in *Drosophila* reproduce observed gene expression with high precision and temporal resolution and reveal a dynamic mechanism for the control of positional information through shifts of gap gene expression domains. My group is extending this approach to a comparative study of the gap gene network between different species of dipterans. No such quantitative systems-level analysis of an evolving developmental gene regulatory network has been achieved to date. I will present results concerning data quantification and modeling of gap genes in the scuttle fly *Megaselia abdita*, and the moth midge *Clogmia albipunctata*. We have created and analyzed quantitative data sets for gap gene expression in both of these species, which are now used for model fitting. Our approach yields precise, quantitative predictions of how changes of gene regulatory feedback affect the timing and positioning of expression domains in these species. These predictions are now being tested experimentally using RNA interference.

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Consequences of enhancer architecture for gene expression dynamics and fitness. Manu Manu[1], Michael Ludwig[1,2], Ralf Kittler[2,3], Kevin White[2,3], Martin Kreitman[1,2]. 1) Ecology and Evolution, University of Chicago, Chicago, IL; 2) Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL; 3) Human Genetics, University of Chicago, Chicago, IL.

During the past decade or so, investigators have identified a number of regulatory features that confer robustness to gene networks. Here we present evidence that the *cis*-regulatory architecture of genes, that is, the number and placement of transcription-factor binding sites (TFBS), promotes the reproducibility of gene expression and buffers genetic and environmental perturbation. Eukaryotic *cis*-regulatory regions often contain TFBS outside the boundaries of enhancers defined by reporter assays. In *Drosophila*, the *cis*-regulatory element driving expression in the second stripe of the *even-skipped* (*eve*) pattern has evolutionarily-conserved binding sites outside the minimal stripe element (MSE). The conservation of these sites suggests that they play a functional role in development. We used recombineering to make constructs that rescue *eve*$^{-}$ lethality and that could be imaged live to investigate the effect of these sites on gene expression dynamics and fitness. We used rescue crosses and quantitative time-course data to show that these binding sites are 1) dispensable for viability but are necessary for 2) precise placement of the stripe and 3) temperature compensation. Our investigation also led to a surprising discovery: we found that *eve*, thought to be a stereotypically-expressed morphogen, is expressed differently in male and female embryos during early development. We traced *eve*'s sex-specific expression to an incomplete compensation of *giant* dosage. However, segmentation itself was found to be sex-independent. This result implies that later autosomal regulation in the segmentation system can correct the deleterious effects of incomplete dosage compensation. Our results show that enhancer architecture is optimized not just to turn genes "on" or "off" but to do so robustly across different environments and genetic backgrounds.

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Epithelial folding during eggshell morphogenesis. Miriam Osterfield[1], XinXin Du[2], Trudi Schüpbach[4,5], Eric Wieschaus[4,5], Stanislav Shvartsman[1,3]. 1) Lewis-Sigler Institute, Princeton Univ, Princeton, NJ; 2) Department of Physics, Princeton University, NJ; 3) Department of Chemical and Biological Engineering, Princeton University, NJ; 4) Department of Molecular Biology, Princeton University, NJ; 5) Howard Hughes Medical Institute.

The formation of tubular structures is central to the development of many types of organs. To investigate the mechanisms of tubulogenesis, we examine dorsal appendage formation, the process in which the follicular epithelium surrounding a Drosophila oocyte develops from a simple ovoid surface to a structure with two protruding tubes. Dorsal appendage tube formation is thought to occur by the process of wrapping. Our analysis shows that during this process, the apical surface of follicle cells remains continuous. The dorsal appendage tubes form by lateral cell rearrangements in a spatially organized process of intercalation, coupled to deformation of the sheet. E-cadherin, Bazooka, and myosin proteins all show non-uniform localizations that suggest specific patterns of tension along cell edges within the apical domain. Using a computational vertex model accounting for cell elasticity and tension in epithelial sheets, we show that these experimentally predicted patterns of tension are sufficient to account for the three-dimensional deformation of the apical surface during early stages of tube formation.

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Quantifying the consistency of interactions in the NADP(H) enzyme network across varying environmental conditions. Teresa Rzezniczak, Thomas J.S. Merritt. Department of Chemistry & Biochemistry, Laurentian University, Sudbury, Ontario, Canada.

Interactions between members of biological networks are often quantified under a single set of conditions, however cellular behaviours are dynamic and interactions can change in response to molecular contexts. The extent which environment plays a role in governing these interactions is still unclear. To determine the consistency of network interactions, we examined the enzyme network responsible for the reduction of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH across three different conditions of stress: oxidative stress, starvation and desiccation. We used synthetic, activity-variant alleles in *Drosophila melanogaster* for *glucose-6-phosphate dehydrogenase*, cytosolic *isocitrate dehydrogenase* and cytosolic *malic enzyme* as well as seven different genetic backgrounds to lend biological significance to the data. The response of the NADP-reducing enzymes as well as two downstream phenotypes (triglyceride and carbohydrate concentration) was quantified spectrophotometrically between the control and stressful conditions. Each stressor was found to affect the activities of NADP-reducing enzymes differently: oxidative stress resulted in metabolic re-routing to the pentose phosphate pathway, desiccation caused up-regulation of the NADP-reducing enzymes, whereas no response was observed under starvation. In addition, we found significant changes in the response to the reductions in the NADP-reducing enzymes with differing experimental conditions, suggesting that the way in which the enzymes interact and the amount of NADPH they contribute to the cellular pool changes with differing conditions. These findings indicate that biological network interactions are strongly influenced by the molecular context of the cell and underscore the importance of examining network dynamics.

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The GEF Vav regulates collective cell migration downstream of guidance receptors by locally activating Rac at the leading edge. Cecilia H. Fernandez-Espartero[1], Damien Ramel[2], Marganit Farago[3], Malartre Marianne[1,5], Carlos M. Luque[1,4], Shiran Limanovich[3], Shulamit Katzav[3], Gregory Emery[2], María D. Martín-Bermudo[1]. 1) Centro Andaluz de Biologia del Desarrollo (CABD), CSIC, Sevilla, Spain; 2) IRIC, Université de Montréal, Quebec, Canada; 3) IMRID, Jerusalem, Israel; 4) EMBL, Heidelberg, Germany; 5) Université Paris Sud, France.

Cell migration is essential in the development and maintenance of multicellular organisms. Guidance by spatial cues is essential for the migrating cells to reach the correct target tissue. Thus, during wound healing and immune responses cells are guided to site of injury and tumor cells may be guided to target tissues. One of our aims is to understand how guidance signals control cell migration. For this purpose, we use the migration of the border cells (BCs) of the Drosophila ovary as a model. BCs are a group of 6-8 anterior specialized follicle cells that at stage 9 of oogenesis delaminate and migrate between the germline toward the oocyte. This directed cell migration is controlled by two receptor tyrosine kinases, PVR and EGFR, which are activated in BCs in response to their ligands produced by the oocyte. Although there is some evidence suggesting that Rac and its activator Mbc can act as mediators of these guidance signals, the molecular mechanisms by which the activity of these two receptors control Rac are still unclear. Recently, we have identified Vav, a guanine nucleotide exchange factor (GEF) for Rac, as putative downstream target of Pvr activation. Loss of Vav function impairs BC migration. Two hybrid and co-IP experiments show that Vav interacts physically with Pvr. Furthermore, we find that stimulation of Pvr in S2 cells induces Vav activation. Finally, we show by FRET analysis that Rac activity is reduced in the absence of Vav and that ectopic Vav activation leads to a non-polarized distribution of Rac activity. Taken altogether, we propose a model in which Vav acts as a signal transducer that couples signalling downstream of guidance receptors to Rac activation during directed cell migration.

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Signal regulation by Rab5 GEFs in Drosophila melanogaster. Katja L Vogt, Martin P Zeidler, Elizabeth Smythe. Biomedical Sciences, University of Sheffield, Sheffield, United Kingdom.

A fundamental concept of the organisation and regulation of cellular communication is the control of signalling by endocytosis. The process of endocytic membrane trafficking is intimately and bidirectionally linked to cell signalling. Crucially, spatial compartmentalisation enables the cell to shuttle distinct signalling complexes into specific intracellular locations. In order to elicit specific responses not only the separation of signals in early and late endosomes, but also their distinct localisation in micro-/ sub- domains (signalosomes). These signalosomes can influence distinct receptor signalling via a variety of exclusive downstream signalling pathways. The small GTPase Rab5 protein not only controls early endosomal dynamics but is a central hub for unique regulatory signalling platforms. Guanine exchange factors (GEFs) that exchange GDP for GTP act as Rab5 activators and appear to be initial important regulators of Rab5.

We are investigating the interplay of endocytosis and signalling in Drosophila with a particular focus on the JAK/STAT pathway and the role of the four Drosophila rab5GEFs (rabex5, alsin, sprint and dRME-6). Our initial results indicate that modulation of these GEFs differentially affects JAK/STAT signalling outputs. We propose that each GEF establishes and activates distinct signalosomes, allowing discriminated signalling. Current studies centre on structure / function analysis of the GEFs to determine the mechanism underpinning the role of endocytosis in JAK/STAT signalling.

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Endocytic regulation of collective cell migration. Gregory Emery[1], Damien Ramel[1], Xiaobo Wang[2], Denise Montell[2]. 1) IRIC, University of Montreal, Montreal, QC, Canada; 2) Department of Biological Chemistry,Center for Cell Dynamics, Johns Hopkins School of Medicine, Baltimore, MD.

Cell migration is a fundamental process to organize tissues but also in development of diseases such as cancer. Cells can migrate individually but growing evidences suggest that many cells types, including tumorigenic cells migrate collectively. Collective migration implies that individual cells coordinate their signaling to migrate as a coherent structure in response to attractive cues. Polarized activity of the small GTPase Rac has been demonstrated to control collective guidance in vivo, however, nothing is known about the mechanisms that restrict Rac activation and localization during collective migration. Here, we show that Rac activity and polarization is regulated by a trafficking cycle involving the recycling endosome compartment and its regulating small GTPase Rab11. Real time analysis of Rac activity by Fluorescence Resonance Energy Transfert (FRET) in drosophila border cells reveals that expression of a dominant negative version of Rab11 (Rab11SN) induces a complete depolarization of Rac activity. Morevover, Rab11 loss of function induces a redistribution of actin protrusions during migration. We hypothesize that loss of polarity of Rac in Rab11SN background induces incoherent generation of forces thereby blocking migration. To test this model, we used a photoactivable version Rac. We demonstrated that local activation of Rac fails to rescue migration in a recycling deficient group indicating that Rab11 is required for generation of polarized forces that drive migration. Morevover, these results implicate Rab11 in the proper sensing of Rac activation level in neighboring cells. Our work demonstrates that endocytosis and recycling are critical to achieve spatial restriction of Rac activation in collective cell migration. These data implies Rab11 as a master regulator of these processes and provide new insights in the role of endocytosis in the organization of individual cells in a coherent multicellular motile structure.

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Intercellular protein movement in syncytial Drosophila follicle cells. Peter McLean, Stephanie Airoldi, Lynn Cooley. Genetics, Yale School of Medicine, 333 Cedar St, New Haven, CT 06520.

Ring canals are stabilized cytoplasmic bridges formed by incomplete cytokinesis, and thus connect sibling cells. They have an essential role in mammalian spermatogenesis and both male and female gametogenesis in Drosophila. Interestingly, ring canals are also present in Drosophila somatic tissues such as the follicle cells, imaginal discs, and the larval brain. However, little is known about somatic ring canal structure, composition, or function. In contrast to the Drosophila germline, we did not observe strict synchronization of follicle cell mitoses or specific orientation of the mitotic spindles. Quantitation of somatic ring canals throughout ovarian development demonstrated that ~89% of main-body follicle cell divisions in stages 2-6 result in a stable ring canal, but ring canals are not present between stalk cells or polar cells. We demonstrated intercellular exchange between main-body follicle cells by observing the spread of fluorescent photoactivatable GFP (PAGFP) from a single activated cell. A computational analysis of the movement of PAGFP between cells suggests that the observed intercellular movement occurred by simple diffusion. Interestingly, the observed frequency of somatic ring canal formation and the extent of spread of activated PAGFP are consistent with the presence of syncytia averaging eight cells, much smaller than the complete mitotic lineage of ~500 follicle cells. We used Fluorescence Loss in Photobleaching (FLIP) of GFP-tagged proteins to monitor intercellular movement of Drosophila proteins. Our data indicated that ring canals in follicle cells allow rapid equilibration of small, endogenous, cytoplasmic proteins between cells. In contrast, ribosomal and mRNA-associated proteins do not show significant intercellular movement. Together, our results suggest a function for ring canals in equilibrating a subset of cytoplasmic proteins in patches of cells around the egg chamber. This implies a broader significance for syncytial organization of cells outside the germline, and provides insight into possible roles for ring canals in other Drosophila tissues.

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Destabilization of Integrin-dependent adhesion leads to epidermal cell-cell fusion in *Drosophila* larvae. Yan Wang, Michael Galko. Biochemistry and Molecular Biology, University of Texas MD Anderson Cancer Center, Houston, TX.

The molecular basis of cell-cell fusion is not yet well understood despite its importance to development and physiology. In the *Drosophila* larval epidermis, where cells are normally mononuclear, cell-cell fusion can be induced by wounding. Nuclear division followed by failed cytokinesis cannot explain the appearance of the multinucleate cells. We thus performed a reporter-based tissue-specific *in vivo* screen for genes that regulate epidermal cell-cell fusion. RNAi-mediated epidermal knockdown of the focal adhesion components βPSintegrin, integrin-linked kinase (ILK) and PINCH leads to epidermal cell-cell fusion even in the unwounded tissue. This suggests that destabilization of cell adhesion, either by knockdown of focal adhesion components or by physical wounding, triggers epidermal cell-cell fusion. Consistent with this model, ILK and PINCH are relocalized from the plasma membrane to the nucleus and the cytoplasm, respectively, in wound-proximal cells where fusion occurs. RNAi-mediated knockdown of focal adhesion components in patches of epidermal cells (genetically mimicking a wound) induces both autonomous (within the patch) and non-autonomous (involving neighboring cells not targeted by the RNAi) syncytium formation. In the *UAS-PINCH*^{RNAi}-expressing larval epidermis we observed that the level of a Jun N-terminal kinase (JNK) activity reporter is elevated preferentially near sites where fusion tends to occur. This resembles the JNK hyperactivation that occurs normally in the proximal cells that are most likely to fuse following wounding. Indeed, we found that local JNK hyperactivation can also drive relocalization of focal adhesion components and cell-cell fusion. This work provides mechanistic insight into the molecular basis of cell-cell fusion in epidermal tissues and provides a platform for further identification of genes that both positively and negatively regulate fusion.

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Wunen, a *Drosophila* lipid phosphate phosphatase, is required for septate junction mediated barrier function. Andrew D. Renault[1], Kristina E. Ile[1], Ratna Tripathy[1], Valentina Goldfinger[1,2]. 1) Max Planck Institute for Developmental Biology, Tübingen, Germany; 2) Department of Microbiology/Biotechnology, University of Tübingen, Tübingen, Germany.

Lipid phosphate phosphatases (LPPs) are integral membrane enzymes than can regulate the levels of bioactive lipids such as sphingosine 1-phosphate and lysophosphatidic acid. The *Drosophila* LPPs *wunen* (*wun*) and *wunen2* (*wun2*) have a well-established role in regulating the survival and migration of germ cells. We now show that Wun has an independent and essential cell autonomous role in development of the trachea. In particular Wun is required to maintain septate junction (SJ) paracellular barrier function, loss of which causes failure to accumulate critical luminal components. We find the integrity of the blood brain barrier is also lost in *wun* mutants indicating a general role for LPPs in SJ function. Furthermore by comparing the rescue ability of different LPP homologs we show that Wun function in the trachea is distinct from its role in germ cell migration, although in both cases, catalytic activity is essential for function.

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Macroglobulin complement related is a secreted core septate junction protein whose localization is mediated through the transmembrane protein Neuroglian. Sonia Hall, Robert Ward. Molecular Biosciences, University of Kansas, Lawrence, KS.

Polarized epithelia play critical roles as barriers to the outside environment and enable the formation of specialized compartments for organs to carry out essential functions. Barrier functions are mediated by cellular junctions, principally tight junctions in vertebrates and septate junctions (SJs) in invertebrates, that line the lateral plasma membrane between cells. Over the last two decades, more than twenty genes have been identified to function in SJ biogenesis. We recently identified mutations in *Macroglobulin complement related* (*Mcr*) that are embryonic lethal and show reduced epithelial cuticle, chitinous deposits in the salivary glands, tracheal length and width control defects, and incomplete dorsal closure. These phenotypes suggest that *Mcr* has a role in SJ formation or maintenance. *Mcr* encodes a protein with α-2-Marcroglobulin and LDL receptor A domains. Previous work has indicated a role for *Mcr* in the phagocytosis of *Candida albicans*, suggesting a role for *Mcr* in innate immunity. Here, we demonstrate the first essential developmental role for *Mcr*. We found that *Mcr* localizes to SJs in ectodermally derived epithelial tissues, and that loss of *Mcr* results in structural and functional defects in SJs. Surprisingly, RNAi of *Mcr* in embryos and imaginal discs suggests a cell autonomous role for *Mcr* in SJ structure and function. We hypothesized that *Mcr* localizes to the SJ by binding to a core SJ transmembrane protein. To identify this protein, we expressed RNAi against twelve SJ transmembrane genes in the dorsal wing compartment using *Ap-Gal4*, and observed a dramatic decrease in *Mcr* expression and localization in cells expressing *Neuroglian-RNA*i. Consistent with this finding, *Mcr* expression is reduced and not membrane associated in *Neuroglian* mutant embryos.

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In a variable thermal environment selection favors greater plasticity of cell membranes in *Drosophila melanogaster*. Brandon S. Cooper[1], Loubna A. Hammad[2], Nicholas P. Fisher[1], Jonathan A. Karty[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.

Variable environments should favor the evolution of generalists that maintain performance across environmental gradients. Antagonistic pleiotropy and mutation accumulation, however, can cause negative genetic correlations in fitness across environments leading to decreased performance of generalist relative to specialist genotypes. When genetic variation for plasticity (i.e., the capacity to change phenotypic trajectory within a lifetime) exists within a population, alleles that enable an organism to match their physiology to the current environment should be maintained at a high frequency. Theory predicts that developmental plasticity should evolve when the environment varies sufficiently among generations, due to temporal (e.g., seasonal) variation or to migration among environments. To test this prediction we characterized plasticity of cell membranes during development in populations of *Drosophila melanogaster* experimentally evolved for over three years in either constant or temporally variable thermal environments. We used two measures of the lipid composition of cell membranes as indices of physiological plasticity (a.k.a. acclimation): (1) change in the ratio of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) and (2) change in lipid saturation in cool (16ºC) relative to warm (25ºC) developmental conditions. We found that flies evolved under variable environments have a significantly greater capacity to acclimate the PE/PC ratio compared to flies evolved in constant environments, supporting the prediction that environments with high among-generation variance favor greater developmental plasticity. Our results are consistent with the selective advantage of a more environmentally sensitive allele which may have associated costs in constant environments.

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Epigenetics and evolution of TE control by piRNA: The significance of dose. Justin P. Blumenstiel[1], Dean M. Castillo[1], Mauricio Galdos[1], Chris Harrison[1], Michelle Wickersheim[1], Kim S. Box[1], Alex Abdullayev[1], Dan Brown[1], Jianwen Fang[2]. 1) Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS; 2) Applied Bioinformatics Lab, University of Kansas, Lawrence, KS.

Transposable elements (TEs) are generally harmful genetic parasites that can cause mutation, shape genomes, and contribute to the architecture of gene expression networks. Historically, natural selection has been considered to play the key role in limiting TE proliferation in populations. However, recent studies have demonstrated that an adaptive system of genome defense by piRNA also limits TE proliferation. Using hybrid dysgenesis in *Drosophila virilis* as a model, we are examining how asymmetric inheritance of maternally provisioned piRNAs determines patterns of TE induced hybrid sterility. Our studies indicate that TE dosage plays a key role both in the induction of TE mediated hybrid sterility and in maternally provisioned protection against it. Furthermore, TE instability can be a general genomic property the can be propagated across generations, but repressed epigenetically. In light of the key role that dosage plays, we will present studies on the molecular evolution of the piRNA machinery that suggest a complex co-evolutionary dynamic between TEs and the machinery of genome defense. In particular, the dominant evolutionary response to increasing TE burden across the *Drosophila* genus seems to be improved translational efficiency in the piRNA machinery, not an increased rate of evolution.

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Probing Developmental Networks via Compensatory Evolution. Sudarshan Chari, Ian Dworkin. Zoology & EEBB, Michigan State University, East Lansing, MI.

Developmental networks though generally conserved can be flexibly utilized during evolution resulting in diverse phenotypes. A deleterious mutation influencing the network output can shift a population from the phenotypic optimum leading to a fitness decline. There are several possible fates for such a mutation and one of those is amelioration by compensatory mutations. While most studies of compensatory evolution focus on *de novo* mutations during the evolutionary process, standing genetic variation for mutational effects may also be important. Furthermore, for a developmental system, network rewiring by compensatory mutations has not been well studied. In order to understand aspects of flexibility of developmental systems via rewiring by compensatory mutations, we have fixed a mutation in the *vestigial* gene, vg^1, in a large natural population of *Drosophila melanogaster*. This mutation severely perturbs wing development leading to wing tissue loss and an associated fitness decline. Using vg^1 populations, we have performed both experimental evolution (with natural selection altering the population) and artificial selection directly for phenotypic compensation of the wing. In artificial selection lineages we have observed almost a complete compensation of the wing phenotype (i.e. phenotypically wild-type) in only 13 generations. Interestingly, there has been no phenotypic compensation in experimental evolution lineages. We postulate a behavioral compensation in these lineages, specifically, influencing courtship. We are currently quantifying the differences in phenotypes and will detail some of the developmental and behavioral processes that have facilitated compensation. Our results clearly show that despite considerable segregating compensatory genetic variation in natural populations, the two selection regimes have exploited different optima to compensate for phenotypic and fitness loss by the same mutation. Our study not only explores the extent of developmental flexibility but also has implications in the interpretation of adaptive landscapes and gain of phenotypes.

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Emergence and diversification of a Drosophila pigmentation pattern through the assembly and evolution of a novel gene regulatory module. Benjamin Prud'homme. IBDML, CNRS, Marseille, France.

The typical pattern of morphological evolution associated with the radiation of a group of related species is the emergence of a novel trait and its subsequent diversification. From butterfly eyespots and their various colorful rings1 to the diversity of shapes assumed by vertebrate teeth2, seashells3 or horn beetles4, this pattern of emergence-diversification holds for countless characters across most animal groups. Yet, the genetic mechanisms associated with these two evolutionary steps are poorly characterized. Here we show that a spot of dark pigment on fly wings has first evolved from the assembly of a novel gene regulatory module whereby pigmentation genes fell under the regulation of a common transcriptional activator. The primitive wing spot pattern subsequently diversified through the sole changes in spatial distribution of this activator. These results suggest that the genetic changes underlying the emergence and the diversification of the wing pigmentation patterns are partitioned within genetic networks. More generally, this two-step model accounts at the gene regulatory level for the general pattern observed in animals and plants where morphological diversification mostly results from occasional novelties and infinite variations on these new themes.

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The genetic architecture of hybrid inviability between Drosophila melanogaster and D. santomea. Daniel R. Matute, Jerry Coyne. Ecology & 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. Hybrids between species experience hybrid breakdown because the long-diverged genomes of their parents cause developmental problems when they come together in a single individual. I have identified ten genes that cause hybrid inviability between two species of fruit flies, Drosophila melanogaster and D. santomea. Interestingly, five of these genes are involved in early embryogenesis, a developmental process that is very similar in all Drosophila species. Additionally, the majority of these genes (9 out of 10) have experienced episodes of accelerated evolution at some point in their phylogenetic history. These results constitute evidence that hybrid incompatibility can evolve as a by-product of adaptive protein evolution, even in processes that have been thought to be highly conserved.

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Mutations in the *neverland* gene turned *Drosophila pachea* into an obligate specialist species. Virginie Orgogozo[1], Michael Lang[1], Sophie Murat[1], Géraldine Gouppil[1], Luciano Matzkin[3], Catherine Blais[2], Émilie Guittard[2], Takuji Yoshiyama-Yanagawa[4,5], Hiroshi Kataoka[5], Ryusuke Niwa[4,6], René Lafont[2], Chantal Dauphin-Villemant[2]. 1) Institut Jacques Monod, CNRS UMR7592, Paris, France; 2) UPMC, Univ Paris 06, Paris, France; 3) University of California San Diego, Section of Ecology, Behavior and Evolution, La Jolla, CA; 4) Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennoudai, Japan; 5) Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Japan; 6) Initiative for the Promotion of Young Scientists' Independent Research, University of Tsukuba, Tsukuba, Japan.

Most living species exploit a limited range of resources. However, little is known on how tight links build up during evolution between such specialist species and their host. We have unraveled the genetic basis of the dependence of *Drosophila pachea* on its single host, the senita cactus *Lophocereus schottii*. *D. pachea* has lost the capacity to transform cholesterol into 7-dehydrocholesterol (first reaction in the steroid hormone biosynthetic pathway, catalyzed by the Neverland oxygenase) and requires uncommon plant sterols. We found that four amino acid changes in the Neverland protein have rendered *D. pachea* dependent on the sterols of its cactus host. This study illustrates how relatively few genetic changes in a single gene may restrict the ecological niche of a species.

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Seasonal variation in life history traits in two species of Drosophila. Emily Behrman, Katherine OBrien, Paul Schmidt. University of Pennsylvania, Philadelphia, PA.

Drosophila is used widely as a genetic model in evolutionary biology, yet there is little information regarding age structure and trait variation in wild populations. Such information is critical in evaluating the basic evolutionary and population dynamics in this species. The rapid generation time of Drosophila allow for both selection and demography to vary over seasons within years; previous work has documented cyclical, seasonal change in the frequency of specific alleles and phenotypes associated with fitness. Here, the age structure in natural populations of both D. melanogaster and simulans is examined at four distinct time points across the growing season. In addition, patterns of variation in basic life history traits for the sibling taxa are investigated and partitioned into effects associated with seasonal change in environmental quality (e.g., phenotypic plasticity) and temporal change in the genetic composition (e.g., response to environmentally mediated selection). The results show that age structure varies greatly across sampling intervals with later collections exhibiting an increasing skew towards older age classes. The taxa have distinct age structure patterns with different trajectories; D. melanogaster is present throughout the entire season while D. simulans is absent in the spring but grossly outnumbers D. melanogaster by the fall. Development time and stress tolerance also vary with season, indicating pervasive genetic changes in the population composition over time. Overall, the data demonstrate that wild Drosophila routinely survive to an age where senescence should be evident if not pronounced; age-specific performance is thus predicted to have significant effects on organismal fitness, but selection on age-specific parameters would covary with observed temporal changes in the underlying age structure of the population. The observed variance in age structure and life histories across seasonal time is predicted to have a significant impact on the evolutionary dynamics of life histories and associated traits in natural populations of this genetic model.

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Synthetic Genetic Interactions of Cell Cycle Modulators in *Drosophila*. Maximilian J. Billmann[1], Thomas Horn[1], Bernd Fischer[2], Thomas Sandmann[1], Wolfgang Huber[2], Michael Boutros[1]. 1) German Cancer Research Center (DKFZ), Division Signaling and Functional Genomics, Heidelberg, Germany; 2) EMBL, Genome Biology Program, Heidelberg, Germany.

To identify novel modulators of the cell cycle and understand their interactions, we performed a sensitive multi-parametric analysis of mitotic phenotypes on a single cell level. To this end, we depleted almost every gene in the *Drosophila* genome and imaged *Drosophila* cells stained for Tubulin, DNA and phospho-Histone 3 levels by automated fluorescent microscopy. Multi-parametric image analysis identified the vast majority of previously described cell cycle regulators and pinpointed potential novel regulators displaying strongly aberrant mitotic ratio or nuclear size phenotypes. A time-course analysis provided complementary insights into the dynamic manifestation of phenotypes. Novel candidates were validated for their *in vivo* role during *Drosophila* wing development and intestinal stem cell maintenance. To derive specific predictions of candidate gene function, we conducted synthetic genetic interaction experiments by combinatorial RNAi with putative novel and well-known cell cycle regulators. We found e.g. the uncharacterized cell cycle modulator *l(2)NC136* to cluster with known regulators of mitosis.

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The Transgenic RNAi Project at Harvard Medical School, The TRiP, is expanding the collection and is establishing the "Digital Red Book of RNAi". LA Perkins[1,2], L Holderbaum[1], D Yang-Zhou[1], L Jiang[1], R Tao[1], C Hu[1], R Sopko[1], S Ball[1], M Foos[1], A Miller[1], S Randklev[1], I Flockhart[1], B McElvany[1], S Mohr[1], JQ Ni[1,4], LP Liu[1,4], S Kondo[1,5], N Perrimon[1,3]. 1) Dept Genetics, Harvard Medical School, Boston, MA; 2) MGH, Boston, MA; 3) HHMI; 4) Tsinghua U Stock Center, China; 5) DGRC, Japan.

In *Drosophila* expression of RNAi constructs using the Gal4/UAS system has emerged as the method of choice to determine the functions of all genes. To facilitate *in vivo* RNAi studies, a number of large-scale resources have been developed that rely on long doublestranded RNAs (dsRNAs). These dsRNAs are problematic as they do not work in the germline and many have poor knockdown efficiencies. The goals of the **TRiP (http://www.flyrnai.org)**, funded by NIH/NIGMS, were to improve methods of transgenic RNAi and to generate RNAi lines for the community. During our first funding period we have optimized vectors for transgenic RNAi and shown that small hairpins (shRNAs) are better reagents than dsRNAs for *in vivo* RNAi, they're more effective in somatic tissues and work in both germlines. Based on the growing need for shRNA fly lines, we have generated >6,000 fly stocks that are distributed by the BDSC. **During our second funding period, we will continue expanding the collection of TRiP shRNA lines**, by generating lines ourselves (using GSI for injections) as well as coordinating the production of lines by a number of outside groups (DGRC, Japan; Tsinghua U, China; and individual labs) that are interested in helping build the resource. **In addition, as information on the efficacy of RNAi lines is currently not being tracked, we are establishing the "Digital Red Book of RNAi"** by collecting information on existing lines and performing validation experiments (qPCR) to evaluate the performance of existing and new lines. Well-organized public availability of this information will not only help individual researchers select the best lines for experiments but also help us identify additional shRNA lines that need to be produced.

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Building a community resource of GFP tagged *Drosophila melanogaster* transcription factors. Rebecca F. Spokony[1], Alec Victorsen[1], Stacy L. Holtzman[2], Sarah El Mouatassim Bih[1], Rebecca Cholst[1], Nader Jameel[1], Koen J.T. Venken[3], Michael Z. Ludwig[1], Jennifer Moran[1], Nicolas Negre[1], Matthew Slattery[1], Hugo J. Bellen[3], Thomas C. Kaufman[2], Kevin P. White[1]. 1) Institute for Genomics & Systems Biology, University of Chicago, Chicago, IL; 2) Department of Biology, Indiana University, Bloomington, IN; 3) HHMI, Baylor College of Medicine, Houston, TX.

Using *recombineering* of BAC genomic fragments, we developed a set of transgenic *Drosophila melanogaster* lines expressing EGFP tagged transcription factors (TTFs) regulated by their endogenous cis-genomic regions. These lines enable the study of proteins that would have previously been difficult due to a lack of or limited reagents. The lines can be used to study a wide variety of biological questions including expression, regulation and protein-DNA binding. We replaced the endogenous stop codon with EGFP within large fragments of genomic DNA (~30-100 kb) and integrated these into the genome at well-characterized locations containing PhiC31 attP sites. We currently have 60 lines for 45 different transcription factors plus alternatively-spliced DNA-binding isoforms and different genomic contexts. We are validating expression of the TTFs by comparing EGFP expression with published RNA and protein expression for the various transcription factors . We are determining TTF function for a subset of the lines with mutant rescue. Almost all of the genomic insertions can be maintained as homozygotes and a small portion of them show mild overexpression phenotypes. For example, *Notch*-EGFP adults display the well-characterized *Notch* overexpression ectopic wing vein phenotype in a dose and temperature dependent fashion. Additionally, we are discovering new expression patterns for genes with little or no published expression data that are consistent with known mutant phenotypes as well as new overexpression phenotypes that are consistent with known expression patterns. We are currently using the validated lines for regulatory element dissection (e.g., Jra) and ChIP-seq (e.g., Hox genes and nuclear receptors).

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Tissue-Specific Translation State Array Analysis in Drosophila melanogaster. Patrick W-L Li, Artem Zycovich, Guiping Du, Marysia Kolipinski, Pankaj Kapahi. Buck Institute for Research on Aging, Novato, CA.

Drosophila melanogaster is one of the most widely used model organisms in biology. The powerful genetic tools and the complex body plan have played a key role in its popularity. Up till now, gene expression studies have been done in whole organism or dissected tissues, which either overlook any tissue-specific changes or are constrained by the limitation of dissection methods. Furthermore, it has been shown that the correlation between mRNA and protein levels is rather modest, likely due to differences in post-transcriptional events, a major one being translation. To overcome these shortcomings, we have developed a method to capture mRNAs bound to tagged ribosomes in Drosophila melanogaster. Using the GAL4-UAS system, we can isolate ribosome bound mRNAs from a variety of tissues or a subset of cells. This technique, combine with other transcriptomic tools such as microarray or RNASeq, can generate insight into the translatome in a spatial (tissue-specific drivers) or temporal (inducible driver) manner. Here we demonstrated that overexpression of this tagged ribosomal protein has no adverse effect on life history traits of the flies. In addition, data generated with our method is consistent with that obtained from dissected tissues, and our method can even capture mRNA from specific cells that are difficult to dissect. In summary, we have established a method to examine cell specific changes in mRNA translation which will be useful in examining the role of spatial and temporal influences on a given phenotype in Drosophila melanogaster.

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Accurate genome-wide identification of dynamic transcriptional enhancers during *Drosophila* development. Daniel J. McKay[1], Jason D. Lieb[1,2]. 1) Dept of Biology, UNC Chapel Hill; 2) Carolina Center for Genome Sciences, UNC Chapel Hill, Chapel Hill, NC.

A longstanding goal in biology is to understand how a diversity of cell types is created from a single genome. Previous work has identified *cis*-regulatory modules (CRMs) associated with nearly 5% of the genes in the *Drosophila* genome. However, the inability to accurately predict the location of regulatory DNA within the genome leaves us with an incomplete understanding of the mechanisms controlling cell fate specification during development. To address this gap, we have generated genome-wide open chromatin profiles at five developmental stages using a technique termed FAIRE-seq (Formaldehyde-Assisted Isolation of Regulatory Elements, followed by high throughput sequencing). Although less than 4% of the genome is open at any particular stage, we find that these regions behave dynamically over time, and cumulatively, over 12% of the genome is open across all samples. To test the ability of open chromatin regions to function as CRMs, we performed transgenic reporter assays. Strikingly, all 26 of the cloned regions drive accurate reporter activity, including regions from genes that have been heavily studied such as *hunchback*, *paired*, and *Distalless*. We conclude that open chromatin regions are highly effective predictors of functional CRM activity. To examine the regulatory network underlying appendage development, we generated open chromatin profiles of three different imaginal discs. While differences exist at key appendage regulators, the overall genome-wide profiles are nearly identical. To test whether these similarities were a consequence of insufficient cellular determination, we generated open chromatin profiles from fully differentiated appendages. To our surprise, we again found the profiles were nearly identical, while being distinct from imaginal discs. Taken together, these data suggest that a core *cis*-regulatory network exists to control appendage development that is nevertheless capable of generating dramatic morphological differences.

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High resolution association mapping in an outbred *Drosophila melanogaster* population using Pool-Sequencing (NGS speed mapping). Christian W. Schloetterer, Héloïse Bastide, Martina Visnovska, Raymond Tobler, Andrea Betancourt. Inst f Populationsgenetik, Vetmeduni Vienna, Wien, Austria.

Next Generation Sequencing (NGS) techniques provide powerful tools for the identification of the genetic basis responsible for variation in quantitative phenotypes (QTLs). However, most of the methods to date only focus on inbred populations of laboratory organisms which poorly reflects natural variation. Here, we test the performance of NGS for association mapping studies in an outbred *Drosophila melanogaster* population. Since, *D. melanogaster* has extremely low levels of linkage disequilibrium, we reasoned that GWAS should be extremely powerful at an unprecedented level of resolution. To test our approach, we focused on an extremely well-studied trait, abdominal pigmentation variation in *D. melanogaster* females. About 5,000 F1 females obtained from naturally inseminated flies were scored for pigmentation and two replicates each of the 100 most extreme phenotypes were sequenced. Our results confirm the efficiency of our approach. In addition to several genes with a proven role in pigmentation (e.g. bab), we identified additional candidates. Most importantly, our analyses suggest that levels of linkage disequilibrium may be low enough to identify causative SNPs . Hence, we propose that our new approach (NGS speed mapping) provides an excellent tool for GWAS studies, in particular for species with low levels of linkage disequilibrium.

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Super-Resolution Imaging of Regulatory Chromatin Dynamics in Developing Embryos. Alistair N. Boettiger, Xiaowei Zhuang. Chemistry and Chemical Biology, Harvard University, Cambridge, Ma.

The differentiation of embryonic cells into their appropriate developmental fates is mediated in part by fine scale structural changes to chromatin. Developmental specific transcription factors drive these changes through the repositioning of histones. This can facilitate or restrict access to transcriptional machinery to the underlying genes or facilitate looping of distal regulatory sequences to target sites. These fine scale structural changes are mostly too small (10s of nanometers) to be observed with conventional microscopy techniques (limited to several hundred nanometer resolution), and have so far evaded in vivo observation in intact embryonic tissue.

We present super-resolution imaging techniques which allow for the detection of regulatory changes in chromatin on the scale of tens of nanometers in developing embryos by imaging particular genomic regions of interest and chromatin associated proteins. These analyses provide a detailed view of regulatory modifications at the single cell level. This allows for a direct causal relations between expression states and modification. It also allows for variation between identical populations to be measured and the frequency of each state within the population to be determined. Additionally, because they are applied within the intact embryo, cell identity and the spatial relation of the cell to its neighbors and embryonic signals are still maintained.

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Functional redundancy of the *Drosophila* p38 MAP kinases probed by mass spectrometry-based interaction proteomics. Vladimir Belozerov[1,2], Zhen-Yuan Lin[3], Anne-Claude Gingras[3,4], Michael Siu[1], John McDermott[2]. 1) Department of Chemistry and Centre for Research in Mass Spectrometry, York University, Toronto, Ontario, Canada; 2) Department of Biology, York University, Toronto, Ontario, Canada; 3) Centre for Systems Biology, Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada; 4) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

The p38 MAPK pathway is a key evolutionarily conserved mediator of an organism's response to stressful environmental stimuli. In mammals four p38 kinases form a robust signaling module believed to be supported by considerable functional redundancy. In *Drosophila* the p38 MAPK family consists of two highly homologous kinases, Mpk2 and p38b, and the third putative kinase p38c. Recent genetic analyses of various Mpk2 and p38b alleles suggest that the two kinases are at least partially redundant. However, the extent of this redundancy, and possible non-overlapping roles remain to be defined. To reveal common and unique molecular functions of individual p38 kinases we generated a high-resolution protein interaction map of Mpk2, p38b, and p38c in S2 cells. The use of an optimized single-step affinity purification procedure followed by gel-free LC-MS/MS analysis allowed us to detect both stable, and more transient, lower-affinity interactions. *In vitro* kinase reactions with a panel of recombinantly expressed interactors and activated p38 were used to identify likely kinase substrates. The results of our study suggest limited functional overlap between Mpk2 and p38b, primarily restricted to the regulation of mRNA processing. Another finding is a previously unacknowledged link between the p38 pathway and the regulation of carbohydrate metabolism. Validation of these new functional connections in the context of larval muscle and gut will also be presented. More broadly, our results illustrate the use of mass spectrometry-based interaction mapping for assigning shared and unique molecular functions to individual members of redundant protein families.

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Pre-meiotic SOLO is required for sister chromatid cohesin, chromosome segregation, synaptonemal complex assembly, and DSB repair in Drosophila meiosis. Rihui Yan[1], Bruce McKee[1,2]. 1) Dept Biochem, Cell, Molec Biol, Univ Tennessee, Knoxville, TN; 2) Genome Science and Technology Program, University of Tennessee, Knoxville, TN.

Pre-meiotic SOLO is required for sister chromatid cohesin, chromosome segregation, synaptonemal complex assembly, and DSB repair in Drosophila meiosis Rihui Yan and Bruce D. McKee Department of Biochemistry, Cellular and Molecular Biology, University of Tennessee, Knoxville, Tennessee 37996 Recombination between homologous chromosomes and cohesion between sister chromatids are jointly required for chromosome segregation in meiosis in many organisms. However, the role and mechanism of cohesion in Drosophila meiosis are not well understood as yet. Mutations of solo, which encodes a cohesion protein involved in Drosophila male meiosis, cause severe homolog and sister chromatid nondisjunction of both sex chromosomes and autosomes in female meiosis. solo mutations also caused reduced frequencies of homologous recombination and the loss of inhibition of sister chromatid recombination as well as delayed repair of double-stranded DNA breaks (DSB). Synaptonemal complex assembly and chromosomal localization of the cohesin protein SMC1 are defective in solo ovaries. SOLO appears on chromosomes prior to meiosis and colocalizes with the cohesin component SMC1 and the synaptonemal complex (SC) component C(3)G in meiosis. Moreover, SOLO physically interacts with SMC1 in vivo. SOLO induced before meiosis completely rescued solo phenotypes, however, it does not restore solo functions when it is expressed after meiosis. Our studies demonstrate that SOLO prior to meiosis is required for its association with meiotic cohesion protein that contains SMC1 and is required for DSB repair, homologous recombination, SC assembly and chromosome segregation during Drosophila meiosis.

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Control of centriole replication by centrosomin proteins. Timothy Megraw[1], Ling-Rong Kao[1], Paul T. Conduit[2], Jordan W. Raff[2]. 1) Biomedical Sciences, Florida State University, Tallahassee, FL. USA; 2) Sir William Dunn School of Pathology, University of Oxford, Oxford, UK.

Centrosomins are conserved centrosome proteins. In Drosophila, centrosomin (Cnn) is required for PCM assembly and for the MTOC activity of mitotic centrosomes. In humans the centrosomin *Cdk5rap2* is mutated in MCPH, a neural stem cell disease that effects brain growth. We recently reported that in *Cdk5rap2* mutant mouse cells the mitotic centrosomes have apparently normal MTOC activity but have amplified centrioles. The mechanism appears to involve loss of centriole engagement.

Consistent with the role for Cdk5rap2 in centriole replication control, we found mutations in Drosophila *cnn* that also cause unrestricted centriole duplication. One cluster of mutations causes centriole amplification in testis, while another mutation causes amplification in neuroblasts. Mutant spermatocytes frequently show eight or more pairs of centrioles per cell in intact sixteen-cell cysts. In contrast to *cnn* null mutations, which severely disrupt cytokinesis in spermatocytes, the new mutations reported here are male fertile despite the frequent assembly of multipolar spindles and spermatids with multiple flagella. These mutations knock out the expression of one of two centrosomal isoforms of Cnn in testes. The remaining isoform is localized to functional centrosomes. By expressing a set of rescue constructs, we show that the level of Cnn expression, rather than a specific role(s) for Cnn isoforms, is responsible for restricting centriole replication. Therefore, loss of expression of one of the centrosomal isoforms causes unrestricted centriole replication, which can be rescued by expression of either centrosomal isoform in early, but not late, spermatocytes.

These results show that, while Cnn is not essential for centriole replication, the control of Cnn levels is critical to restrict centriole replication to once per cell cycle.

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Spindle misorientation does not cause tumor-like phenotypes in the follicle cell epithelium. Daniel T Bergstralh, Daniel St Johnston. Gurdon Inst, Univ Cambridge, Cambridge.

The incorrect orientation of mitotic spindles has been heavily implicated in tumorigenesis in mammals. It has also been suggested to underlie tumor-like phenotypes in the Drosophila follicle cell epithelium (FCE), a single layer of epithelial cells in which spindles are normally oriented in parallel to the place of the epithelium. While studies in Drosophila have contributed greatly to our understanding of this process in several tissues, spindle orientation in the FCE has not been extensively examined. In the neuorepithelium, spindles orient through interaction with adherens junctions. We show that this interaction is not at work in the FCE. However, we demonstrate that Pins is expressed in the ovary and, as in other tissues, participates in orienting spindles in the FCE. We further show that exogenous expression of Inscuteable, another spindle orientation factor, promotes a 90 degree reorientation of the spindle. Surprisingly, neither the loss of Pins nor the expression of Inscuteable cause tumor-like phenotypes. Live imaging reveals that cells dividing outside the plane of the epithelium reintegrate into the monolayer. These results indicate that although spindle orientation is under control in the FCE, the loss of that control is not sufficient to promote loss of epithelial integrity, suggesting a checkpoint process whereby that integrity is actively monitored and maintained.

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Endocrine hormonal effects on neoplastic tumorigenesis. Thu H. Tran, Katherine Pfister, Adrian Halme. Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA.

Tumor formation is a multi-factorial process that involves contributions from both genetic mutations within tumor cells as well as inputs from surrounding signals. In *Drosophila*, several tumor suppressor genes have been identified, enhancing our understanding of the role of autonomous gene mutations in tumor formation. In contrast, relatively little is known in *Drosophila* about the non-autonomous effects of surrounding signals on mutant cells during tumorigenesis. To address this, we have begun to examine the role of developmental signals in regulating tumor formation and progression. The endocrine signals ecdysone and juvenile hormone are critical coordinators of *Drosophila* developmental transitions. Using several different neoplastic tumor models, we have characterized the initiation of tumorigenesis in these models and observed a correlation between the timing of tumor formation in imaginal tissues and the expression of *juvevenile hormone esterase* (*Jhe*). *Jhe* expression initiates a developmentally important transition in larval hormone signaling, where juvenile hormone levels drop and ecdysone signaling begins to rise, eventually leading to pupation. Furthermore, we have shown that the manipulation of larval endocrine signals alters tumor development, suggesting that the larval endocrine signals play an important role in regulating tumorigenesis. Ongoing experiments are examining the effects of specific endocrine hormone signals on tumor formation and development, and exploring the molecular mechanisms by which hormone signals regulate tumorigenesis.

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Polyploidy as a mechanism of tissue repair. Vicki P. Losick[1], Don T. Fox[2], Allan C. Spradling[1]. 1) Dept Embryology, HHMI, Carnegie Institution for Science, Baltimore, MD; 2) Department of Pharmacology and Cancer Biology Duke University Medical Center, Durham, NC.

Wound healing is essential for all organisms to survive. It protects against infection and restores tissue integrity following injury or damage. Many adult tissues lack active stem cells and in these cases, the cellular mechanisms of tissue repair are not well understood. Adult fruit flies can also recover rapidly from penetrating injury or genetically induced tissue damage. In response to tissue damage, adult cells reenter the cell cycle and express S phase markers in at least three distinct tissue types. However, these cells appear to help heal the damaged tissue not by cell division, but by increasing ploidy through endoreplication. Interestingly, a similar response has been shown to occur in mammalian tissues, particularly the liver, but the mechanism and importance of endoreplication to the repair program remains poorly understood. The adult fruit fly response may provide insight into a neglected aspect of mammalian tissue repair where a pre-existing cell can fill the space and function of cells lost to damage.

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Homeodomain-interacting protein kinase inhibits Hippo signaling to promote growth during *Drosophila* development. Joanna Chen, Esther Verheyen. Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada.

The Hippo (Hpo) pathway regulates tissue size by controlling cell proliferation and apoptosis. The core components of the Hpo pathway, Hpo, Salvador, Warts (Wts) and Mats, form a kinase cascade to inhibit the activity of Yorkie (Yki), the transcriptional regulator of the pathway. Inhibition or loss of Hpo signaling results in massive overgrowth. Here, we identified *homeodomain-interacting protein kinase* (*hipk*) as the first kinase to promote Yki activity in the Hippo pathway. Hipk encodes a member of a novel family of nuclear protein kinases. Changes in Hipk protein levels affect cell proliferation and apoptosis, but not cell size, during *Drosophila* wing development. Hipk modulates expression of Hippo targets, such as *ex*-lacZ, *DIAP*-lacZ, Cyclin E and Wg. Our genetic interaction studies suggest that Hipk functions downstream of Wts and Ex. Moreover, Hipk activity in the Hippo pathway appears to be Yki-dependent. Our biochemical studies indicate that Hipk interacts with and phosphorylates Yki. Our findings suggest that Hipk is a positive regulator of Yki to regulate Hpo signaling during *Drosophila* development.

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Tumor suppression by cell competition through regulation of the Hippo pathway. Molly C. Schroeder[1,2], Chiao-Lin Chen[2], Madhuri Kango-Sing[3], Chunyao Tao[2], Georg Halder[2]. 1) Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; 2) The University of Texas MD Anderson Cancer Center, 1515 Holcombe, Houston, TX 77030; 3) University of Dayton, Dayton, Ohio.

Homeostatic mechanisms can eliminate abnormal cells to prevent diseases such as cancer. However, the underlying mechanisms of this surveillance are poorly understood. Here we investigated how clones of cells mutant for the neoplastic tumor suppressor gene *scribble* (*scrib*) are eliminated from Drosophila imaginal discs. When all cells in imaginal discs are mutant for *scrib*, then they hyperactivate the Hippo pathway effector Yorkie (Yki), which drives growth of the discs into large neoplastic masses. Strikingly, when discs also contain normal cells, then the *scrib* cells do not overproliferate and eventually undergo apoptosis through JNK-dependent mechanisms. However, induction of apoptosis does not explain how *scrib* cells are prevented from overproliferating. We report that cell competition between *scrib* and wild-type cells prevents hyperproliferation by suppressing Yki activity in *scrib* cells. The suppression of Yki activation is critical for the elimination of *scrib* clones by cell competition and experimental elevation of Yki activity in *scrib* cells is sufficient to fuel their neoplastic growth. Thus, cell competition acts as a tumor suppression mechanism by regulating the Hippo pathway in *scrib* cells.

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The cell adhesion molecule Echinoid functions as a tumor suppressor and upstream regulator of the Hippo signaling pathway. Tao Yue, Aiguo Tian, Jin Jiang. Developmental Biology, University of Texas Southwestern Medical Center, Dallas, TX.

How multi-cellular organisms control their growth to reach final organ size during development is a fascinating problem in Biology. Recent studies, initially from Drosophila, have identified an evolutionarily conserved pathway, the Hippo tumor suppressor pathway, as a key mechanism that controls tissue growth and organ size by simultaneously inhibiting cell growth/proliferation and promoting cell death. The Hpo signaling pathway has also been implicated in cell contact-dependent growth inhibition, and deregulation of the Hpo pathway has been connected to a wide range of human cancers. The core pathway consists of the Hpo/Warts (Wts) kinase cassette that phosphorylates and inactivates the transcriptional coactivator Yorkie (Yki). Here, we report that Echinoid (Ed), an immunoglobulin domain-containing cell adhesion molecule, acts as an upstream regulator of the Hpo pathway. Loss of Ed compromises Yki phosphorylation, resulting in elevated Yki activity that drives Hpo target gene expression and tissue overgrowth. Ed physically interacts with and stabilizes the Hpo-binding partner Salvador (Sav) at adherens junctions. Ed/Sav interaction is promoted by cell-cell contact and requires dimerization of Ed cytoplasmic domain. Overexpression of Sav or dimerized Ed cytoplasmic domain suppressed loss-of-Ed phenotypes. We propose that Ed may link cell-cell contact to Hpo signaling through binding and stabilizing Sav, thus modulating the Hpo kinase activity.

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Muscle size and myonuclear position are independently regulated by distinct Dynein pathways. 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 10065; 2) Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, NY 10065.

Morphogenesis, through modulation of the cytoskeleton, dictates cell size, shape, and organization, and informs cellular function. Patients with muscle diseases present with aberrant muscle cell morphologies characterized by smaller myofibers with mispositioned nuclei. However, the mechanisms that control these processes and their contribution to muscle weakness in disease are not known. To understand how the cytoskeleton impacts muscle form and function, we examined the role of Dynein during *Drosophila* muscle development and found that regulation of muscle size and myonuclear positioning are mechanistically distinct. Several factors including the Dynein heavy chain (Dhc64C), the Dynein light chain (Dlc90F), and Partner of Inscuteable (Pins) contribute to both muscle growth and myonuclear positioning. However, Lis1 contributes only to Dynein-dependent muscle size, whereas CLIP-190 and Glued only contribute to Dynein-dependent myonuclear positioning. Moreover, Lis1 and CLIP-190 do not functionally interact. Mechanistically, there is a decrease in the density of microtubules at the muscle poles in *clip190* mutants, suggesting that microtubule interactions with the cortex are required for proper nuclear positioning. In *lis1* mutants, Dynein hyper-accumulates at the muscle poles, suggesting that retrograde trafficking away from the poles is required for proper muscle growth. Importantly, all mutants had the correct number of nuclei present within each myofiber, suggesting that fusion alone is not sufficient to regulate myofiber size. The effects of both Lis1 and CLIP-190 are downstream of Dynein arriving at the muscle pole, suggesting that these proteins specify separate Dynein functions within a single localization. Finally, defects in muscle size or myonuclear positioning impair muscle function *in vivo*. These findings indicate that muscle size and myonuclear positioning are essential for muscle function, yet regulated by distinct Dynein-dependent mechanisms.

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MiR-92b is a heart and muscle specific microRNA that regulates Mef2 level through a negative feedback loop in *Drosophila*. Zhimin Chen[1], Shanshan Liang[1], Ying Zhao[1], Zhe Han[1,2]. 1) Department of Internal Medicine, Division of Molecular Medicine and Genetics, University of Michigan Medical School, Ann Arbor, MI; 2) Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI.

Level of Mef2 transcription factor must be precisely controlled since Mef2 is essential for heart and muscle differentiation by activating hundreds of target genes. Since Mef2 can self-activate, a negative feedback loop must present to keep its expression level stable. Using microarray and genetic analysis, we have identified miR-92b, a highly conserved microRNA, to be a post-transcriptional regulator of Mef2 in *Drosophila*. MiR-92b is expressed in a heart and muscle specific pattern similar to that of Mef2 and is directly activated by Mef2 through conserved Mef2 binding sites in its *cis*-regulatory region. On the other hand, miR-92b represses Mef2 translation through binding to conserved miR-92 binding sites in the 3' UTR of Mef2, forming a negative feedback loop that maintains Mef2 level in a proper range. Perturbing this negative feedback loop, either by deleting or over-expressing miR-92b, causes corresponding up or down changes of Mef2 protein level and affects muscle development. A microRNA usually has many targets and its level needs to be tightly controlled as well. Our data suggest that a microRNA and a transcription factor can form a negative feedback loop to maintain the stable expression level of both efficiently.

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Mitotic Cell Rounding Accelerates Invagination of the *Drosophila* Tracheal Placode. Takefumi Kondo, Shigeo Hayashi. Lab. for morphogenetic signaling, RIKEN CDB, Kobe, Hyogo, Japan.

Animal cells change their shape to sphere upon entry into mitotic phase. Mitotic cell rounding is a conserved process governed by extensive rearrangement of actin cytoskeleton, and is necessary for proper cell division. In addition, mitosis is also coupled with microtubule reorganization to form spindle. During development, since the regulation of cytoskeletal structure in interphase is involved in cell shape change and modulation of cell-cell adhesion, mitotic entry must be precisely controlled to avoid interference of tissue morphogenesis and integrity. On the other hand, whether mitotic cell rounding itself plays an active role in epithelial morphogenesis is unknown.

Invagination is one of the key morphogenetic processes, which converts flat epithelial sheets into three-dimensional structures. To understand cellular mechanisms that accomplish the full process of epithelial invagination, we performed three-dimensional live imaging of *Drosophila* tracheal placode as a model system, and found that this morphogenetic event is divided into two distinct phases by speed. First, the slow invagination phase proceeds with a wave of circular Myosin concentration formed in the placode, causing a series of cell intercalation and apical constriction of central cells. In the second phase, speed of invagination was suddenly increased. This acceleration was associated with entry of one of the central cells into mitosis and rounding at the basal side, resulting in the fast basal movement of the apical surface of the placode. Genetic block of mitotic entry and cell rounding interrupted transition to the second phase. On the other hand, microtubule inhibitor colchicine, which prevents spindle formation and cell division, but not cell rounding, did not interfere the phase transition. These results indicate that cell rounding accompanied by mitotic entry actively drives fast invagination of tracheal placode.

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Without Children (Woc) affects cystoblast differentiation and proper soma-germ line association by modulating Stat target gene expression. Lilach Gilboa, Iris Maimon, Malka Popliker. Biological Regulation, Weizmann Institute of Science, Rehovot, Israel.

In the Drosophila ovary, somatic cells and germ cells are tightly associated. How this association is achieved and its biological consequences remain poorly understood. We show that the putative transcription/chromatin-binding factor, Without Children (Woc), is required for proper expression of Stat target genes, which in turn affects soma-germ line association and the differentiation of germ line stem cell (GSC) daughter cells.

Woc is a zinc-finger transcription factor, which was also shown to bind and protect telomeres. It is expressed in both somatic cells and germ cells. We find that Woc is required within somatic cells for correct cyst development. Removing Woc from somatic cells by tissue specific RNAi or in clones results in loss of association between soma and germ line. In larval ovaries, the somatic Intermingled Cells fail to intermingle with primordial germ cells. In the adult germarium, Escort Cells (ECs) fail to send cellular extensions that contact differentiating germ cells. Within such germaria, cystoblasts (the immediate daughters of GSCs) do not form cysts, filling the ovary with single germ cells.

We find that the full range of phenotypes exhibited by Woc-deficient larval ovaries and germaria resemble ovaries in which Stat function or its target gene *zfh-1* have been removed. Indeed, in *woc*-mutant cells, the Stat target gene *zfh-1* is not properly expressed. Upd over-expression cannot rescue *woc* phenotypes. However, expression of Zfh-1 from a heterologus promoter does rescue cyst formation in *woc* mutant ovaries.

Our data suggest a novel role for Stat in regulating cystoblast differentiation and demonstrate a requirement for a transcription/chromatin factor, previously associated with telomere integrity, in regulating ovarian Stat function.

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Regulation of pachytene checkpoint in *Drosophila* ovaries via Polo-mediated phosphorylation of Maelstrom. Jun Wei Pek, Toshie Kai. Temasek Life Science Laboratory, 1 Research Link National University of Singapore Singapore 117604.

In *Drosophila*, Maelstrom is a conserved component of the perinuclear nuage, a germ-line-unique structure, which appears to serve as a site for piRNA production to repress deleterious transposons. Maelstrom also functions in the nucleus as a transcription regulator to repress the expression of *microRNA-7*, a process which is essential for proper differentiation of germ-line stem cells. Here, we report a novel function of Maelstrom in regulating the pachytene checkpoint independent of its transposon silencing and germ-line stem cell differentiation activities. *Drosophila* Maelstrom is phosphorylated at a conserved Serine 138 residue, and this phosphorylation event is required for repression of the pachytene checkpoint through repressing the checkpoint protein Sir2, but not transposon silencing and germ-line stem cell differentiation. We identify Polo as a kinase which mediates phosphorylation of Maelstrom to regulate the pachytene checkpoint. Therefore, our results suggest that Polo-mediated phosphorylation of Maelstrom may be a novel mechanism that controls the pachytene checkpoint through repressing Sir2 in the *Drosophila* ovaries.

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Tribbles is a kinase that directs a switch in gene expression to trigger follicle cell migration. Leonard L. Dobens, Rahul Das, Venessa Masoner, Laramie Pence. School of Biological Science, University Missouri-Kansas City, Kansas City, MO.

tribbles (*trbl*) encodes the founding member of the Trb family of candidate pseudokinase molecules required for cell proliferation, growth and migration throughout the metazoan lineage. During cell migration in flies, *trbl* drives protein turnover, notably of (1) the String cdc25 phosphatase in the mesoderm during gastrulation and (2) the C/EBP homolog Slow Border Cells (Slbo) during border cell migration in the ovary. Here we re-examine the role of *trbl* in follicle cell (FC) migration by testing (1) specific Trbl antisera, (2) site-directed mutants in the Trbl kinase-like domain and (3) a *trbl* null allele generated by FRT-mediated deletion. During late stages of oogenesis, Trbl protein accumulates at high levels in the nuclei in non-migrating FC and at low levels in both the migratory border cells and centripetal FC. During border cell migration, low levels of Trbl are maintained by *slbo* repression, but ectopic expression of Slbo in posterior FC is not sufficient to repress Trbl expression. It has been shown previously that WT Trbl misexpression is sufficient to both block border cell migration and enhance Slbo protein turnover and here we show that misexpression of a Trbl molecule bearing a site-directed mutant in the catalytic loop of its kinase-like domain results in the opposite phenotype: a failure to block border cell migration and enhanced stability of the Slbo protein. Thus Trbl is a bonafide kinase that mutually represses Slbo during border cell migration. During centripetal FC migration, clones of a *trbl* null allele lead to (1) a loss of FC apico-basal polarity, (2) the disintegration of organized structures resembling actin cables and (3) reduced expression of the Slbo target Cut. These data suggest that the *trbl* kinase regulates both the supracellular organization of the cytoskeleton and a gene expression switch from Slbo to Cut to coordinate the migration of FC tissue sheets.

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Cell-type-specific translational control of *cycB* in the *Drosophila* male germline. Catherine C. Baker, Byung Soo Gim, Margaret T. Fuller. Dept Developmental Biol, Stanford Univ Sch Medicine, Stanford, CA.

In the *Drosophila* male germline, the majority of the mRNAs required for post-meiotic spermatid differentiation are transcribed in spermatocytes, the male germ cells undergoing meiotic G2 prophase. This spermatocyte transcription program and a 20-fold increase in cell volume require that G2 prophase be extended for a total of 3.5 days before the meiotic divisions occur. We have found that the delay of meiotic division is directed in part by cell type-specific regulation of Cyclin B (CycB), a member of the core cell cycle machinery. The mRNA for CycB is expressed throughout spermatocyte development, but CycB protein does not appear until just before meiotic division. We have found that this delay in appearance of CycB is due to translational repression by Rbp4, an RNA-binding protein. When *rbp4* is knocked down by RNAi, CycB protein appears in early spermatocytes where it is normally absent. Rbp4 binds to a 35-nucleotide (nt) conserved sequence within the short (130nt) spermatocyte 3'UTR of *cycB*, and that same conserved sequence is also required for translational repression of a CycB-eYFP reporter in early spermatocytes. With the help of BioGRID and FlyAtlas, we identified a potential co-factor: CG9975 (nicknamed Fest). Fest and Rbp4 co-immunoprecipitate from S2 cells, and both proteins are expressed in early spermatocytes onwards. Surprisingly, Fest does not function with Rbp4 to repress translation of *cycB*, but acts instead in opposition: CycB does not accumulate when *fest* is knocked down in spermatocytes by RNAi. To test whether (a) Fest antagonizes Rbp4 in late spermatocytes to allow translation of *cycB*, or (b) Rbp4 antagonizes translation-promoting activity of Fest until late spermatocytes, we expressed the Rbp4-resistant CycB-eYFP reporter (Δ35nt) in a *fest* RNAi background. The CycB-eYFP(Δ35nt) reporter was derepressed in early spermatocytes in the wild-type control, but expression was not detected in *fest* RNAi spermatocytes. This suggests that Fest is directly required for *cycB* translation, with Rbp4 likely repressing its activity in all but the most mature spermatocytes.

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Barriers to Male Transmission of Mitochondrial DNA in Sperm Development. Steven A. DeLuca. Dev Biol, UCSF, San Francisco, CA.

The mitochondrial genome (mtDNA) is maternally inherited in most animals, and studies in a few animals have proposed that paternal mtDNA elimination is coupled to zygote formation. We investigated the molecular mechanisms responsible for eliminating paternal mtDNA in Drosophila, and unexpectedly found that paternal inheritance is prevented prior to zygote formation. Using a genetic tool developed in our lab (Xu et al. Science, 2008), we isolated mtDNA mutations that allowed us to follow paternal mtDNA through a cross. We discovered that fertilized embryos lacked paternal mtDNA, and mtDNA was also absent in mature sperm. We then documented two distinct processes that eliminate mtDNA from developing sperm. We visualized the abrupt disappearance of mtDNA nucleoids during the last 100 microns of sperm tail elongation, and identified a mitochondrial nuclease, Endonuclease G, that is required for this disappearance. In Endonuclease G mutants, persisting mtDNA nucleoids were collected and eliminated by a second process that trims and shapes spermatid tails during sperm individualization. Our results document the primary mechanisms enforcing a unique inheritance strategy in which the father restricts the transmission of his own mtDNA.

Poster Full Abstracts - Cell Biology and Signal Transduction

Poster board number is above title. The first author is the presenter

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Regulation of epithelial polarity by the E3 ubiquitin ligase Neuralized and the Bearded inhibitors in Drosophila. soline chanet, françois schweiguth. Inst Pasteur - CNRS URA 2578, Paris.

Understanding how epithelial polarity is established and regulated during tissue morphogenesis is a major issue. Here, we identify a novel regulatory mechanism important for mesoderm invagination, germ-band extension and trans-epithelial migration in the Drosophila embryo. This mechanism involves the inhibition of the conserved E3 ubiquitin ligase Neuralized by the proteins of the Bearded family. First, Bearded mutant embryos displayed a loss of epithelial polarity associated with an early loss of the apical domain. Bearded regulated epithelial polarity by antagonizing neuralized. Second, repression of Bearded gene expression by Snail was required for the Snail-dependent disassembly of Adherens Junctions in the mesoderm. Third, neuralized was strictly required to promote the down-regulation of the apical domain in the midgut epithelium and to facilitate the trans-epithelial migration of primordial germ cells across this epithelium. This function of Neuralized was independent of its known role in Notch signalling. Thus, Neuralized has two distinct functions in epithelial cell polarity and Notch signalling.

158B

Wild-type Planar Cell Polarity requires the spatially restricted activity of Prickle protein isoforms. Simon Collier, Meagan Valentine, Andrea Belalcazar. Dept Biological Sci, Marshall Univ, Huntington, WV.

Two isoforms of the Prickle protein, Pk and Sple, are active in Frizzled Planar Cell Polarity (Fz PCP) signaling during adult fly development. Loss-of-function phenotypes reveal that the two Prickle isoforms play different roles in the development of adult PCP, and gain-of-function experiments show that the two isoforms have different cellular activities. Both Prickle isoforms contain a PET domain and three LIM domains, but differ in their N-termini. Specifically, the 13 N-terminal amino acids in the Pk isoform are replaced by 349 amino acids in the Sple isoform. Using the Gal4-UAS system, we have generated flies that express just one Prickle protein isoform (either Pk or Sple) ubiquitously throughout development. We have compared the PCP of these flies in numerous tissues including wing, abdomen, thorax, leg and eye. In most regions examined, one Prickle isoform (either Pk or Sple) confers wild-type polarity, while the other confers an alternate polarity. Our findings suggest that wild-type PCP in the adult fly is generated using a patchwork of Fz signals that incorporate either the Pk isoform or the Sple isoform. We refer to as the Prickle Isoform Code. We are currently attempting to correlate the spatial restricted activity of Prickle isoforms with local signaling gradients, to try to define the underlying logic of the code.

159C

***aPKC* regulates localization but not function of Numb during neuroblast asymmetric divisions.** Jill Haenfler[1], Chaoyuan Kuang[1,2], Cheng-Yu Lee[1,3,4,5]. 1) Program in Cell and Molecular Biology; 2) MSTP; 3) Dept of Cell and Dev Biology; 4) Div of Mol Med & Genetics, Dept of Int Med; 5) Center for Stem Cell Biology, Life Sciences Inst, Univ of Michigan, Ann Arbor, MI.

Cortical cell polarity regulates unequal partitioning of the cell fate determinants to functionally distinguish stem cells from progenitor cells during asymmetric cell divisions. Whether polarity proteins specify stem/progenitor cell potential by regulating segregation and function of the fate determinants remains unknown. The *Drosophila* larval brain contains steady populations of type I and II neuroblasts, which undergo repeated asymmetric divisions to self-renew and to generate progenitor cells with restricted potential. Both populations of neuroblasts became aberrantly expanded in larval brains lacking the tumor suppressor Lgl. To understand how Lgl suppresses formation of ectopic neuroblasts, we investigated their cellular origin using lineage analysis. Surprisingly, we found the ectopic neuroblasts in *lgl* mutants arise due to a failure to maintain restricted potential in progenitor cells. In *lgl* mutants, Numb failed to localize asymmetrically in mitotic neuroblasts and intermediate neural progenitors and increased function of *numb* or inactivation of *Notch* signaling suppressed the ectopic neuroblast phenotype. Exclusive asymmetric inheritance of Numb into the progenitor cells requires the ACBD3 binding (AB) domain, which is also essential for Numb to suppress ectopic neuroblasts in *lgl* mutants. Although the non-phosphorylatable form of the Numb transgenic protein at two conserved aPKC phosphorylation sites within the AB domain, serines 48 and 52, symmetrically segregated into both daughter cells, unexpectedly, both the phosphomimetic and non-phosphorylatable form suppressed ectopic neuroblasts in *lgl* mutants, strongly suggesting that Numb can suppress reversion of progenitor cells back into neuroblasts independently of the regulation by aPKC. We propose that Lgl antagonizes aPKC to ensure that a critical threshold of Numb is reached in the future progenitor cells where Numb maintains restricted potential via an aPKC-independent mechanism.

160A

Functions of a helix-loop-helix transcription factor, Extramacrochaetae, in development of left-right asymmetry in the *Drosophila* embryonic hindgut. Ryo Hatori, Kiicrhio Taniguchi, Takashi Okumura, Naotaka Nakazawa, Reo Maeda, Kenji Matsuno. Tokyo University, Department of Biological Science and Technology Noda, Chiba Yamazaki 2641.

Left-right asymmetrical morphogenesis is a critical aspect of many animal organogenesis. To understand the mechanisms involved in left-right asymmetrical morphogenesis, we are using the embryonic hindgut of *Drosophila*. During development, the hindgut rotates left handedly 90 degrees and this rotating direction genetically determined. In our genome wide genetic screen, we previously found that *DE-Cadherin* (*DE-Cad*)and *Myosin31DF* (*Myo31DF*) mutants show randomized and reverse laterality of the hindgut, respectively. More recently, we identified *extramacrochaetae* (*emc*), as a gene involved in regulating the laterality of the embryonic hindgut. *emc* encodes a negative helix-loop-helix transcription factor involved in a diverse range of biological processes such as cell-fate determination. Our epistatic analysis implies that Emc functions upstream of Myo31DF and *D*E-Cadherin. In wildtype embryos, *D*E-Cadherin tended to be localized in a planar left-right asymmetrical manner at cell boundaries. However, in *emc* mutants, there was no left-right bias in the planar localization of *D*E-Cadherin. Next, we hypothesized that this planar left right bias of *D*E-Cadherin localization might regulate left right asymmetrical cell shape through differential cell adhesion. Indeed, the shape of epithelial cells of the hindgut were chiral with respect to the anterior-posterior axis. This new type of cell chirality was named, planar cell chirality (PCC). PCC tended to be slanted to the left in wild-type flies, but *emc* mutants showed no left-right bias in PCC. Moreover, our *in silico* simulation suggested that PCC is sufficient for the left-right asymmetric morphogenesis of the hindgut. In summary, we have shown that *emc* might regulate the left-right asymmetrical cell shape of the hindgut by controlling the left-right asymmetrical *D*E-Cad distribution and adhesion.

161B

Epithelial cell polarity in the hindgut of the *Drosophila* embryo. Alexandra Kumichel, Elisabeth Knust. Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, Dresden, Germany.

The Crumbs complex, which consists of the core components Crumbs (Crb), Stardust, *D*Patj and *D*Lin-7, is localized in the subapical region of ectodermally derived epithelial cells, apical to the zonula adherens (ZA), where it is essential for the maintenance of apico-basal cell polarity. In the *Drosophila* embryonic hindgut epithelium, the large intestine is subdivided into a dorsal and a ventral domain, which are separated by two lateral cell rows called boundary cells. Boundary cells differ in their shape and organization of the apical surface from the remaining hindgut cells. Strikingly, *crb* mRNA and protein levels are strongly upregulated in these cells in comparison to the other cells of the hindgut, and both are localized apically. In order to investigate the role of Crb in the altered phenotype of the boundary cells, we studied the localization of the other core components of the Crb complex in the boundary cells by immunohistochemistry and found that none of them showed upregulated protein levels or an altered localization compared to the cells of the dorsal or ventral domain. We also studied the localization of other polarity markers as well as the effect of loss or overexpression of Crb on cell morphology of the boundary cells.

162C

Drosophila Planar Polarity Gene Multiple Wing Hairs Interacts with formin to Locally Inhibit Actin Cytoskeleton. Qiuheng Lu, Paul Adler. University of Virginia, Charlottesville, VA.

The frizzled pathway has been extensively studied in wing planar cell polarity (PCP); however, it remains unclear how the PCP signal is read out as single distally pointing actin-rich hair. The downstream PCP gene multiple wing hairs (mwh) is thought to encode an inhibitor of the actin cytoskeleton. The amino half of Mwh shows similarity to the same region of Diaphanous (dia), a protein that promotes actin polymerization. The amino terminal part of Dia is key for regulating Dia activity and it also mediates dimerization. This suggested the possibility that Mwh might inhibit the activity of the actin cytoskeleton by acting as a “dominant negative Dia” (i.e. by forming inactive heterodimers). Consistent with this model we found that expression of a constitutively active Dia leads to multiple hair cells. We have also found genetic interactions between mwh and dia, between the amino half of Mwh and dia, as well as between Mwh and constitutively active Dia that are consistent with the two proteins acting antagonistically. Further we established that the two proteins could be co-immunoprecipitated from wing discs and that they colocalized in pupal wing cells. Further studies of this model are in progress.

163A

Separating planar cell polarity and Hippo signaling activities of the protocadherins Fat and Dachsous. Hitoshi Matakatsu, Seth Blair. Dept Zoology, Univ Wisconsin, Madison, WI.

The protocadherins Fat (Ft) and Dachsous (Ds) are required for several biological processes in the development of *Drosophila*, including controlling growth via the Hippo signaling pathway, planar cell polarity (PCP) and the proximodistal patterning of appendages like as wing and eye. Recently it has been suggested that mammalian homologue Ft and Ds also regulates PCP and growth. Ft and Ds binds in a preferentially heterophilic fashion. It has thus been suggested that Ft and Ds serve not as adhesion molecules, but as receptor and ligand in a poorly understood signaling pathway. To understand Ds-Ft signaling pathway on PCP and growth control, we performed a structure-function analysis of Ft and Ds, separating their adhesive and signaling functions. We found that the extracellular domain of Ft is not required for its activity in growth control, PCP and proximodistal patterning (Matakatsu and Blair, 2006). To identify the domains which are responsible for PCP and growth control activities in Ft’ intracellular domains (ICD), we extend analysis for Ft’ ICD. Surprisingly, the effects of Ft’ICD on PCP and the growth control are largely separable, suggesting that PCP and growth control is mostly independent pathway. In contrast with Ft, the extracellular domain of Ds is necessary and sufficient to mediate its effects on PCP, consistent with the model that Ds acts as a ligand during PCP. However, we would provide evidence that Ds can regulate growth independently of Ft, and that the intracellular domain of Ds can affect growth control and proximodistal patterning.

164B

Short stop in rhabdomere terminal web is essential for Drosophila photoreceptor morphogenesis. Sang-Chul Nam, Uyen Ngoc Mui, Christina M. Lubczyk. Dept Biol, Baylor Univ, Waco, TX.

Crumbs (Crb), a cell polarity gene, has been shown to provide a positional cue for the apical membrane domain and adherens junction (AJ) during Drosophila photoreceptor morphogenesis. It has recently been found that stable microtubules in developing Drosophila photoreceptors were linked to Crb localization. Coordinated interactions between microtubule and actin cytoskeletons are involved in many polarized cellular processes. Since Spectraplakin (Short stop, Shot) is able to bind both microtubule and actin cytoskeletons, the role of Shot was analyzed in the regulations of apical Crb domain in developing Drosophila photoreceptors. The localization pattern of Shot in developing pupal photoreceptors showed a unique intracellular distribution. Shot localized at rhabdomere terminal web which is at the basal side of the apical Crb or rhabdomere, and in between the AJs. The shot mutant photoreceptors showed dramatic mislocalizations of Crb, AJs, and the stable microtubules. This role of Shot in Crb and AJ regulation was further supported by Shot’s gain-of-function phenotype. Shot overexpression in photoreceptors caused a cell polarity defect including dramatic mislocalization of Crb, AJs and the stable microtubules in the developing photoreceptors. Furthermore, a strong genetic interaction between shot and crb was found using a genetic modifier test. In summary, we found a unique localization of Shot in photoreceptors, and identified the role of Shot in the regulation of the apical Crb domain and AJs through genetic mutational analysis. Our data suggest that Shot, an actin-microtubule cross-linker, is essential in the apical and AJ controls during the photoreceptors morphogenesis.

165C

Impact of retinal disease-causing missense mutations in the extracellular domain of Crumbs on photoreceptor development and survival in *Drosophila*. Milena Pellikka, Ulrich Tepass. Dept Cell & Systems Biology, University of Toronto, Toronto, ON.

The apical transmembrane protein Crumbs (Crb) is a critical regulator of epithelial polarity and apical membrane morphogenesis in photoreceptor cells (PRCs). *Drosophila* and vertebrate Crb proteins localize to corresponding apical membrane domains, the stalk membrane in *Drosophila* and the inner segment of vertebrate PRCs. Crb is required for the maintenance of rhabdomere shape, zonula adherens integrity, stalk membrane length, and cell survival of *Drosophila* PRCs. Similarly, mutations in one of the three human orthologs of Crb (CRB1) are linked to eye degenerative conditions such as Leber’s

Congenital Amaurosis (LCA) and Retinitis Pigmentosa type 12 (RP12). Work in vertebrate models suggests that a loss of epithelial integrity precedes PRCs degeneration in Crb compromised retinas. Over 40 distinct disease-causing missense mutations that map to the extracellular region have been reported for human CRB1. To gain insight into how these missense mutations affect CRB1 function and to elucidate the function of the large extracellular region of Crb/CRB1 we have recreated four disease-causing missense mutations that affect conserved amino acids in *Drosophila* Crb. Our analysis of these Crb mutant isoforms in both wildtype and *crb* mutant PRCs identified specific residues/domains in Crb that are required for the normal localization of Crb at the stalk membrane. Moreover, each of the four missense mutations tested so far shows a unique cell biological profile including mutations that cause mislocalization of Crb to the rhabdomere, which leads to a displacement of Rhodopsin. Loss of Rhodopsin function is a known cause of RP, thus suggesting a potential disease mechanism that may be independent from the role of Crumbs in supporting polarity for these particular mutant alleles. In contrast to expression of normal Crb in a *crb* mutant background, none of the four Crb missense mutants can prevent PRC degeneration.

166A

Crumbs supports apical delivery in the developing photoreceptor. Rhian F. Walther, Franck Pichaud. MRC LMCB, University College London, London, United Kingdom.

Epithelial polarity remodeling is key for allowing epithelial-derived organ formation. Typically it involves a conserved set of factors, including the apically localized Par6-DaPKC, Crumbs (Crb), Sdt and PATJ, the adherens junction associated Bazooka (Baz) as well as the laterally localized Lethal giant larvae (Lgl), the Serine-Threonine kinase Par1 and the recently identified Yurt/Coracle pathway. However, the molecular nature underlying the genetic interactions between these factors remains for the most part elusive. In order to tackle this question, we are making use of a striking step of apico-basal polarity remodeling occurring during photoreceptor morphogenesis. In this context, we find that Par1 localizes at the lateral membrane of the cell where it limits lateral clustering of Baz. In this cell, par1 function is required to limit Baz-dependent apical membrane morphogenesis. In parallel, Lgl is required to prevent basal, but not lateral accumulation of the apical factor Crb. In this situation, Crb is sufficient to support the Rab11-MyoV apical trafficking route required to build the apical organelle, called the rhabdomere, but it is also sufficient to redirect apical secretion of the luminal factor eyes-shut toward the basal side of the cell. Our work therefore presents Crb as a main effector of directed apical trafficking during apico-basal epithelial polarity remodeling.

167B

An shRNA screen for genes involved in epithelial polarity identifies a novel member of the Par polarity complex. Frederik Wirtz-Peitz[1], Dong Yan[1], Takashi Nishimura[2], Norbert Perrimon[1]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) RIKEN Center for Developmental Biology, Kobe, Japan.

In the Drosophila embryo, epithelial cells are polarized towards the end of cellularization by the concerted activity of a conserved set of polarity proteins. How these proteins interact to establish and maintain distinct membrane domains is poorly understood. We screened for novel polarity genes using a library of shRNA constructs, which permit the analysis of nearly protein-null embryos by knockdown of a gene's maternal and zygotic expression. Thus, we identified a previously uncharacterized protein, which, when knocked down, causes a defect similar to mutants in the Par complex. This polarity complex, consisting of atypical protein kinase C together with its regulators Par-6 and Bazooka, localizes to the apical cell cortex where it stabilizes the apical domain and inhibits determinants of the basolateral domain. Our data reveal that the protein identified in our screen participates in the Par complex by virtue of a direct interaction with Bazooka, and we present an initial characterization of its molecular role in epithelial polarity.

168C

Determining the mechanisms of CTP synthase filament (cytoophidia) formation. Gabriel N Aughey, Ji-long Liu. MRC Functional Genomics Unit, University of Oxford, Oxford, United Kingdom.

Cytidine triphosphate synthase (CTPS) is the rate limiting enzyme for the de novo synthesis of cytidine triphosphate (CTP), a fundamental component of DNA and RNA, as well as a precursor to fatty acid synthesis. It has recently been observed that CTP synthase (CTPS) can be compartmentalised into discrete filamentous cytoplasmic structures that have been termed cytoophidia. This feature of CTPS is highly conserved throughout evolution and has been reported in single celled organisms (the bacteria C. cerescentus, and budding yeast, S. cerevisiae) as well as complex eukaryotes including Drosophila and humans, suggesting that the compartmentation of CTPS in this way is a fundamental feature of all cells. The rate limiting enzyme for GTP biosynthesis, inosine monophosphate (IMPDH), also colocalises to this structure in Drosophila melanogaster and has been shown to form filaments in human cells. Expression of GFP tagged CTPS in Drosophila S2 cells results in increased abundance of cytoophidia. This system will be used for the development of a high throughput, genome-wide RNAi screen with Drosophila S2 cells in order to identify factors that regulate CTPS compartmentalisation.

169A

Coordination between stable and dynamic microtubule networks determines and maintains Drosophila bristle shape. Amir Bitan, Uri Abdu. Department of life sciences, Ben Gurion University, Beer Sheva, Israel.

Within interphase cells, microtubules (MT) are organized in a cell-specific manner to support cell shape and function. Here, we report that coordination between stable and dynamic MTs determine and maintain the highly elongated bristle cell shape. By following MT-decorating hooks and by tracking EB1, we identified two MT populations within bristles, namely a stable MT population polarized minus-ends-distal toward the bristle tip and a dynamic MT population that exhibits mixed polarity. Manipulating MT dynamics by klp10A down-regulation demonstrates that MTs can initiate new shaft extensions, thus possessing the ability to determine growth direction. Actin-filament bundling subsequently supports the newly-formed shaft extensions. Established by elongation defects in the Drosophila ikk-epsilon homologue, ik2 mutant bristles, we report that stable and dynamic MT orientation and polarized organization are important for proper bristle elongation. Thus, we demonstrate for the first time that coordination between stable and dynamic MT sets that are both axially-organized but differently polarized, drives cell elongation.

170B

JNK signaling regulates the actin-binding protein Profilin in *Drosophila* larval wound closure. Amanda R. Brock[1,2], Yan Wang[1], Susanne Berger[3], Violet C. Han[1], Yujane Wu[1,2], Renate Renkawitz-Pohl[3], Michael J. Galko[1,2]. 1) Biochem & Molec Bio, UT MD Anderson Cancer Ctr, Houston, TX; 2)

Genes & Development Graduate Program, UT GSBS, UTMDACC, Houston, TX; 3) Fachbereich Biologie, Philipps-Universitat Marburg, Germany.

Wound healing is essential for the comfort and survival of most organisms. Epidermal closure requires that cells can recognize tissue damage, alter their behavior to migrate across the gap, and eventually reestablish tissue continuity. Two signaling pathways are known to regulate these behaviors in *Drosophila* larvae, the Jun N-terminal kinase (JNK) signaling pathway and the receptor tyrosine kinase, Pvr. Currently, it is uncertain how these signaling pathways regulate the actin cytoskeletal rearrangements that are a prerequisite for efficient cell migration. Here we show that *chickadee*, the *Drosophila* Profilin, is necessary for larval wound closure. After wounding, Profilin protein levels increase in cells around the wound gap. This increase is likely a result of the transcriptional activation of the chic locus. We show here that wound-induced chic transcription is regulated by the JNK signaling pathway, and not by Pvr. The canonical JNK pathway has two downstream transcription factors, Jun and Fos, but of these, only Fos regulates chic transcription. Lastly, we also show that larvae lacking epidermal Profilin are unable to properly concentrate actin at the wound edge and are unable to extend filopodia and lamellipodia into the wound area. Thus, we show a connection between JNK signaling pathway activation and expression and function of an important cytoskeletal regulator during wound closure.

171C

Cytoskeletal polarization during collective cell migration in the Drosophila egg chamber. Maureen P. Cetera, Sally Horne-Badovinac. DRSB, University of Chicago, Chicago, IL.

Collective cell migration is critical for proper morphogenesis of developing organisms. The Drosophila egg chamber provides a novel system in which to study collective cell migration of a continuous epithelial cell layer. During oogenesis, the egg chamber elongates from a spherical precursor to a mature elliptical egg. At this time, the follicular epithelium migrates circumferentially around the egg chamber's anterior-posterior axis along an extracellular matrix. Follicle cell migration is hypothesized to contribute to egg chamber elongation but the molecular mechanisms underlying this morphogenetic event are currently unknown (Haigo and Bilder, 2011). We predict the migrating epithelium would require planar polarization of its cytoskeleton and we have shown each follicle cell has a distinct leading and trailing edge. The leading edge consists of actin-based protrusions that are sensitive to Integrin and Enabled levels and the back of the cell displays Myosin II accumulation that may serve to relieve focal adhesions to allow forward migration. We have developed techniques to observe the dynamics of follicle cell crawling along the extracellular matrix and by manipulating protrusion formation, adhesion or Myosin II activity, we can determine their role in collective cell migration of the follicular epithelium.

172A

Distinguishing spectrin gain-of-function and loss-of-function effects in the larval fat body of Drosophila. Bianca Diaconesea, Ron Dubreuil. Dept. of Biological Sciences, University of Illinois Chicago, Chicago, IL.

Lethal mutations affecting α and β spectrin have been described in many different systems. Phenotypes have also been observed with overexpression of full-length β spectrin or spectrin fragments in model systems. The latter phenotypes are often categorized as dominant negatives, although this can be difficult to demonstrate directly. Here we gain new insight into this issue by comparing the effects of β spectrin overexpression and knockdown in a single cell type. We find: 1) RNAi knockdown of α or β spectrin in the larval fat body of *Drosophila melanogaster* altered plasma membrane morphology, but did not otherwise affect growth or viability of the organism. 2) β spectrin overexpression was lethal at the highest levels and produced milder phenotypes at lower levels. In contrast, overexpression of α spectrin was never lethal. 3) Excess β spectrin accumulated specifically at the plasma membrane, resulting in an apparent block in lipophorin secretion from the fat body. This block led to abnormal dietary lipid accumulation in the midgut, identical to that observed with lipophorin RNAi. 4) Lethality and lipid transport defects were overcome when α spectrin was co-expressed with β spectrin. Rescue did not noticeably change the abundance or distribution of overexpressed spectrin, suggesting that the β phenotype depends upon misregulation of β spectrin function in the absence of α, as well as overexpression. There are a number of implications of this work for assessing spectrin function. While there are some dominant negative effects of β overexpression in the fat body, there also appear to be hypermorphic gain-of-function effects that may not be related to normal spectrin function (e.g. on secretion of lipophorin). We speculate that gain of function effects could play a prominent role in spectrin genetics in other systems as well.

173B

The Putative Ena Interacting Protein, SKIP, is Required for Border Cell Migration. Julie Gates, Kate Bowen, Lindsay Regruto, Kara Weichler. Biology Dept., Bucknell University, Lewisburg, PA.

During development the actin cytoskeleton must be remodeled to accommodate the remarkable changes in cell shape, cell rearrangements and cell migrations that occur as tissues and organs are formed. If actin dynamics are not properly regulated, morphogenesis is disrupted and normal development fails. Numerous proteins have been identified that influence actin dynamics including members of the Ena/VASP protein family. *Drosophila* has a single Ena/VASP family member, Ena. Previous work has shown that Ena is required during multiple morphogenetic processes including dorsal closure, nurse cell dumping and border cell migration. To gain additional insight in to how Ena may be regulated, we have chosen to examine the role of a putative Ena binding partner, SKIP (Shal K^+ Channel Interacting Protein). SKIP was found to bind to Ena in a large-scale yeast two-hybrid screen carried out by Giot and colleagues in 2003. There are three SKIP protein isoforms. The longest isoform, SKIP1, contains an N- and C-terminal SAM (sterile-alpha-motif) domain and an SH3 domain. While we are currently carrying out biochemical experiments to verify this interaction, the presence of an SH3 domain in SKIP1 and SKIP2, and a proline-rich region in Ena that has been shown to bind SH3-containing proteins, made SKIP a putative binding partner worth further characterization. We have used tissue-specific expression of SKIP RNAi to reduce SKIP protein levels in either all somatic follicle cells (T155-Gal4) or specifically in border cells, centripetal and posterior follicle cells (slbo-Gal4). In both cases a reduction in SKIP protein levels results in delayed border cell migration and the occasional failure of a subset of border cells to remain within the border cell cluster. Preliminary data suggests that the reduction in SKIP protein levels may also result in defects in the follicle cell basal actin network. We are currently examining the localization of Ena in follicle cells with reduced SKIP protein levels to determine whether SKIP functions by regulating the localization of Ena.

174C

Narrowing regions of chromosome two that genetically interact with Abl kinase during embryonic development. Terri Hale[1], Daniella Kawa[1], Adam O-Neil[1], April Peterson[1], Andrew Simmons[1], Traci Stevens[2]. 1) Cosby High School, Midlothian, VA; 2) Biology Dept, Randolph-Macon College, Ashland,

VA.

Signaling by Abl kinase is important in normal cell development, differentiation, and migration. Abl protein regulates the assembly of actin, a globular protein that is a major component of the cytoskeleton. In humans, Abl activation occurs in cells that have the Philadelphia chromosome, which carries the mutant *Bcr-Abl* fusion gene, an oncogene that forms as a result of a translocation between chromosomes 9 and 22. As a result of this translocation, Abl kinase activity is increased, and the structure of actin and cell migration are altered. The goal of our laboratory is to identify genes that interact with Abl during cell migration, using *Drosophila* embryos that express Bcr-Abl as a model system. Embryos expressing Bcr-Abl die, with defects in developmental processes that require cell migration. To identify genetic interactions with Abl, the phenotypes of embryos expressing Bcr-Abl were compared to the phenotypes of embryos expressing Bcr-Abl and carrying a heterozygous deficiency. In this study, four deficiencies previously shown to interact with Bcr-Abl were examined. Within each region, we tested smaller deficiencies and mutant alleles of candidate genes. For example, we dissected a region of the second chromosome defined by two overlapping deficiencies that suppressed phenotypes associated with Bcr-Abl expression (Df(2R)ED1673 and Df(2R)ED1715). We found that a mutant allele of *didum*, which lies in the region of overlap, suppressed Bcr-Abl associated phenotypes, while five smaller deficiencies and mutant alleles of two other candidate genes in this region did not modify phenotypes associated with Bcr-Abl expression. We analyzed three other interacting deficiencies in a similar fashion, and overall, we identified three genes that are likely to play a role in Abl signaling pathways. This research was a collaborative study between Randolph-Macon College and four students and a biology teacher from Cosby High School over two summers.

175A

The integrin effectors, PINCH and RSU1, modulate actomyosin contractility in mutants of the myosin phosphatase *flapwing* via independent mechanisms. Julie L. Kadrmas[1,2], Stephen M. Pronovost[2]. 1) Oncological Sci; 2) Huntsman Cancer Institute, Univ Utah, Salt Lake City, UT.

Contractility of the actomyosin cytoskeleton is essential for cell shape changes and cell migration underpinning many biological processes. Actomyosin contraction is driven by kinase activity on serine-21 and threonine-20 of non-muscle Myosin Regulatory Light Chain, encoded by *spaghetti squash* (*sqh*). Actomyosin relaxation requires the corresponding dephosphorylation of Sqh. *flapwing* (*flw*) encodes Protein phosphatase 1β, with the single essential function of Sqh dephosphorylation. Strong loss-of-function mutants in *flw* have hyper-phosphorylated Sqh, with lethality due to larval muscle detachment in a majority of animals. This phenotype of *flw* mutants overlaps with that of PINCH, a 5 LIM domain scaffolding protein encoded by *steamer duck* (*stck*). PINCH functions to stabilize actin-integrin linkages in muscle. In *stck* mutants, embryonic lethality arises from cytoskeletal detachment from the muscle cell membrane at sites of integrin attachment. PINCH functions as part of a stabilized protein complex that includes its direct binding partner RSU1, encoded by *ics*. We tested for a genetic interaction between *flw* and both *stck* and *ics*. We demonstrate that ectopic expression of PINCH-Flag using the native *stck* promoter fully suppresses the larval lethality of *flw* mutants, but does not reduce aberrantly high levels of phospho-Sqh as a means to promote pupariation. Additionally, we show that elimination of RSU1 enhances the larval lethality of *flw* mutants. In the absence of RSU1, *flw* larvae exhibit an additional 3-fold increase in levels of phospho-Sqh. Together, this supports a model in which both PINCH and RSU1 regulate actomyosin contractility in larvae, but do so via distinct mechanisms: RSU1 can influence the phosphorylation state of Sqh, whereas PINCH acts by an alternative mechanism, perhaps by stabilizing a constitutively contracted cytoskeleton. Future work will further define these regulatory pathways.

176B

Dissecting the regulation, interactions, and activity of the APC2-Dia complex in the formation of actin pseudocleavage furrows in the Drosophila syncytial embryo. Ezgi Kunttas Tatli[1], Vince Stepanik[1], Richa Jaiswal[2], Bruce L. Goode[2], Brooke M. McCartney[1]. 1) Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts.

Many cellular and developmental processes like cell division, cell shape change and cell migration require precise cytoskeletal rearrangements, which are orchestrated via numerous actin and microtubule-associated proteins. The colon cancer tumor suppressor Adenomatous polyposis coli (APC) is a poorly understood regulator of the actin cytoskeleton. One powerful model system to study the role of APC in actin organization in vivo is the Drosophila syncytial embryo. During this stage, the embryo undergoes synchronous nuclear divisions without cytokinesis, and actin-based pseudocleavage furrows act as physical barriers between neighboring nuclei during each division to ensure mitotic fidelity. We have previously shown that a complex between APC2 and the formin Diaphanous (Dia) regulates the formation and extension of pseudocleavage furrows. However, the regulation of this complex, the molecular basis for their interaction, and APC2's affects on Dia's actin nucleation and elongation activity are not well understood. To answer these questions, first we tested the hypothesis that the activity of the APC2-Dia complex is regulated by APC2 phosphorylation. Drosophila APC2 is a phospho-protein and APC activity is regulated by phosphorylation in other contexts. We show that phosphorylation of the 20 amino acid repeats (20Rs), a region known to interact with Armadillo (Arm), plays a role in actin furrow extension. Interestingly, Dia binds to both a region of APC2 containing the 20Rs and to the SAMP repeats, suggesting a complex interaction between APC2 and Dia that may be regulated by phosphorylation. Lastly, using in vitro assays, we are investigating the effects of both APC2 and APC1 on the actin nucleation and elongation activity of Dia.

177C

Analysis of cell overstretching induced by microtubule depolymerization during tracheal morphogenesis. Pierre-Marie LE DROGUEN, Antoine GUICHET, Veronique BRODU. Institut Jacques MONOD, Paris, Paris, France.

Microtubules (MTs) are essential for many cell features such as cell shape, polarity, motility and vesicle trafficking. Through these processes, MTs are involved in establishing epithelial structure. Notably they play a central role in directing Adherens Junction (AJ) assembly during cellularization of Drosophila embryo. During gastrulation onwards, AJs have to be reorganized and maintained but the role of MT is poorly characterized. We have addressed the question of MT requirement during embryonic tracheal morphogenesis. Tracheal branches arise in part from the migration of cells at the tip of branch buds. This migration induces a pulling force on following tracheal cells as cells remain attached to each other. Hence this force triggers cell intercalation followed by cell elongation leading to the total branch extension. Throughout this branching process, AJs are remodeled and maintained between tracheal cells, ultimately giving rise to a branched tubular network organized around a lumen. We have established that during tracheal morphogenesis MTs are important for the branching process as depolymerization of MT network leads to branch breaks. We first characterized more precisely this phenotype and show that secretion of lumen products is not affected. Furthermore, the overall cell polarity seems to be maintained, and reorganization of AJ during

intercalation events occurs without obvious defects. In order to precisely monitor the MT requirement leading to branch break, we have developed a live imaging approach in which MT are depolymerized by over expressing the MT severing protein Spastin. We revealed that breaks induced in tracheal branches appear in a progressive instead of abrupt manner, with cells that overstretch, as seen in DE-Cadherin hypomorph mutant embryos. This overstretched phenotype occurs after intercalation when cells have to elongate their apical part. As tracheal cells fail to maintain tension during apical extension, we are currently investigating a link between MT and myosin II whose activity has already been involved as a tension driving force.

178A

Both Capulet and Slingshot restrict actin polymerization through regulating Twinstar activity during Drosophila eye morphogenesis. Chiao-Ming Lin, Pei-Yi Lin, Yu-Chiao Li, Yu-Huei Ho, Jui-Chou Hsu. Institute of Molecular Medicine, Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan 30034, Republic of China.

In response to extracellular signaling cues, the cortical actin cytoskeleton within a cell reorganizes to regulate various cell activities, including morphogenesis, cell movement, and cell division. Dynamic turnover provides cells with the plasticity necessary to remodel actin networks rapidly and replenish the pool of ATP-bound actin monomers available for new growth. A striking example of a transient shape change is the morphogenetic furrow (MF) that progresses across the eye disc from posterior to anterior during Drosophila eye morphogenesis. CAP/Capulet (Capt), Slingshot (Ssh) and Cofilin/Twinstar (Tsr) are all involved in restriction of actin polymerization. Loss of capt, ssh and tsr in the Drosophila eye epithelia similarly cause accumulation of F-actin and enlarged apical area. By comparing their phenotypes, at single cell resolution, in eye epithelia, we found that capt and ssh, but not tsr, mutant cells within and posterior to the morphogenetic furrow (MF) shared similar phenotypes. These include capt/ssh mutant cells possessed (1) largely complementary accumulation of excessive F-actin and phosphorylated myosin light chain (p-MLC) at apical cortex, (2) hexagonal cell packing with discontinuous adherens junctions (AJs) and (3) increased Ci155 accumulation in the eye epithelial cells. Similar phenotypes could be observed in capt/ssh mutant cells anterior to the MF, upon activation of Hh pathway. We further found that the accumulation of F-actin, but not p-MLC, depended on the removal of Ci75. Conversely, Capt/Ssh negatively regulated Ci155 levels within the MF, at a step upstream of Protein kinase A (PKA)-mediated Ci155 proteolysis. Significantly, overexpressing a constitutively active form of cofilin and N-terminal region of Capt that recycle cofilin respectively rescued the ssh and capt mutant phenotypes. Together, we conclude that Capt and Ssh act at distinct steps to modulate cofilin-mediated F-actin remodeling during eye morphogenesis.

179B

Dissecting the domains of APC2 required for cortical actin association. Olivia Molinar[1], Molly Berntsen[2,3], Paige Davison[2,3], Terrence Wong[2,3], Ezgi Kunttas-Tatli[1], Gordon Rule[1,2], Brooke McCartney[1]. 1) Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) HHMI-Summer Research Institute; 3) equal contribution.

Adenomatous polyposis coli (APC) proteins regulate the actin and microtubule cytoskeletons, and negatively regulate the Wnt signaling pathway. While APC's role in Wnt signaling and microtubule function are well studied, the mechanisms by which APC proteins interact with and regulate the actin cytoskeleton are not well understood. APC2 localizes to actin in the Drosophila syncytial embryo, where together with the formin Diaphanous, it is required for the extension of actin pseudocleavage furrows that form during metaphase. To understand how APC2 promotes actin furrow extension, we are investigating the mechanisms that affect the localization of APC2 to the actin cortex. Previously we reported that the N-terminal Armadillo (Arm) repeats and the C-terminal 30 amino acids (C30) together are necessary and sufficient for the cortical localization of APC2. Deletion of C30 alone results in loss of cortical localization in the syncytial embryo and defects in furrow extension. Within C30 is a 15 amino acid sequence (amino acids 1048-1063) that is highly conserved within Drosophila species suggesting functional significance. Computational structural analysis of this conserved region predicts a coiled-coil. We found that the 15 amino acid conserved region together with the Arm repeats are necessary and sufficient for cortical localization of APC2 in S2 cells. A point mutation targeting one of the key hydrophobic residues in the predicted coil (V1050T) abolished cortical localization, strongly suggesting that the coil is necessary for APC2 localization to the actin cortex. Because the Arm repeats of APC2 promote its self-association and cortical localization, we predict that oligomerization of APC2 via the Arm repeats may be necessary for cortical localization. We are currently testing whether the self-association of APC2 is required for C30 function.

180C

Does *htsN4* RNA localization matter for developing oocytes? Nancy J. Pokrywka, Lita Sacks, Huadi Zhang, Kathleen M. Raley-Susman. Dept. of Biology, Vassar College, Poughkeepsie, NY.

The cytoskeleton plays a key role in the establishment of cell polarity, a process that is crucial for such biological processes as cell motility, neuronal function and embryonic patterning. A prime model of cell polarity is the localization of mRNAs during oogenesis. *Swallow (swa)* is required for the proper localization of several RNAs, including the N4 splicing variant of *hu li tai shao* (*htsN4*). The significance of *htsN4* mRNA localization during late oogenesis and early embryogenesis is unclear, but it encodes an adducin-like protein, and like mammalian adducins, may play a role in the regulation of the actin-spectrin cytoskeleton. This is of interest because *swa* mutations, in addition to disrupting *htsN4* RNA localization, also result in actin defects during mid-to-late oogenesis. However, recent evidence suggests that Swa protein may itself be membrane associated and interact with actin filaments. Thus, we were interested in distinguishing between two models for *swa* function; one that posits a direct role for *swa* in organizing actin structures, and an alternate model that argues *swa* affects actin organization indirectly by localizing *htsN4* RNA (and presumably Hts protein). We tested these models in several ways. First, we looked for actin defects under other conditions that interfere with *htsN4* RNA localization, such as microtubule disruption, to see if loss of *htsN4* RNA localization correlates with actin defects. In order to determine if *htsN4* RNA localization defects are sufficient to induce actin aberrations, we also designed variants of *htsN4* that lack some or all of the sequences necessary for correct RNA localization. We find that actin defects are often a consequence of microtubule disruption, but are not necessarily induced by a loss of *htsN4* RNA localization. Finally, we will also present evidence that the distribution of Ovhts-RC protein in mid- and late-stage oocytes is independent of *htsN4* RNA localization, suggesting that *htsN4* RNA localization may be unnecessary for oocyte organization.

181A

Genetic interactions between Clic, Cdc42 and Cdc42 effectors in filopodia formation. Regan Price[1], Soichi Tanda[1], Mark Berryman[2]. 1) Biological Sciences, Ohio University, Athens, OH; 2) Biomedical Sciences, Ohio University, Athens, OH.

Cell motility is driven by membrane protrusion, which begins with the projection of filopodia and lamellipodia. Actin dynamics at the cell cortex are regulated by the small GTPase Cell Division Cycle 42 (Cdc42) and its downstream effectors, the actin nucleators Diaphanous (Dia) and Wiskott-Aldrich syndrome protein (WASp). We seek to understand how Chloride intracellular channels (Clics) contribute to cell motility. To study Clic's role in filopodia formation, gene dosage and protein activity of Cdc42, Dia, and WASp were altered in the presence and absence of Clic function. Larval hemocytes were stained with the F-actin marker phalloidin, and scored for filopodia formation. We found that a constitutively active form of Cdc42 (Cdc42-CA), stimulates filopodia formation in larval hemocytes, consistent with previous studies. When Cdc42-CA was expressed in a Clic null mutant background, filopodia formation was suppressed, suggesting that Clic functions downstream of Cdc42. Therefore, we tested for genetic interactions between Clic and Dia, another protein important in filopodia formation. Overexpression of wild-type Dia resulted in an increase in the number of cells forming filopodia. This phenotype was dependent on Clic, as the number significantly decreased when wild-type Dia was expressed in the Clic mutant background. When a constitutively active form of Dia was overexpressed, a greater increase in the number of cells forming filopodia was observed as compared to wild-type Dia. However, this phenotype was not altered in the Clic mutant background. Next, we tested for interactions between Clic and WASp. Overexpression of wild-type WASp in hemoctyes led to an increase in the number of cells forming filopodia. This phenotype was unchanged when WASp was overexpressed in the Clic mutant background, suggesting that Clic does not interact with this protein in filopodia formation. Together these results suggest that Clic functions downstream of Cdc42, and that it interacts with the autoinhibited form of wild-type Dia, possibly promoting its activation during filopodia formation.

182B

Drosophila nurse cell dumping reveals a novel interaction between prostaglandin signaling and Fascin. Tina Tootle, Christopher Groen, Andrew Spracklen, Tiffany Fagan. Anatomy and Cell Biology, University of Iowa, Iowa City, IA.

While actin cytoskeletal dynamics are known to be regulated by prostaglandins, lipid signals produced downstream of cyclooxygenase (COX) enzymes, the mechanisms by which PGs mediate this remain unknown. Drosophila oogenesis provides a model for studying how prostaglandin signaling affects actin remodeling. During oogenesis, a process called nurse cell dumping occurs. This process requires active remodeling of the actin cytoskeleton to allow the nurse cells to squeeze their cytoplasmic contents into the growing oocyte. Using this model, we have previously shown that prostaglandins are required for actin remodeling during dumping, and that Pxt is the Drosophila COX-like enzyme. A screen utilizing our in vitro follicle maturation assay (Spracklen, Meyer, and Tootle, unpublished data) identified Fascin (*singed*), an actin bundling protein, as a downstream target of prostaglandin signaling. Here, we show that fascin and pxt mutants display similar actin remodeling defects in nurse cells. Reduced Fascin levels enhance the dumping, and thus actin remodeling, defects of both COX inhibition and reduced Pxt levels. Additionally, over-expression of Fascin in the germline, using the UAS-Gal4 system, suppresses the effects of COX inhibition. Importantly, Fascin levels, both mRNA and protein, are not affected by alterations in prostaglandin signaling. Additionally, prostaglandin signaling does not appear to globally affect actin bundling, as another actin bundling protein Villin (*quail*) fails to interact with Pxt or COX inhibition. These data indicate that one role of prostaglandin signaling in regulating actin remodeling is to specifically modulate Fascin activity. Current efforts are focused on determining the mechanism by which prostaglandins regulate Fascin. This is the first link between prostaglandins and Fascin, and is particularly intriguing as both are implicated in mediating cancer progression and metastasis.

183C

Polymerization, Metabolic Regulation, and the Origins of the Cytoskeleton. James E. Wilhelm, Chalongrat Noree, Dane Samilo, Risa Broyer, Brian Sato. Section on Cell and Developmental Biology, Univ California San Diego, La Jolla, CA.

In eukaryotes, four major classes of filament forming proteins are known to play a role in cellular organization and function: septins, tubulin, actin, and intermediate filament proteins. However, while no new filaments have been discovered in over 20 years, it has been unclear whether all of the filaments that comprise the cytoskeleton have been found. In order to address this question, we have conducted visual screens of both yeast and Drosophila GFP strain collections to identify proteins that form novel intracellular filaments. This screen identified a large number of intracellular structures including four novel filament systems comprised of glutamate synthase, GDP-mannose pyrophosphorylase, CTP synthase, or subunits of the eIF2/2B translation factor complex. Given the novelty of these structures, we have focused our efforts on characterizing CTP synthase filaments. By combining structure function analysis with a novel in vitro polymerization assay, we have found that regulation of enzyme activity is intimately connected with the regulation of polymerization. CTP synthase filaments are also present in all species examined from bacteria to humans. Interestingly, CTP synthase filaments are restricted to axons in neurons. This spatial regulation suggests that these filaments have additional functions separate from the regulation of enzyme activity. The identification of four novel filaments doubles the number of known intracellular filament networks and has broad implications for our understanding of how cells organize biochemical activities in the cytoplasm. This work also has implications for the evolution of the cytoskeleton since it suggests that the classic cytoskeleton may have evolved from metabolic enzymes that used polymerization as a mechanism for regulating enzyme activity.

184A

The role of CTP Synthase during CNS development. Omur Y. Tastan, M. Ghows Azzam, Kemian Gou, Mayte Siswick, Ji-Long Liu. MRC Functional Genomics Unit Department of Physiology, Anatomy and Genetics, University of Oxford, United Kingdom.

CTP Synthase is an enzyme that is involved in pyrimidine biosynthesis which converts UTP to CTP. When expressed at high levels, CTP Synthase forms filaments called cytoophidia. CTP Synthase has been shown to be enriched in certain cancer types. In addition, several inhibitors of CTP Synthase, e.g. DON (6-diazo-5-oxo-L-norleucine), has shown promising potential as therapeutic drugs. Recent studies show that cytoophidium is found in many cell types. Expression analysis showed that CTP Synthase can be found in the cytoplasm, nucleus and also in the cytoophidium at different cell types and at different stages of development in various tissues. Mutant analysis showed that CTP Synthase mutations result in larval lethality with pupation defects which is fully rescuable with a CTP Synthase transgene. In an effort to identify CTP Synthase interactors we performed a yeast two hybrid screen and recovered three potential CTP Synthase interactors among 20 hits. The verification of interactions by co-immunoprecipitation experiments is ongoing. Expression analysis showed that all three proteins form filaments in DON treated human cell lines. We are in the process of generating transgenic animals and antibodies to characterize these genes further in vivo.

185B

A BMP-dependent feedback loop regulates *dpp* expression by direct and indirect mechanisms in the *Drosophila* wing imaginal disc. Maryanna M. Aldrich, Lorena Soares, Kristi Wharton. MCB Department, Brown University, Providence, RI.

Positional information across a field of unspecified cells is often established by a gradient of morphogen activity that results in threshold-specific transcriptional responses. As the tissue develops, regulatory mechanisms likely exist to ensure robustness within signal transduction pathways that generate such morphogen activity gradients. In the *Drosophila* wing disc, the graded distribution of the phosphorylated BMP signal transducer, phospho-Mad (pMad), directs the transcriptional response of distinct sets of target genes critical for patterning along the anterior-posterior axis. BMP signaling is thought to maintain robustness as a result of feedback mechanisms involving regulation of the pathway modulators, *dad* and *pent*. The work presented here characterizes an additional feedback mechanism that directly impacts transcriptional regulation of the BMP ligand, *dpp*. We show that a negative feedback loop mediated through canonical Mad-mediated BMP signaling regulates *dpp* transcription within its endogenous wing pouch expression domain. Through a combined analysis of binding sites within the *dpp cis*-regulatory sequences and of requirements for candidate transcription factors, we determined that Shn is required for negative feedback but Ci is not essential. Furthermore, we find that BMP signaling exhibits a domain-specific effect on transcription of *dpp*. In the lateral region of the wing disc, BMP signaling positively regulates *dpp* expression. Curiously, this effect is blocked by action at a putative Mad binding site within the control regions of *dpp*. Within the medial region of the wing pouch, the endogenous expression domain of *dpp, dpp* is regulated in a pMad dose-dependent manner. Taken together, we propose that this complex regulation of *dpp* serves to fine tune BMP signaling should fluctuations in signaling output be encountered, thus, ensuring robustness during patterning of the wing pouch.

186C

A *Drosophila* cell culture model for Dpp-induced epithelial plasticity. David J. Casso[1], Björn Gärtner[2], J. Alex Rondon[1,4], Aiguo Tian[1,3], Rik Derynck[1], Julia Zeitlinger[2], Katja Brückner[1]. 1) University of California San Francisco, San Francisco, CA; 2) Stowers Institute for Medical Research, Kansas City, MO; 3) Present Address: Univ Texas Southwestern Medial Center, Dallas, TX; 4) Present Address: Genentech, South San Francisco, CA.

Epithelial plasticity, which reflects changes of epithelial cells regarding their morphology and differentiation state, is an essential program in normal development, and underlies life-threatening pathologies such as fibrosis and tumor metastasis. In vertebrates, members of the TGF-β/BMP family are potent inducers of epithelial plasticity. *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 adult fly. Based on an RNAi screen comprising all *Drosophila* kinases and phosphatases, expression profiling, and ChIP analyses, we identified Mad (mothers against Dpp) transcriptional 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. We now focus on the mechanism of cooperation between the Dpp and Akt/Tor pathways, and investigate the role of these pathways and their transcriptional targets during thorax closure in *Drosophila* in vivo.

187A

A Molecular Competition between Wingless and BMP Signaling Controlled by Mad Phosphorylations. Edward V Eivers[1], Hadrien Degmany[2], Edward DeRobertis[2]. 1) Department of Biological Sciences, California State University, Los Angeles, CA 90032-8201; 2) Howard Hughes Medical Institute, University of California, Los Angeles, CA 90095-1662.

Bone morphogenetic proteins (BMPs) and Wnts are growth factors that provide essential patterning signals for cell proliferation and differentiation. Here we describe a novel mechanism of action for the transcription factor Mad in Wingless signal transduction. Traditionally, Mad has been shown to transmit BMP signals in response to phosphorylation of its C-terminal domain. We now propose a novel role for Mad in Wg signaling, independent of its C-terminal phosphorylation by BMP receptors. By applying both genetic and biochemical approaches we demonstrate that Mad binds to the Wg transcriptional complex and is required for signaling in the wing imaginal disc and cell culture assays. Wg signaling is inhibited by phosphorylation of Mad by the BMP receptor resulting from activation of the BMP pathway. The results presented here show that Mad has distinct signal transduction roles in the BMP and Wg signaling pathways, with the outcome depending on its phosphorylation state.

188B

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

Signaling pathways are important to several life processes including development, differentiation, growth, homeostasis, and apoptosis. One major family of signaling pathways is the Mitogen-activated protein kinase (MAPK) cascade. The MAPK family includes ERKs, JNKs/SAPKs, and p38/HOG; these kinases activate transcription factors in response to cell growth and/or stress signals. The positive regulation of the MAPK pathways is well characterized; however, less is known about their negative regulation. Our lab studies the novel gene *raw*, an antagonist of JNK signaling, using embryonic dorsal closure as a model. *raw*'s embryonic loss of function phenotype includes dorsal closure defects, hypotrophy of ventral denticle belts, and ectopic expression of the JNK target gene, *dpp*, in cells beyond the leading edge (LE) epidermis. Using genetics, we have previously shown that *raw* is widely expressed during embryogenesis and is required to suppress Basket (JNK)-independent AP-1 activity in the lateral epidermis of embryos undergoing dorsal closure. Therefore, Raw functions to silence basal levels of the epidermal AP-1 transcription factor. Furthermore, we show biochemical data that activated phospho-Jun accumulates to high levels in *raw* and *raw basket* mutant embryos, about 2.5 fold higher than wild type. Since Jun is active in *raw* mutants even in the absence of *basket*, this indicates that another kinase is responsible for activating the AP-1 transcription factor. As Raw does not act through the JNK signaling negative feedback loop involving the MKP, Puckered, our findings indicate Raw functions in a previously unrecognized JNK/AP-1 regulatory system. To better understand this regulatory system, here we test: (1) whether the ectopic *dpp* expression in *raw* mutants is due to Jun mislocalization, and (2) that a MAPK other than the *basket*-encoded JNK activates Jun in the epidermis of *raw* mutants.

189C

Eggshell patterning by Wishful thinking: signaling with positive feedback. 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*), in regulating the 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 non-uniformly expressed in the follicle cells (FCs), which are a mono-layer of epithelial cells engulfing the developing oocyte. This pattern is spatially conserved in the FCs of multiple *Drosophila* species, and it correlates with the domains of BMP signaling activity. We found WIT to be required for BMP signaling. In addition, targets of signaling were lost in cells null for *wit*. Furthermore, we established wit as a transcriptional target of BMP signaling, and is thus maintained in a positive feedback regulatory loop. Of importance, we demonstrate that WIT is essential for proper eggshell morphology. Previously, studies have limited WIT's role to neurogenesis; however, we demonstrate a role for WIT in non-neuronal tissue to control patterning and morphogenesis of the *Drosophila* eggshell.

190A

Connections between dorsal closure and head involution. Matthew J. Moulton, Anthea Letsou. Department of Human Genetics, University of Utah, Salt Lake City, UT.

The Drosophila embryo undergoes several important physiologic processes before transitioning to a larva. Among these processes are dorsal closure and head involution. Both occur at approximately the same time during embryogenesis (8-12 hrs. AEL) and involve epithelial cell migration. Dorsal closure occurs when leading edge cells signal to adjacent epithelial cells inducing them to change shape and cover the amnioserosa. Similarly, head involution requires cellular signals to evoke changes in the epithelium to cover the head. Many mutants of the dorsal-open class not only fail to complete dorsal closure but also fail to complete head involution. In many cases, it is known that head involution defects are secondary to defects in dorsal closure. Therefore, even though both dorsal closure and head involution are morphologically similar, it is not clear whether they utilize the same or different molecular signals. Our lab studies a series of embryonic lethal *mummy* (*mmy*) mutants that exhibit variably expressed dorsal closure and head involution defects. *mmy* encodes a UDP-*N*-acetylglucosamine diphosphorylase which is responsible for producing sugars used to modify proteins, thus modulating their function. Some *mmy* mutants are able to complete dorsal closure but fail to head involute, revealing independent roles for *mmy* in controlling the molecular components involved in these two processes. Here, we present our results from an analysis of embryonic cuticle phenotypes of several *mmy* alleles. We also present data using *engrailed* as a marker to track dorsal ridge formation and migration throughout development in *mmy* mutants. Our data indicate that *mmy* has independent roles in dorsal closure and head involution. Identification of the Mmy-modified products in dorsal closure and head involution will enhance our molecular understanding of these critical morphogenetic processes.

191B

Identification of genes that interact with *Drosophila auxilin*. Susan M.L. Banks, William R. Stoutt, Janice A. Fischer. ICMB, MCDB, University of Texas at Austin, Austin, TX.

Notch signaling is important for cell-cell signaling during development and is highly conserved across all multi-cellular organisms. Failure in Notch signaling is causative in many human diseases. Studies in our laboratory are elucidating the signaling pathway and describing more general internalization components functioning during signaling. In the eye, activation of the Notch pathway requires lqf(*Drosophila* Epsin)-dependent and clathrin-dependent internalization of the Notch receptor ligands, Delta or Serrate, by the signaling cells. However, it is unclear exactly how and why ligand must be internalized to activate Notch signaling. Recently, our laboratory found that in addition to clathrin and Epsin, Auxilin is essential for signaling and internalization of the Notch ligand Delta. We showed that Auxilin is required for uncoating clathrin-coated vesicles to maintain a pool of free clathrin and Epsin in the cell. Using *auxilin* mutants as an entryway, I am attempting to identify previously unknown components of the Notch signaling pathway. An F1 EMS screen was performed and seventeen complementation groups were identified as enhancers of the *auxilin* mutant phenotype. Among the seventeen genes identified are *Delta, lqf, hsc70, and faf*. Delta and epsin have known roles in the Notch pathway, specifically in terms of sending a signal. Hsc70 is an ATP-ase that binds Auxilin to function in uncoating clathrin-coated vesicles. Faf maintains levels of active Epsin in the cell. These results suggest that I have isolated mutants in genes closely tied to Notch signaling. Two mutants, previously undescribed in Notch signaling in the developing *Drosophila* eye, have been identified and soon the identities of two more complementation groups will be known. When Notch signaling fails during eye development, it results in a rough eye phenotype due to aberrant photoreceptor number and patterning. Preliminary results suggest the newly identified mutants are playing a role in Notch signaling during eye development, as the photoreceptor mutant phenotype is enhanced in the screen mutants.

192C

Friend of Echinoid (Fred) and Echinoid (Ed) regulate EGFR trafficking. Qian Nie, Susan Spencer. Department of Biology, Saint Louis University, St 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. Our findings suggest that Ed's PDZ-binding domain promotes endocytosis of both Ed and EGFR from the cell surface. A possible model of how Fred and Ed regulate EGFR internalization will be discussed.

193A

A transmembrane RING ubiquitin E3 ligase, Godzilla, regulates endosomal trafficking. Yasuo Yamazaki, Gaurav Varshney, Christina Schönherr, Murat Dogru, Bengt Hallberg, Ruth Palmer. Department of Molecular Biology, Umeå University, Umeå, Sweden.

Endocytosis is a common cellular process, regulating cell signaling mediated by various types of membrane receptors. Recent studies have emerged that endocytosis is crucial for several developmental processes such as the regulation of proper cell division and the formation and interpretation of morphogen gradient. Here we characterize two transmembrane-type RING ubiquitin E3 ligases, Goliath and Godzilla, on endosomal trafficking. Both Goliath and Godzilla is specifically localized on early endosomal membranes and when manipulated causes enlargement of endosome vesicles, a phenotype typically observed upon disruption of endosomal trafficking. Mutation of the RING domain, abrogates ligase activity and results in a loss of the enlarged endosome phenotype; suggesting that endosome enlargement dependent upon ubiquitin ligase activity. Moreover, endogenous Godzilla protein is localized on endosomes and mutants display an enlarged endosome phenotype *in vivo*. This enlarged endosome phenotype can be rescued by a *godzilla* genomic transgene. Our data strongly suggests that this family of E3 ubiquitin ligases are critical regulators of endosomal trafficking in *Drosophila*.

194B

Expression and Function of Glutactin in Drosophila Larvae and Adults. Pedro Alvarez-Ortiz, Bryan Ballif, Shawna Guillemette, Rachel Humphrey, Jim Vigoreaux. Biology, University of Vermont, Burlington, VT.

The basal lamina is a specialized extracellular matrix (ECM) that plays an essential role in tissue organization and integrity. One component of the Drosophila basal lamina is glutactin, a highly acidic and sulfated glycoprotein with calcium binding activity and sequence similarity to serine esterases, but lacking a critical catalytic serine residue. During embryogenesis, glutactin has been shown to be expressed in the basement membranes enclosing the gut, brain, nerve cord, and sensory bodies. Here we show that glutactin is abundantly expressed in Drosophila larva and adults. The protein is highly resistant to non-ionic detergent extraction and remains associated with larval body wall. Immunostaining of larval sections show glutactin localizes to regions enveloping the body wall musculature and, to a lesser extent, visceral muscle. The protein is expressed in all regions of the adult fly, including the head, thorax, legs, and abdomen. Immunostaining is found predominantly along the alimentary canal and digestive tract, including the proventriculus (a specialization of the anterior alimentary canal), midgut, and the rectal ampulla. The presence of the protein in the pericellular matrices surrounding muscles of the thorax and legs, but not of the large indirect flight muscles, suggest specialized differences in matrices associated with adult muscles. Differences are also detected in adult female and male gonads, with staining being more prominent along the reproductive tract in females. The functional consequences of up-regulating and down-regulating the expression of glutactin will be presented.

195C

Gon1 is a matrix metalloproteinase required for migrating cells to detach from the ECM. Afshan Ismat, Alan Cheshire, Deborah Andrew. Dept Cell Biol, Johns Hopkins Sch Med, Baltimore, MD.

Proper migration of cells through the three-dimensional extracellular matrix (ECM) requires clearing a path at the cell front or leading edge, and detachment from the ECM at the rear or trailing edge. Among the extracellular enzymes that could modify the ECM to allow for proper cell migration is the ADAMTS family of matrix metalloproteinases. The Drosophila genome encodes three ADAMTS genes, including *gon1 (CG14869)*, which is expressed in many migratory tissues. Loss of *gon1* causes migration defects in tissues that express *gon1*, the caudal visceral mesoderm and tracheal visceral branches, and in tissues that do not, the germ cells. Tissue-specific rescue experiments show that *gon1* functions both cell autonomously and non-cell autonomously to rescue migration defects. The salivary gland (SG) migrates as an intact organ, and comprises adherent polarized epithelial cells surrounding an inner, matrix-filled lumen. The basal surfaces of SG cells face outward, acting as the leading edge of this migrating collective, and the apical surfaces face inward toward the lumen, potentially acting as the trailing edge. In the absence of *gon1*, the SG exhibits severe apical membrane retractions resulting from an apparent failure of the apical membrane to detach from the apical ECM of the lumen. The distal-most SG cells are more elongated than in wild type, and the lateral membranes appear stretched and torn. Thus, without *gon1*, migratory forces are pulling the SG forward, but the trailing edge of individual SG cells cannot detach from the apical ECM. This apparent failure in trailing edge detachment in the SG brings up the issue of whether *gon1* functions the same in other cell types. To address this, I am exploring where Gon1 localizes in individually migrating cells, the germ cells. I hope to learn if *gon1* functions at the leading edge to clear a path for forward movement or at the trailing edge to allow cells to detach from the ECM. Through this work, I expect to learn how the ADAMTS family of extracellular proteinases contributes to cell migration and tissue morphogenesis during development.

196A

Genetic interaction between POSH and Zasp52. Ashley Lennox, Rebecca Garlena, Beth Stronach. Univ of Pittsburgh Sch Med, Pittsburgh, PA.

The POSH protein consists of a RING domain with ubiquitin E3-ligase activity and four SH3 domains. Evidence supports a proapoptotic role for POSH as a scaffold protein linking Rac GTPase and the Jun Kinase pathway. Yet POSH has been tied to other processes including innate immunity, longevity, apoptotic resistance, synaptic growth, and virus trafficking. Though Drosophila POSH mutants are viable, our previous studies have shown that overexpression of POSH in the embryo leads to loss of amnioserosa integrity and caspase-dependent cell death. In these studies, we noted that the phenotypic consequences of POSH overexpression were enhanced by inclusion of a GFP protein trap line, zcl423, which labels dorsal ectodermal cells. We find that the genetic interaction between POSH overexpression and zcl423 is recapitulated in several tissues, including the larval eye and wing discs. While zcl423 expression is predominant in muscle tissue, the phenotypes we observe are manifest in epithelial tissues, prompting us to explore the expression of zcl423 more closely. Zcl423 is detectable at relatively reduced levels in many non-muscle tissues of the larva, including the imaginal discs, salivary glands, hemocytes, central nervous system, and ring gland, though it is absent from fat body, for instance. Within epithelial cells, zcl423 localizes in discrete foci at the basal membrane, alternating with patches of βPS integrin staining, and in relatively punctate fashion at cell junctions in association with E-cadherin. To determine what protein is labeled with GFP in the zcl423 line, we performed plasmid rescue and sequenced the flanking DNA. Zcl423 is a GFP protein trap in Zasp52, the Z-band alternatively-spliced PDZ protein at position 52 on chromosome 2R. It is likely that zcl423 is a viable hypomorph, because we can recapitulate the genetic interaction with POSH expression using a Zasp52 RNAi line. To get at the mechanism underlying the genetic interaction, we are pursuing studies to determine whether POSH is altering Zasp52 levels or localization to bring about epithelial tissue dysmorphology.

197B

Gliotactin functionally interacts with Discs-Large through phosphotyrosine signaling and a PDZ binding motif. Mojgan Padash-Barmchi, Kendra Sturgeon, Vanessa J. Auld. Dept. of Zoology, Health Science Institute, University of British Columbia, Vancouver, Canada V6T1Z3.

Establishment and maintenance of permeability barriers (PB) is one of the most important functions of the polarized epithelial cells. PB in Drosophila epithelia is established by septate junctions (SJs) between two adjacent cells and by tricellular junctions (TCJs) at the contact site of three epithelial cells. The transmembrane protein Gliotactin (Gli) is uniquely localized to the TCJ and is necessary for PB maintenance. Tight control of the level and localization of Gli by tyrosine (Y) phosphorylation is important for localization of Gli to the TCJ and survival of epithelial cells. We are interested in understanding of the importance of this tight regulation in the epithelial cells. We used site directed mutagenesis as well as the UAS-Gal4 expression system to investigate the importance of precise localization of Gli to the TCJ. We have previously shown that blocking endocytosis causes mislocalization of Gli to the SJ and results in cell delamination and death. Here, we show that this mislocalization results in downregulation of the tumor suppressor protein, Discs Large (Dlg). Blocking the downregulation of Dlg causes severe tissue overgrowth and apoptotic cell death. We show that Gli functionally interacts with Dlg and this interaction requires Y phosphorylation and the PDZ binding motif of Gli. The tissue overgrowth as well as apoptosis caused by coexpression of Gli and Dlg requires phosphorylation of Dlg at serine 797. Blocking this phosphorylation completely prevents those phenotypes. We further find that Drosophila JNK acts downstream of Gli and Dlg to mediate the overgrowth phenotype caused by coexpression of Gli and Dlg. Our results suggest that correct localization of Gli to the TCJ is important and there is a cellular mechanism that compensates for the presence of ectopic Gli at the SJ by reducing the level of Dlg to diminish the severe defects resulted from interaction of Gli with Dlg at the SJ.

198C

Spatial and temporal regulation of cell adhesion in Drosophila is mediated by the bHLH transcription factor Delilah. Adi Salzberg, Atalya Nachman, Nirit Egoz, Naomi Halachmi, Moran Toder. Rappaport Fac Medicine, Technion-Israel Ins Technology, Haifa, Israel.

How transcription factors and signaling networks specify cell fates is a central question in developmental biology. Although we have a conceptual picture of how differential gene expression is used to generate different types of cells, we still lack a full understanding on how any cell is specified and how it acquires its unique properties. In a recent work we have identified the bHLH transcription factor Delilah (Dei) as an important regulator of cell adhesion in Drosophila. We have demonstrated that in organs in which sub-groups of cells differentiate into more adhesive ('sticky') or less adhesive cell types, Dei is expressed in the stickier cell types. In these cells Dei is required for inducing proper level of expression of βPS integrin. Based on these observations we think of Dei as a molecular switch that turns on βPS integrin expression wherever a sticky cell has to develop. Since such a switch needs to be turned on in different tissues and different developmental and physiological contexts, it is predicted that the *dei* gene would be able to respond to various signaling pathways and transcription factors. Indeed, when we analyzed the regulatory region of *dei*, using *lacZ* reporter constructs, we found that the regulatory region of the *dei* locus harbors multiple discrete regulatory modules that drive expression in different subsets of the *dei*-expressing cells and respond to different transcription factors. This observation supports the idea that spatial and temporal regulation of cell adhesion in Drosophila is mediated, at least in part, by the regulated expression of Dei.

199A

Regulation of integrin turnover by force in vivo. Guy Tanentzapf[1], Mary Pines[1], Stefan Czerniecki[1], stephanie Ellis[1], Raibatak Das[2], Daniel Coombs[2]. 1) CPS Dept, Univ British Columbia, Vancouver, BC; 2) Mathematics Dept. Univ British Columbia, Vancouver, BC.

We are interested in understanding how complex tissue architecture forms during development and once formed how it is maintained throughout the life of the organism. In particular our lab studies the role of cell adhesion and the cytoskeleton in the context of development with an emphasis on integrins, the major receptors for the ECM in the fly. A fundamental question in developmental biology is how tissue structure is stabilized at the completion of embryogenesis. During embryonic development tissues undergo substantial remodeling and adhesion must be dynamic but post-embryonically stable adhesion maintains tissue architecture over the long term and must be stable. Our work is designed to address the question of how the transition from dynamic to stable adhesion is mediated. To this end we have developed methodologies that allow us to study the dynamics of the adhesion complex in vivo in live embryos and larva using Fluorescence Recovery After Photobleaching (FRAP). In addition we are utilizing mutations that make it possible to conditionally decrease or increase the force imposed on integrin-mediated adhesions. Using these approaches we have uncovered an essential role for mechanical force in regulating integrin turnover and importantly in stabilizing cell adhesion at the completion of embryogenesis. Finally, using a set of point mutations in integrins and their associated proteins we have been able to specifically address the mechanisms that underlie the stabilization of adhesion at the completion of embryogenesis.

200B

Expression and functions of Drosophila Mmp1 and Mmp2 in Drosophila oogenesis. Xiaoxi Wang, Kimberly LaFever, Andrea Page-McCaw. Department of Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, TN.

Matrix metalloproteinases (MMPs) are matrix-degrading proteinases. They play crucial roles in many physiological processes by remodeling extracellular matrix (ECM) and by regulating signaling pathways. Drosophila melanogaster provides an excellent model for studying the physiological functions of MMPs because of its superb genetics and because there are only two evolutionary conserved, non-redundant MMPs in the genome. Here we address the functions of Drosophila Mmp1 and Mmp2 in Drosophila oogenesis. Mmp2 temperature sensitive mutants are female-sterile and display egg chamber degeneration, indicating an indispensable role of Mmp2 in oogenesis. By using a partially-rescuing genomic construct expressing C-terminal EGFP fusion, we localized Mmp2 to the terminal filament cells and cap cells, which are the niche cells for both germline stem cells and somatic stem cells. Interestingly, Mmp1 is also expressed in those cells, as well as on the basement membrane underlying follicle cells in the germarium and early stage egg chambers. Mmp1 expression diminishes starting from stage 6 egg chambers and is restricted to stalk cells thereafter, which is concurrent with the switch of follicle cells from mitosis to endoreplication. We hypothesized that Drosophila Mmp1 and Mmp2 play important roles in oogenesis by regulating stem cells niche signaling or/and by regulating the remodeling of extracellular matrix of proliferating pre-follicle cells.

201C

The extracellular loops of Smoothened play a regulatory role in Hedgehog signaling. Candace E. Carroll, Marada Suresh, Daniel Stewart, J. Xiaoxi Oyang, Stacey K. Ogden. Biochemistry, St Jude Children's Research Hospital, Memphis, TN.

The Hedgehog (Hh) signaling pathway plays a critical role during metazoan development and is frequently inappropriately activated in cancer. Smoothened (Smo), a transmembrane protein that is a member of the G-protein coupled receptor (GPCR) superfamily, is absolutely required for the transduction of ligand-induced pathway activation. The extracellular loops (EC) of canonical GPCRs harbor cysteine residues that form disulfide bonds that affect both the active and inactive state by regulating receptor confirmation, dimerization and/or ligand binding. In the present study, we mutated the conserved cysteine residues of Smo and tested for effects on Hh signal transduction. While mutating EC3 cysteines did not reveal altered signaling compared to wild type Smo, investigations of EC1 and EC2 mutants reveal ligand-independent activation of the Hh pathway in both mammalian and Drosophila in vitro systems. Indirect immunofluorescence reveals altered sub-cellular localization of both the EC1 and EC2 mutants, suggesting that these mutants are signaling in a manner inconsistent with canonical Hh signaling. Further biochemical studies reveal a possible disulfide bond between an EC1 and the EC2 cysteine, which is seen in canonical GPCRs. Our studies reveal previously uncharacterized functional roles for Smo EC1 and EC2.

202A

A sensitized suppressor/enhancer screen for Hedgehog pathway components identifies the kinase *Darkener of apricot* as a direct regulator of the transcription factor Ci. Ryan R Hurtado[1], Cheng Du[2], Leonard Rabinow[3], Robert Holmgren[1]. 1) Northwestern University, Evanston, IL; 2) Univ. Nebraska Medical Center, Omaha, Nebraska; 3) Université de Paris XI, Paris, France.

As a key mediator of embryonic development, the Hedgehog (Hh) morphogen acts in a signaling gradient to specify the fates of nearby cells. In adults, aberrant Hedgehog signaling is involved in a variety of carcinomas. While much is known about Hh signaling, many gaps of understanding in the pathway still remain.

Using the *Drosophila* wing as a model for Hh signaling we performed an RNAi based suppressor/enhancer screen in a fu^1 sensitized genetic background looking for novel proteins involved in the Hh pathway. UAS-RNAi lines for all available kinases, phosphatases, acetylases and deacetylases as well as 1590 randomly selected genes were screened. Using the wing specific MS1096-Gal4 driver individual genes were knocked down and the distance between the 3rd and 4th wing vein, which is directly regulated by Hh, was assessed. Our screen was able to successfully identify previously known pathway members as well as 21 novel suppressors and 59 novel enhancers of fu^1. Many hits have shown interesting effects on Hh target gene expression and follow-up experiments are being performed.

Knockdown of the kinase Darkener of Apricot (doa) enhanced fu^1 in adult wings and reduced expression of the Hh target gene *dpp* in wing discs. Genetic mutations in *doa* rescue the wing expansion phenotype as well as reduce ectopic target gene expression in wing discs of a Hh overexpression mutant Hh^{mrt}. A highly conserved *doa* consensus site at S199 of the Hh transcription factor Ci, known to be phosphorylated *in vivo* exists. In a S2R+ cell luciferase assay, glutamate substitution at S199 results in higher Ci activity. Current experiments are investigating whether this increased activity can be recapitulated by overexpression of *doa* both by luciferase experiments *in vitro* as well as with transgenics *in vivo*.

203B

Altered localization of activating Smoothened mutants. Suresh Marada, Candace Carroll, Daniel Stewart, Jessica Ouyang, Stacey Ogden. Biochemistry, St. Jude Children's Research Hospital, Memphis, TN.

Hedgehog (Hh) signal transduction plays an essential role in patterning and organizing tissues during metazoan development. Smoothened (Smo), the obligate signal transducing molecule of the Hh pathway, is predicted to function as a G-protein coupled receptor. In the absence of Hh, Smo localizes to intracellular vesicles, and upon Hh stimulation, translocates to the plasma membrane and undergoes a conformational shift of its intracellular domains to facilitate signaling to downstream effectors. Numerous studies have examined the contribution of the Smo intracellular tail to signaling. However, few studies have examined the contribution of Smo extracellular domains to its regulation and signaling. In this study we generated a set of novel Smo mutants by replacing highly conserved extracellular loop cysteines with alanines. These Smo mutants, when expressed in Drosophila wings and embryos, induce Hh gain-of-function phenotypes. Flip-out clonal analysis revealed that these mutants trigger ectopic stabilization of the Hh pathway transcriptional effector cubitus interruptus (Ci), and ectopic expression of Hh target genes *patched (ptc)* and *decapentaplegic (dpp)*, suggesting that these mutants are constitutively active. Based on these phenotypes we predicted that these activating Smo mutants would localize to the plasma membrane in a Hh-independent manner. On the contrary, subcellular localization studies in Clone 8 and S2 cells revealed that these activating Smo mutants are not localized to plasma membrane, either in the absence or presence of Hh, but are instead retained in the endoplasmic reticulum (ER). These results argue that the activating Smo mutants can induce high-level signaling from the ER.

204C

The Hedgehog-induced Smoothened conformational switch assembles a signaling complex that activates Fused by promoting its dimerization and phosphorylation. Qing Shi[1], Shuang Li[1], Jianhang Jia[2], Jin Jiang[1]. 1) Department of Developmental Biology, UT Southwestern Medical Center at Dallas, Dallas, TX 75390, USA; 2) Markey Cancer Center and Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY 40536, USA.

Hedgehog (Hh) transduces signal by regulating the subcellular localization and conformational state of the GPCR-like protein Smoothened (Smo) but how Smo relays the signal to cytoplasmic signaling components remains poorly understood. Here, by colocalization study and fluorescence resonance energy transfer (FRET) analysis, we find that Hh-induced Smo conformational change recruits Costal2 (Cos2)/Fused (Fu) and promotes Fu kinase domain dimerization. By performing mutagenesis analysis and using phospho-specific antibodies, we show that induced dimerization through the Fu kinase domain activates Fu by inducing multi-site phosphorylation of its activation loop (AL) and that phospho-mimetic mutations of AL activate the Hh pathway. Interestingly, we observe that graded Hh signals progressively increase Fu kinase domain dimerization and AL phosphorylation, suggesting that Hh activates Fu in a dose-dependent manner. Moreover, we find that activated Fu regulates Cubitus interruptus (Ci) by both promoting its transcriptional activator activity and inhibiting its proteolysis into a repressor form. We provide evidence that activated Fu exerts these regulations by interfering with the formation of Ci-Sufu and Ci-Cos2-kinase complexes that normally inhibit Ci activity and promote its processing. Taken together, our results suggest that Hh-induced Smo conformational change facilitates the assembly of active Smo-Cos2-Fu signaling complexes that promote Fu kinase domain dimerization,

phosphorylation and activation, and that Fu regulates both the activator and repressor forms of Ci.

205A

A dsRNA Based Screen Identifies Novel Proteins Involved in Drosophila Hedgehog Signaling Pathway. Maggie Sledd, Ryan Hurtado, Robert Holmgren. Molecular Biosciences, Northwestern University, Evanston, IL.

A screen has been performed identifying potential novel members of the Hedgehog (Hh) signaling pathway. The Hh signaling pathway is responsible for the patterning of many different organs during development. In mammals, aberrant Hh signaling is associated with a number of different cancers, including basal cell carcinoma and prostate cancer, and therefore, a further understanding of Hh could lead to new treatments. While many proteins involved in the Hh signaling pathway have been discovered, there are still many that remain unknown. In *Drosophila melanogaster*, the Hh signaling pathway is responsible for the patterning of the wing, so a UAS-Gal4 RNAi screen was devised using the MS1096-Gal4 driver to specifically knock down gene expression in the wing. The screen was performed in a sensitized *fused (fu^1)* mutant background, looking for both suppressors and enhancers of the fu^1 phenotype. By observing wing vein patterning, it was possible to determine whether loss of a particular gene had a significant effect on Hh signaling. Thus far, the screen has covered 11% of the *Drosophila* genome. Out of 1590 genes knocked down, 29 were strong enhancers and 11 were suppressors of the fu^1 phenotype. Several of these hits were followed up using RNAi in larval wing discs and visualizing gene expression with immunofluoresence. One gene of particular interest, *megator* (*mtor*), was a strong enhancer of the fu^1 phenotype. Knockdown of *mtor* with RNAi resulted in a decrease in expression of the Hh target genes *decapentapleigic* and *collier*. Future research will include testing for genetic interactions with a mutation in the endogenous mtor gene to validate its involvement, and elucidating the function of Mtor in the Hh signaling pathway.

206B

USP8 promotes Smoothened signaling by preventing its ubiquitination and changing its subcellular localization. Ruohan Xia, Hongge Jia, Junkai Fan, Yajuan Liu, Jianhang Jia. Department of Molecular and Cellular Biochemistry, Markey Cancer Center,University of Kentucky, Lexington, KY 40536, USA.

The seven transmembrane protein Smoothened (Smo) is a critical component of the Hedgehog (Hh) signaling pathway and is regulated by phosphorylation, dimerization, and cell-surface accumulation upon Hh stimulation. However, it is not clear how Hh regulates Smo accumulation on the cell surface or how Hh regulates the intracellular trafficking of Smo. In addition, little is known about whether ubiquitination is involved in Smo regulation. In this study, we demonstrate that Smo is multi-monoubiquitinated and that Smo ubiquitination is inhibited by Hh and by hosphorylation. Using an in vivo RNAi screen, we identified ubiquitin-specific protease 8 (USP8) as a deubiquitinase that downregulates Smo ubiquitination. Inactivation of USP8 increases Smo ubiquitination and attenuates Hh-induced Smo accumulation, leading to decreased Hh signaling activity. Moreover, overexpression of USP8 prevents Smo ubiquitination and elevates Smo accumulation, leading to increased Hh signaling activity. Mechanistically, we show that Hh promotes the interaction of USP8 with Smo aa625-753, which covers the three PKA and CK1 phosphorylation clusters. Finally, USP8 promotes the accumulation of Smo at the cell surface and prevents localization to the early endosomes, presumably by deubiquitinating Smo. Our studies identify USP8 as a positive regulator in Hh signaling by downregulating Smo ubiquitination and thereby mediating Smo intracellular trafficking.

207C

Rab GTPase mediated Golgi trafficking as a potential major orchestrator of Autophagy in *Drosophila melanogaster*. Carlos I Ayala-Navarro, Thomas P Neufeld. Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

Autophagy is a catabolic housekeeping mechanism employed by cells to achieve quality control of organelles and proteins, cope with stress and combat starvation. The process occurs by sequestration of cytoplasmic material into autophagosomes to ultimately fuse with lysosomes to degrade cargo, replenish nutrients and maintain cellular health. Vesicular traffic has been shown to be a major player in this process from the beginning to the end stages. Among the latter, a small number of Rab GTPases have been identified as important regulators of autophagy. Nonetheless, our understanding of the role of traffic is still incompletely understood in regards to autophagy and autophagosomal dynamics. It should be highlighted that most of the focus and research has been exhaustively researched in mammal and yeast models. Therefore, we decided to perform a screen of the ~30 Rab GTPases in the genetic workhorse Drosophila melanogaster to elucidate their role in autophagy. The screen consisted of knockdown (dsRNA) and over-expression of constitutively active, dominant negative and wild-type versions of each Rab protein. Our results highlight the value of Drosophila as powerful biological model in the study of autophagy and reveal novel Rab GTPases involved in the regulation of this process. Of special interest is a selected group of Rab proteins, 2, 6, 19 and 39, involved in traffic towards, within and from the Golgi network. The fact that these GTPases were detected as candidates regulating golgi trafficking prompted us to develop an interest for dAtg9, a golgi localized transmembrane protein in mammals, which has been proposed to deliver membrane to nascent autophagosomes. However, traffic regulation of Atg9 is incompletely understood and exhaustively being researched. Whether our Rab candidates regulate traffic of Atg9 under Fed or starvation induced autophagy will be explored.

208A

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

Phosphatidylinositol (PI) phosphates (PIPs) are membrane lipids that play essential roles within the cell. PIPs, such as PI 4-phosphate (PI4P), are strictly regulated by various kinases and phosphatases. PI4P localizes to the Golgi where it recruits important regulators of intracellular trafficking. In addition, PI4P has also been implicated in the biogenesis of lysosome-related organelles (LROs). LROs are a class of organelles that include pigment granules (required for *Drosophila* eye pigmentation), melanosomes, and dense and alpha granules in platelets. A collection of heritable diseases that disrupt LRO function lead to hypopigmentation, prolonged bleeding, neurodegeneration, immunodeficiency and lung fibrosis. These diseases are often a result of mutations in genes encoding trafficking complexes, such as the clathrin adaptor protein complex AP-3 and the homotypic fusion and vacuole protein sorting complex (HOPS); however, the cellular mechanisms underlying LRO biogenesis are not well understood. By manipulating PI4P levels in the cell using *Drosophila* mutants for the type II PI 4-kinase (PI4KII) and the PI4P phosphatase Sac1, our lab has identified PI4P as a critical player in LRO formation. *PI4KII* and *sac1* mutants have defects in red and brown eye pigment levels due to an altered number and distribution of eye pigment granules. Additionally, we have shown that

PI4KII and *sac1* mutants genetically interact with other trafficking complex mutants, such as those affecting AP-3 and HOPS. Indeed, we have found that Sac1 is required for proper localization of the AP-3δ subunit Garnet. This suggests that altering PI4P levels affects multiple LRO trafficking pathways and that correct PI4P levels is required for LRO formation.

209B

Notch signaling from *vps25* mutant cells confers apoptotic protection to neighboring cells via Yorkie activation in *Drosophila*. Hillary K. Graves[1], Sarah E. Woodfield[1,2], Georg Halder[1,2], Andreas Bergmann[1,2,3]. 1) Biochemistry & Molecular Biology, MD Anderson Cancer Center, Houston, TX, USA; 2) Baylor College of Medicine, Graduate Program in Developmental Biology, Houston, TX, USA; 3) Department of Cancer Biology, University of Massachusetts Medical School, Worcester, MA, USA.

The microenvironment in which epithelial carcinomas arise can influence their progression. Often, epithelial cells with oncogenic transformations are eliminated by apoptosis, suggesting that signaling between oncogenic and normal cells is a mechanism by which organisms eliminate aberrant cells from epithelia. In light of this fact, untransformed cells likely have molecular cues that identify that they are normal and need not be eliminated, though little is known about these cues. In *Drosophila* imaginal epithelia, clones of cells mutant for the endocytic neoplastic tumor suppressor gene *vps25* are eliminated by cell death, but induce nearby untransformed cells to express Diap1, an important inhibitor of apoptosis. Here, we show that the non-autonomous apoptotic resistance induced by *vps25* mutant cells is mediated at the transcriptional level by Yorkie, the conserved downstream effector of Hippo signaling. Furthermore, we show that inhibiting ectopic Notch signaling from the vps25 mutant cells prevents the non-autonomous induction of Yorkie signaling. Finally, we show that overactivation of Notch signaling is sufficient to induce non-autonomous apoptotic resistance via Diap1 expression. Our data indicate that Notch signaling from cells mutant for enodcytic neoplastic tumor suppressor genes could be part of a mechanism by which animals protect untransformed cells in tissues while eliminating epithelial malignancies.

210C

The molecular basis of airway maturation in Drosophila. Chie Hosono, Rho Matsuda, Christos Samakovlis. Developmental Biology, The Wenner-Gren Institute, Stockholm, Sweden.

The respiratory tubes of mammalian lungs and the Drosophila tracheal system undergo a series of maturation events at the end of embryogenesis. During this period the nascent tubes acquire their mature size, clear the luminal liquid and transform into functional respiratory networks. Our laboratory has recorded three precisely controlled transitions of cellular activities during airway maturation in living fly embryos: First, a secretion burst deposits extracellular matrix into the lumen and expands tube diameter. Second, the activation of a massive apical endocytosis wave clears the matrix. Finally, luminal liquid is evacuated and the network is filled with a gas within ten minutes. The mechanisms underlying the precise spatial and temporal regulation of epithelial activities during airway maturation are unknown. We used a tracheal specific driver and ~20000 transgenic UAS-RNAi strains to first describe all protein-coding genes involved in the process. We found 1461 genes, involved in airway maturation. Tracheal inactivation of 1935 of the remaining genes caused lethality or adult phenotypes in >50% of the animals. To identify the developmental regulators of airway maturation, we preselected about 600 genes encoding putative regulators like kinases, channels, GPCRs, transcription factors and proteins of unknown functions. We further classified these into 12 groups based on the defects in tube morphologies, apical secretion and protein clearance events caused by RNAi. The combination of this phenotypic analysis with the data from protein interaction databases revealed a new gene regulatory network that controls tube planar cell polarity, and integrity during airway liquid clearance.

211A

Searching for substrates of the MAST kinase homolog Drop out using SILAC based phospho-proteomics. Alistair J Langlands, Daniel Hain, H.-Arno J. Muller. Division of Cell & Developmental Biology, University of Dundee, Scotland, UK.

The maternal effect mutant *drop out* (*dop*) causes severe defects during cellularisation: inward growth of the cleavage membrane is slowed, protein polarity complexes are mislocalised and the nuclei drop out during mid-cellularisation. We have established that *dop* encodes the single microtubule-associated serine/threonine (MAST) kinase homolog in *Drosophila*. Although MAST kinases have been implicated in a number of human diseases such as breast cancer, inflammatory bowel disease and neurodegenerative diseases, their function remains poorly understood. The highly conserved domain structure makes Dop an excellent model to better understand MAST kinase function. We generated a range of EMS-induced *dop* alleles, the majority of which affect the kinase domain, indicating that the kinase domain is essential for Dop function. Therefore, identifying the substrates of Dop will be an important step in understanding its function. We utilise a SILAC (stable isotope labelling of amino acids in cell culture) and a phospho-proteomic approach to identify the substrates of Dop. We are differentially labelling proteins in embryos with heavy isotopes followed by mass spectrometry to detect differences in phospho-peptide patterns between *dop* mutants and wild type. One potential substrate of Dop is Dynein intermediate chain (Dic). We show that the phosphorylation of Dic is dependent on Dop. Furthermore, we identified synergistic genetic interactions between *dop* mutations and mutations in genes encoding components of the Dynein/Dynactin complex. Double mutants of *dop* with either Dic or P150/Glued also exhibit enhanced phenotypes in cellularlisation. These genetic interactions support our biochemical data and indicate that mutations in *dop* affect Dynein-dependent transport processes.

212B

Moesin is required for trafficking of Crumbs in the follicular epithelium. Kristin Sherrard, Richard Fehon. Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Moesin, a FERM domain protein that directly binds actin, has been implicated in the organization of apical actin structures and in crosslinking the plasma membrane to the cortex. Moesin also negatively regulates Rho1 activity in developing imaginal discs and other epithelial cells. The mechanisms of Moesin's activity and identity of its downstream partners have remained elusive, in part due to a large maternal contribution making Moesin hard to deplete in embryonic tissue. In addition, in imaginal epithelia Moesin-depleted cells undergo JNK-mediated apoptosis (Neisch et al. 2010), making it difficult to assess its other cellular functions. Our recent work in the follicular epithelium has uncovered a distinct and apparently Rho-independent trafficking defect in Moesin-depleted cells. We have observed a strong accumulation of Rab5, Rab4, Rab11, and Hrs, as well as excess vesicular Crumbs in Moe deficient cells. Our current working model is that Moesin promotes the recycling and/or degradation of Crumbs, a transmembrane protein which is essential for maintaining

epithelial polarity. We are currently working to assess the specificity and mechanism of Moesin's interaction with Crumbs and its role in trafficking, as well as to probe the role of Crumbs trafficking at particular stages of oogenesis. .

213C

The chocolate and maroon genes are involved in intracellular transport. Rishi Singhal[1], Aminah Wali[1,2], Paaqua Grant[1], Diana Johnson[1]. 1) Biological Sciences, George Washington University, Washington, DC; 2) University of Maryland, Baltimore County.

There are over one hundred genes that affect eye color in Drosophila. A number of them produce products that function in intracellular transport. We used deletion analysis to find candidate genes for both chocolate (cho) and maroon (ma). We used sequencing and transgenic rescue experiments to identify and confirm their gene identities. The cho gene is a vacuolar ATPase, CG2934. The gene has previously been identified as playing a role in Notch signaling and vacuolar acidification. It is expressed at moderate to high levels throughout development. It is also very highly expressed in many adult tissues including the gut, Malpighian tubules and is highly expressed in the brain, head and eye. The ma gene is a vacuolar protein sorting gene, CG8454, Vps16. It was previously shown to be required for trafficking to lysosomes and for the development of pigment granules. It is also expressed throughout development, though at lower levels than the cho gene. Gene expression of cho is at its highest at the late embryo and early larval stages. Expression of ma peaks later in the late larval to mid pupal stages. Like cho, ma's adult expression is at relatively high levels in the brain, head, eye, gut, and Malpighian tubule. Knockouts of both genes have been reported to be lethal. The cho mutant allele contains one base change resulting in a missense mutation while the ma allele shows a deletion of nine bases causing a deletion of three neighboring amino acids. Interactions between these alleles and other trafficking genes are being investigated to determine whether the mutant alleles' effects are limited to development of pigment granules or also affect other functions of the proteins.

214A

PP2A binds to β_H-spectrin and modulates endosomal maturation and *zonula adherens* stability. Graham Thomas[1], Seung-Kyu Lee[1], Elizabeth Klipfell[2], Joanna Sandilos[1]. 1) Dept Biology/BMB, Penn State, University Park, PA; 2) Cleveland Clinic, Cleveland, OH.

β_H-spectrin (β_H) in conjunction with α-spectrin forms the apical membrane skeleton, an F-actin-based cytoskeletal meshwork associated with the apical plasma membrane. β_H (*karst*) mutants result in perturbation of the *zonula adherens* (ZA) without a conspicuous loss of polarity. We have shown that β_H is recruited to the apical membrane *via* the FERM-binding domain of the apical polarity determinant Crumbs, a region that has a specific role in stabilizing the ZA. Multiple lines of evidence suggest that β_H modulates endosomal trafficking, and our current model suggests that β_H remains associated with internalizing vesicles/endosomes until it is released by Annexin B9 at the multivesicular body (MVB) stage. Annexin B9 is required for efficient trafficking through the MVB and to maintain a high-fidelity apical-lateral boundary. *D*E-Cadherin is amongst the cargoes in this pathway. The C-terminal segment 33 of β_H (βH33) appears to be a focal point for these trafficking activities: Overexpression of this domain disrupts endocytosis leading to membrane expansion and is the binding site for Annexin B9. Here we report that βH33 binds to the dual specificity protein phosphatase PP2A, which has demonstrated roles in the regulation of apicobasal polarity. Through knockdown and overexpression experiments and we provide evidence that PP2A sustains the ZA. In addition, levels of the MVB markers Hrs and Vps16 are altered in a tissue specific fashion when PP2A is overexpressed or knocked down. This suggests that the effects of PP2A on the ZA may arise through modulation of the MVB and the recycling of ZA components in the β_H/Annexin B9 pathway. We also demonstrate that PP2A modulation dramatically affects the distribution of cellular F-actin. We hypothesize that Crumbs recruits β_H to act as a scaffold for PP2A to maintain ZA itegrity *via* appropriate recycling of *D*E-Cadherin.

215B

V0-ATPase subunit a1 regulates vesicle sorting through binding to t-SNARE acceptor complexes. Dong Wang[1,4], W.Ryan Williamson[1,4], Sankaranarayanan Srinivasan[2], Daniel Epstein[1], Florante A Quiocho[2], P. Robin Hiesinger[1,3]. 1) Dept Physiology, UT Southwestern Med Ctr, Dallas, Texas 75390; 2) Verna and Marrs McLean Department of Biochemistry and Molecular Biology Baylor College of Medicine, Houston, Texas 77030; 3) Green Center Division for Systems Biology, University of Texas Southwestern Med Ctr, Dallas, Texas 75390; 4) Equal contribution.

The vesicular ATPase is a multi-subunit proton pump whose insertion into specific target membranes is determined by different V0a subunits. Here we show that a SNARE-binding deficient variant of the neuronal V0a subunit V100 fails to restore neurotransmission and reveals intracellular vesicle sorting defects. In neurons, V100 binds to the target SNAREs Syntaxin and SNAP25 under exclusion of the vesicle SNARE Synaptobrevin. However, the V100 N-terminus does not bind to the exposed SNARE domain of ‘open’ Syntaxin, but binds to the helical bundles from by ‘closed’ Syntaxin or a Syntaxin/SNAP-25 acceptor complex. Binding to this t-SNARE acceptor complex is reduced when Synaptobrevin is present. V100 has a low t-SNARE binding affinity and restricts n-Syb function in vivo, suggesting a function prior to v-/t-SNARE complex formation. We propose that the SNARE interaction of V100 exerts an intracellular sorting function and show that the V100 N-terminus fused to the C-terminus of the v-SNARE Synaptobrevin is sufficient to execute this function in vivo.

216C

The interaction between two JAK signaling ligands: Upd and Upd3. Qian Chen, Douglas Harrison. Dept Biol, Univ Kentucky, Lexington, KY.

JAK/STAT signaling pathway is highly conserved between mammals and *Drosophila*. But unlike the mammalian JAK signaling, which can be stimulated by a variety of cytokines and growth factors, the *Drosophila* JAK signaling has only three ligands identified: Upd, Upd2 and Upd3. Upd is the first ligand identified in this pathway and it is so far considered to be the most essential one. While Upd2 and Upd3 are also shown to participate in the fine-tuning of the signaling, how they cooperatively regulate the pathway with Upd is not fully understood. The expression patterns of *upd2* and *upd3* overlap with that of upd during several developmental processes. *upd2* and *upd* express in identical stripes in embryos, while *upd3* and *upd* 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 by forming different ligand complexes. Here we show homotypic and heterotypic interaction between Upd and Upd3 using Biomolecular Fluorescence Complementation (BiFC), and the results were confirmed by yeast two-hybrid and co-immunoprecipitation. To determine the sequence that is essential for the physical interaction, we aligned the three ligands and identified six short consensus domains that are distributed across the proteins. Each of the consensus sequences on Upd3 was substituted by five Alanine residues individually. Each Upd3 substitution variant is being tested for interaction with intact Upd and Upd3 by BiFC. Finally, the signaling intensity stimulated by different ligand complexes and the upd3 substitution variants will be compared by a

luciferase assay.

217A

Refinement of the JAK/STAT Genetic Circuit in Border Cell Recruitment and Detachment. Amanda J. Monahan, Michelle Starz-Gaiano. Department of Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD, 21250.

The genetic regulation that triggers a cell to transition from non-migratory to migratory is incompletely understood despite its role in many processes during normal development and pathological events. The process of border cell specification is an exemplary model to investigate how epithelial cells are converted into a migratory state. In the egg chamber, high levels of the activated transcriptional regulator Signal Transducer and Activator of Transcription (STAT) converts a subset of epithelial follicle cells into a collective migratory cluster, called the border cells, while adjacent cells are left behind. Cells excluded from the border cell cluster initially activate STAT signaling but then exhibit a significant decrease in active STAT relative to their neighbors, and this downregulation is required for proper border cell migration. A proposed STAT-activated genetic circuit employs pro-migratory and inhibitory cues to limit the number of motile cells. *apontic* and its downstream target, *miR-279*, are known components that feedback negatively on STAT signaling. We have identified an additional component to this negative feedback circuit in follicle cells- *suppressors of cytokine signaling (socs)36e*. Members of the highly conserved SOCS family have been shown to be downstream targets and attenuators of STAT signaling. In the egg chamber, a hypomorphic allele of *socs36e* results in extra follicle cells becoming invasive - a phenotype similar to loss of function *miR-279* or hyperactive STAT. Furthermore, we have observed a genetic interaction between *apontic* and *socs36e*, indicating *socs36e* may be a downstream target of both STAT and APT. Thus, our results demonstrate that the combined functions of *miR-279* and *socs36e* may be sufficient to account for APT's negative regulation on STAT activity.

218B

The Distribution of the JAK/STAT Ligand Unpaired (Upd) During Oogenesis. Dustin W. Perry, Travis R. Sexton, Douglas A. Harrison. Department of Biology, University of Kentucky, Lexington, KY.

Morphogens are molecules that can directly specify different cell fates in a concentration dependent manner. During Drosophila development, much focus has been given to the Wnt (Wg), Hedgehog (Hh) and TGF-β (Dpp) ligands for their abilities to behave as morphogens. Using a novel system in which to study morphogens, the Drosophila ovary, we report on another morphogen, Unpaired (Upd). Upd is a member of the Unpaired ligand family that activates the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling in Drosophila. Previous work with Upd showed that it is glycosylated, secreted, and bound to the extracellular matrix. Upd can be released from the ECM with heparin, suggesting Upd may bind to Heparan Sulfate Proteoglycans (HSPGs). In the ovary, the JAK/STAT pathway is activated in a graded fashion and the level of JAK signaling is sufficient to determine anterior follicular cell fates. Antibody staining against Upd shows that once released from its source in the ovary, there is a clear graded distribution along the apical surface of the epithelium. Mosaic clonal analysis has revealed that loss of one of the four known HSPGs, Dally, causes a reduction in JAK signaling and extracellular Upd accumulation in egg chambers in the vitellarium. A stretch of positively charged amino acids near the N-terminus of Upd affects interactions with the ECM. Our data support a role for Dally in stabilization of the extracellular Upd, by retaining it to the ECM, preventing its degradation. The Drosophila ovum presents a novel model for morphogen signaling with distinct advantages for ligand tracking and manipulation of extracellular environment.

219C

Elucidating the mechanism by which Apontic inhibits JAK/STAT activity. Afsoon Saadin, Michelle Starz-Gaiano. University of Maryland Baltimore County, Baltimore, MD.

Cell migration, which is required for various desired biological events, is also the cause of an undesired phenomenon, tumor metastasis. Out of different signaling pathways that contribute to cell migration, the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) is of high interest since this pathway is involved in various biological processes, and dysfunction of the pathway is correlated to different human diseases, including immune disorders and tumorigenesis. Some components of this pathway including DOME, UNPAIRED, JAK and STAT are well characterized, however, other components of the pathway including APT and SOCS are not completely understood. It has been shown that APT and SOCS36E are negative regulators of the pathway and they both affect cell migration, however, the mechanism by which they act is not clear. It is known that APT inhibits STAT activity indirectly. To identify the component/s of the pathway that mediate the regulatory function of APT, we have taken advantage of a STAT Luciferase reporter assay and RNAi technology in S2 cells. We confirmed that knockdown of *socs36e* led to higher STAT reporter expression. We also found that cells change transcription activity levels non-linearly in response to higher or lower levels of APT expression. We also show that cells that over-express APT and have reduced SOCS36E, have increased STAT activity, suggesting that SOCS36E is one of the components through which APT functions. Further experiments are aimed towards understanding the mechanistic relationship between APT and SOCS36E.

220A

The effect of Upd3 on stem cells in Drosophila testes. Lingfeng Tang, Douglas Harrision. Department of Biology, University of Kentucky, Lexington, KY.

The Drosophila testis serves as a perfect model for investigating stem cells. Besides the sheath, the testis is only composed of 3 lineages of cells: hub cells, germline cells and somatic cyst cells. At the tip of testes is the hub, which is composed of 9-12 cells. The germline stem cells (GSCs) and somatic stem cells (SSCs) directly attach to the hub. JAK/STAT activity which is activated by Upd, the ligand of the pathway, serves as the molecular niche of both GSCs and SSCs in the testes. Upd activates JAK/STAT pathway in SSCs directly, then SSCs affect GSCs through TGF-beta signaling. It has been reported that loss of function of Stat92e leads to the loss of both GSCs and SSCs in testes, and ectopic activation of Stat92e leads to ectopic stem cell like germline cells. Mutants for Upd3, another JAK pathway ligand, have recently been found to exhibit reduced lifespan and premature male reproductive senescence. Reduced lifespan could be an indication of accelerated aging of flies. As an alternative, Upd3 mutations may lead to reduced JAK/STAT activity in testes, which may subsequently impair the maintenance of GSCs and SSCs in testes. These alternatives can be distinguished by the phenotypes of upd3 mutant males as they age. If aging is accelerated in testes we would expect thinner testes, less GSCs and SSCs and decreased cell division rate which are normally observed as the fly ages. If Upd3 mutation leads to reduced JAK/STAT activity we would expect more GSCs, less SSCs and lower expression of beta PS integrin which are

the opposite phenotypes of mutation of socs36e, a negative regulator of JAK/STAT pathway. The diameter of testes of both wildtype and Upd3 mutant will be measured at different time points. In addition, using antibody staining to mark different cell types and PH3 staining to mark dividing cells, the number of GSCs and SSCs, as well as division rate of GSCs and SSCs will be determined as animals age. The results of these analyses will be presented.

221B

Transcriptional regulation of the *unpaired3* gene in *Drosophila* development. Yu-Chen Tsai, Hsin-Yi Huang. Dept Life Science, Tung-hai Univ, Taichung, Taiwan.

Unpaired3 (Upd3) is a ligand of Janus Kinase/ Signal Transducers and Activators of Transcription (Jak/STAT) signaling in *Drosophila*. *upd3* is expressed in eye-antenna disc, gonad in the embryonic stage. *upd3* may play a role in eye, gonad, haematopoiesis and immune response. In this study, we focus on the transcriptional regulation of the *upd3* gene and further study the upstream signaling of the *upd3* gene. We analyzed the *upd3* enhancers *in vivo*. The 22.1Kb genomic regions around the *upd3* gene were analyzed. These *upd3* genomic fragments were cloned to enhancer-testing vector, pH-Stinger, which contains a GFP reporter. The enhancer-testing constructs were injected into *Drosophila* and then selected for transgenic lines. The expression pattern of GFP reporter was examined *in vivo*. We found a possible 1.2Kb *upd3* enhancer which may regulate eye, intestine cells, posterior signaling center (PSC) in lymph gland. We further narrowed down this enhancer fragment and examine GFP pattern *in vivo*. To understand the upstream signaling of the *upd3* gene, we predicted the possible transcriptional factor binding sites. Suppressor of Hairless (Su(H)), STAT92E and AP-1 binding sites are found in 22.1Kb genomic regions of the *upd3* gene. We will further analyze the transcriptional regulation of the *upd3* gene in eye, gonad, and lymph gland development in *Drosophila*.

222C

Characterization of tracheal remodeling in third instar larvae through sequential imaging. Alexandru S Denes, Oguz Kanca, Emmanuel Caussinus, Markus Affolter. Biozentrum, University of Basel, Basel, Switzerland.

The tracheal system is an excellent model for the study of tubular organs. Little is known about late third instar and pupal stage remodeling in the tracheal system, at least in part due to a lack of live imaging techniques. This study aims to improve our understanding of remodeling by labeling and tracking cells within the same animal. Unlike embryos, larvae have a fully developed gut and muscular system, making imaging very difficult. Therefore, we cooled down the animals using a custom built metal box and ice. The larvae were mounted on a slide and cover slip that fit on the box. The imaging was done with an inverted confocal microscope. While under anesthesia, the heart continued to beat at a slow rate; other movements were essentially suppressed for the duration of a recording session (around 20 min). Both the tracheal system and the wing disc are accessible to live imaging. Subsequently, the larvae were transferred to food and allowed to continue development: the survival rate was close to 100% even after multiple recordings. Cell labeling was achieved through two methods. First, we generated flies harboring a transgene encoding a nuclear localized, tandem version of mEos2 (tdEos2). Photoconverted cells could be recognized even after multiple rounds of division. Second, we developed a version of Flybow with good spectral separation: up to five different colors could be simultaneously recorded. Larvae expressing tdEos2 in the tracheal system were staged and individual cells in various branches of tracheal metameres were photoconverted. We were able to show there are two temporal gradients with regard to cell division in the dorsal branches: one from the anterior tracheomeres to the more posterior ones and another from proximal to distal with regard to the dorsal trunk. The degree of polyploidy in the dorsal trunk along the antero-posterior axis was quantified using Flybow. Furthermore, we tracked the rounds of cell division in the second tracheomere.

223A

Computer simulation and live-imaging support a stochastic model of ventral furrow formation. Philipp Spahn, Rolf Reuter. University of Tuebingen, Tuebingen, Germany, Interfaculty Institute for Cell Biology, Division of Animal Genetics.

Ventral furrow formation (VFF) in Drosophila is an attractive model system to understand how an epithelium undergoes coordinated morphogenesis. In order to initiate the internalization of ventral tissue, cells of the ventral epithelium constrict their apices leading to a furrow-like invagination into the interior of the embryo. This apical constriction is brought about by an apically localized contracting actomyosin meshwork being tightly coupled to apically positioned adherens junctions. It has been previously reported that the reduction of apical cell area occurs in a stereotypical incremental fashion because phases of actomyosin contraction alternate with phases of stabilization, where the cell has to maintain its constricted state before the next contraction pulse is set off. Here, we present a 2D computer simulation integrating a simple model of a stochastically contracting actomyosin and mechanical cell-cell coupling. We find that rapid random contraction pulses in combination with a ventral-to-lateral gradient of contraction rate are sufficient to cause an epithelial transformation that closely mimics the ventral furrow, as can be validated by confocal live-imaging. Unlike previously stated, we do not find apical constriction to follow a stereotypical incremental pattern. Both simulation and live-imaging show that occasional pauses in area decrease do not need to coincide with pauses of contraction as the cell's contraction can be compensated by contractions of neighboring cells. This can result in a temporary halt of apical constriction or in cell stretching, both showing up as pauses in the area graph. Challenging the notion that apical constriction follows a stereotypical pattern and only depends on the cell's own actomyosin contraction, we propose a model, in which shape change of ventral cells occurs as a stochastic process driven by random contraction pulses and mutual mechanical interaction with neighboring cells.

224B

Candidate-based *in vivo* RNAi Screen to Identify Novel Genes Regulating Collective Border Cell Migration. George Aranjuez[1,3], Elizabeth Kudlaty[2], Jocelyn A. McDonald[3]. 1) Genetics, Case Western Reserve University, Cleveland, OH; 2) Biological Sciences, Northwestern University, Evanston, IL; 3) Molecular Genetics, Cleveland Clinic Foundation, Cleveland, OH.

Border cells are a group of 6-10 cells that migrate during late *Drosophila* oogenesis. These cells move as a cluster that collectively follows guidance cues to the oocyte, their migratory target. Border cell migration serves as a powerful genetic model for collective cell migration, which occurs as part of normal embryonic development, wound healing, and tumor metastasis. To identify new genes required for collective border cell migration, we performed an *in vivo* RNAi screen to knock down genes encoding PDZ domain-containing proteins. The PDZ domain is one of the largest families of protein interaction domains found in eukaryotes. Proteins with PDZ domains are known to regulate apical-basal polarity and signalling cascades, both of which play important roles in border cell migration. Targeting PDZ domain-containing proteins effectively screens a larger number of genes via the protein complexes and pathways through which the PDZ domain-containing proteins work. As a validation of our screening approach, we pulled out *baz* and *par-6*, known regulators of

border cell migration. We identified additional novel genes that likely function in diverse cellular pathways, many of which have not yet been studied in the context of cell migration in any organism. Furthermore, we demonstrated that a targeted, well-designed screen is sufficient to discover new candidate regulators of collective border cell migration with minimal time and effort.

225C

TGF-β/Activin signaling mediates border cell migration during Drosophila oogenesis. Sameen Babur, Maryanna Aldrich, Takuya Akiyama, Kristi Wharton. Department of Molecular Biology, Cell Biology, & Biochemistry , Brown University, Providence, RI.

The development of vastly diverse organisms requires the properly timed migration of both single cells and cohesive clusters of cells. These cells gain invasive properties and are directed to their destinations through various guidance factors and cues. Elucidating this highly complex cellular behavior in the context of both normal development and the misregulated occurrence of cancer metastasis requires understanding the intricate molecular mechanisms controlling cell migration. Border cell migration during Drosophila oogenesis provides an excellent in vivo system to visualize and identify the many interacting factors involved in the phenomenon of collective cell migration. In this process, nonmigratory polar cells that lie at the anterior end of each Drosophila egg chamber induce the surrounding follicle cells to become invasive, migratory border cells through the action of JAK-STAT signaling. A cluster of two polar cells and four to eight border cells subsequently delaminates from the anterior end of the egg chamber and collectively migrates posteriorly to the oocyte. We have identified an important role for TGF-β/Activin signaling in this process, as disruption of this signaling pathway via RNAi in either the border cells or the polar cells leads to a significant migration delay. This effect is specific to the migratory behavior of these clusters, as reduction of TGF-β/Activin signaling does not affect border cell specification, cluster delamination, or cluster cohesiveness. As seen in other cases of delayed cluster migration, we observe a mislocalization of E-cadherin when TGF-b/Activin signaling is reduced. Finally, we find that the effect of TGF-β/Activin signaling on border cell migration appears to be independent of a role for the related BMP signaling pathway.

226A

Genetic screening to identify enzymes affecting the spread of ovarian cancer. Neville Cobbe, Sarah Forrester, Jenny Horend, Hussain Jaffery, Sally Quine, Amy Rothwell, Shaun Speldewinde, Sarah Taylor, Adriana Guillermo Wiesinger, Helen Young, Daimark Bennett. School of Biological Sciences, University of Liverpool, Liverpool.

Ovarian cancer is an aggressive form of carcinoma that is regrettably difficult to detect before metastasis, rendering treatment very difficult. Our goal is to understand the cause and mechanisms of this invasive cell migration by using the fruit fly, *Drosophila melanogaster*. During normal development of the *Drosophila* ovary, a specialized group of epithelial cells (known as the border cell-cluster) migrates through the egg chamber in a manner akin to the invasive migration of ovarian cancer cells. As border cell migration can be readily visualized microscopically, this provides a powerful model system to study the mechanics of ovarian cancer metastasis *in vivo*, with conserved genes previously implicated in both processes alike. Our major focus is on the way cancers are controlled by reversible phosphorylation, by identifying kinases and phosphatases affecting border cell migration. We have compiled an extensive database of conserved *Drosophila* kinases and phosphatases, using this to design genetic crosses in which a short hairpin RNA (shRNA) could be specifically produced under the control of the GAL4 transcription factor expressed ectopically within border cells, ideally leading to depletion of a particular enzyme within the border cells by means of RNA interference (RNAi). Here we will present and discuss the results of two successive genetic screens performed blindly with UAS-shRNA lines from different sources, which have been retrospectively validated by identification of enzymes independently characterized as important for border cell migration.

227B

The role of fd64a in salivary gland migration. Caitlin D. Hanlon, Deborah J. Andrew. Cell Biol, Johns Hopkins Med Inst, Baltimore, MD.

Drosophila fd64a encodes a member of the fork head box family of transcription factors. Most closely related to mammalian FoxL, fd64A is dynamically expressed in embryos in several tissues, including the somatic muscles, the caudal visceral mesoderm, the ventral longitudinal muscles, and the hindgut. fd64A expression in a subset of somatic body wall muscles in thoracic segments T2 and T3 is especially intriguing due to the intimate association of these muscles with the migrating embryonic salivary gland. The goal of this project is to determine if and how fd64a affects salivary gland migration, and to identify and characterize the relevant Fd64a targets. To investigate the role of fd64a in salivary gland migration, two overlapping deficiencies removing the fd64a gene were assayed for defects in salivary gland morphology and placement. Staining with apical membrane and nuclear markers show a range of salivary gland defects in embryos homozygous for each deficiency and in embryos carrying the two deficiencies in trans, including rough, stunted, and mis-positioned glands. We have also created UAS-fd64A transgenic fly lines that allow for expression of the gene in new places. Ectopic expression of fd64a using twist-Gal4, which drives expression in all mesoderm, causes the salivary gland to curl and bend. These data support a role for fd64a in directing salivary gland migration. Based on these promising preliminary findings, we are now creating a null allele of fd64a to fully characterize its role in salivary gland migration and to use as a tool to find the relevant downstream effectors.

228C

Par-1 Controls Non-Muscle Myosin II Activity Through Myosin Phosphatase to Regulate Collective Border Cell Migration. Pralay Majumder[1], Aranjuez George[1,2], McDonald Jocelyn[1,2]. 1) Molecular Genetics, Lerner Research Institute, Cleveland Clinic, Cleveland, OH; 2) Genetics, Case Western Reserve University School of Medicine, Cleveland, OH.

Many cells migrate in collective groups during tissue morphogenesis, wound healing, tumor invasion and metastasis. In single migrating cells, localized actomyosin contraction couples with actin polymerization and cell-matrix adhesion to regulate cell protrusions and retract trailing cell edges. In contrast, we have only a limited understanding of mechanisms that coordinate actomyosin dynamics in collective cell migration. We study the migration of Drosophila border cells (BCs), which move as a cohesive group of 6-10 epithelial-derived cells during late oogenesis. We previously observed that mutants of the cell polarity protein Par-1, a serine-threonine kinase, result in defective border cell epithelial detachment and migration. We now show that these defects are caused by perturbations in cytoskeletal dynamics due to disruption of a previously unknown signaling pathway between Par-1 and non-muscle myosin-II (Myo-II). Using live imaging, we show that Myo-II is required for two critical features of BC migration: detachment of BCs from the surrounding epithelium, and extension of cell protrusions of normal length and lifetime. We identified a robust genetic interaction between the myosin regulatory light

chain spaghetti squash (sqh) and par-1 and, remarkably, found that active Sqh strongly suppresses par-1-dependent mutant phenotypes. We show that Par-1 regulates dynamic subcellular localization of Sqh in live BCs and also regulates Myo-II activity. Specifically, Par-1 phosphorylates and inactivates myosin phosphatase, thus promoting phosphorylation of Sqh and increased Myo-II activation. Finally, Par-1 localizes to and increases active Myo-II at the cluster rear to promote detachment; in the absence of Par-1, spatially distinct active Myo-II is reduced. Our study reveals that Par-1 regulates polarized Myo-II activity by localized inhibition of myosin phosphatase, thus modulating the spatiotemporal actomyosin dynamics required for collective BC migration.

229A

WASH, a Rho1 effector, functions through the Arp2/3 complex in hemocyte migration. James J Watts, Evelyn Rodriguez-Mesa, Susan M Parkhurst. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

The Wiskott Aldrich Syndrome (WAS) family of proteins has been shown to affect the cortical actin cytoskeleton and plasma membrane during endocytosis and cell migration. We recently characterized a new member of the WAS family, WASH, which acts downstream of Rho1, activates the Arp2/3 complex, and crosslinks microtubules and actin filaments. To examine WASH's role in migration, we study the hemocytes of the Drosophila embryo. Hemocytes undergo highly stereotyped developmental migrations in stage 12 to 16 embryos, and they can be induced to migrate towards wounds. We utilize GFP and RNAi constructs driven solely in hemocytes to track their migration and knockdown WASH, respectively. WASH knockdown causes disruptions to both induced and developmental migrations including defects in migration direction and cell polarity. Consistent with our previous study, knockdowns of Rho1 or Arp3 result in similar migration defects, suggesting that WASH acts downstream of Rho1 to activate the Arp2/3 complex in this context. We confirmed this finding by reproducing the migration defects of WASH knockdown in a Rho1 null embryo rescued by a Rho1 point mutant that retains most of its function except for its ability to activate WASH. We plan on determining the mechanism through which WASH affects migration direction and cell polarity utilizing a recently characterized hemocyte cell line.

230B

***Fkbp14* is Required for *Drosophila* Development and Interacts with the Notch Pathway.** Julia M Bonner[1,2], Diana L van de Hoef[1,2], Gabrielle L Boulianne[1,2]. 1) Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto.

FK506 binding proteins (FKBPs) are a large, highly conserved family of proteins involved in a wide array of biochemical processes including protein folding, assembly, and trafficking. Little is known regarding many FKBP *in vivo* functions or binding partners, nor the extent and specificity with which they contribute to metazoan development. We identified a member of this family, *Fkbp14*, in a screen for novel interactors with *presenilin* (*psn*), a key component of the Notch pathway. We demonstrate that *Fkbp14* genetically interacts with members of the Notch signaling pathway, and observed "escaper" mutants display classic Notch phenotypes including reduced adult sensory structures, as well as defects in wing margin specification. Initial investigations indicate that *Fkbp14*-null mutations are homozygous lethal and show dramatically reduced PSN protein levels, while clonal analysis has revealed a severely reduced cell-viability phenotype. These results indicate a novel requirement for *Fkbp14* in *Drosophila* development. Further investigation into the function and regulation of *Fkbp14* will provide valuable insight into FKBPs and their significance in development and disease in multicellular systems.

231C

Interactions between MEF2, SD, VG and the Notch pathway during Indirect Flight Muscle development. Charlotte Caine, Joel Silber, Alexis Lalouette. Develomental And Molecular Biology, Institut Jacques Monod, PARIS, France.

Myogenesis of indirect flight muscles (IFM) in Drosophila melanogaster follows a well defined cellular developmental scheme. During embryogenesis, a subset of cells, the Adult Muscle Precursors (AMPs), are specified. These cells will become proliferating myoblasts during the larval stages which will then give rise to the adult IFM. Although the cellular aspect of this developmental process is well studied, the molecular biology behind the different stages is still under investigation. We are currently working on the interactions required during the transition between proliferating myoblasts to differentiated myoblasts ready to fuse to the muscle fiber. It has been previously shown that proliferating myoblasts express the Notch pathway, and that this pathway is inhibited in developing muscle fibers. On the other hand, it has also been shown that the Myocyte Enhancing Factor 2 (MEF2), Vestigial (VG) and Scalloped (SD) transcription factors are necessary for IFM development and that VG is required for Notch pathway repression in differentiating fibers. Our study focuses on the interactions between Notch and MEF2 and mechanisms by which the Notch pathway is inhibited during differentiation. Here we show that MEF2 is capable of inhibiting the Notch pathway in non myogenic cells. A previous screen for MEF2 potential targets identified Delta and Neuralized, two components of the Notch pathway. Both are expressed in developing fibers where MEF2, SD and VG are expressed. Our preliminary results show that MEF2 is required for Delta expression in developing IFMs and that this regulation is potentially dependent on an enhancer to which MEF2 and SD bind. We have identified a similar Neuralized enhancer that seems to be potentially regulated by MEF2. We are currently studying the effect of MEF2 on these targets in vivo and in vitro to understand the role they play during IFM differentiation.

232A

The role of Notch signaling in primary pigment cell formation. Yu-Huei Ho[1], Jui-Chou Hsu[1,2]. 1) Institute of Molecular Medicine, Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan 30013, Republic of China; 2) Department of Biological Science and Technology, National Chiao Tung University, Hsinchu,Taiwan 30013, Republic of China.

Notch signaling is a highly conserved pathway in regulating cell differentiation. In pupal eye, Notch is required for the differentiation of the primary pigment cell (PPC), whose differentiation is proposed to be induced by receiving Delta from the neighboring cone cells. So far, the detailed mechanism is unknown. Here we show that the activation of Notch signaling during the time window of PPC selection induces extra enlarged cells outside of the PPC, phenocopying PPCs, suggesting that Notch induces cell enlargement to differentiate as PPC. However, through live imaging, we found that the activation of Notch is not sufficient to specify cells as PPCs, implying that Notch needs to combine other signaling to specify PPC. Moreover, we also find that Notch has different contributions, including selection, enlargement and protection, to PPC formation at different development stages by analyzing the temperature sensitive allele of Notch at different time points. Collectively, we had further clarified Notch functions during PPC selection.

233B

Monosaccharide *O*-fucosylation of Notch receptor is required for Notch signaling in *Drosophila*. Akira Ishio[1], Tomonori Ayukawa[1], Naoki Aoyama[1], Hiroyuki O.Ishikawa[1], Tomoko Yamakawa[1], Takeshi Sasamura[1], Tetsuya Okajima[2], Kenji Matsuno[1]. 1) Department of Biological Science and Technology, Tokyo University of Science, Chiba; 2) Nagoya University Graduate School of Medicine.

Notch (N) is a transmembrane receptor with homology to epidermal growth factor (EGF)-like repeats and mediates cell-cell interactions necessary for many cell-fate decisions. These EGF-like repeats are *O*-fucosylated by the protein *O*-fucosyltransferase 1 (*O*-fut1), which is essential for N signaling. However, roles of monosaccharide *O*-fucose modification in N signaling became elusive, because it was proposed that *O*-fucsyltransferase activity-independent functions of *O*-fut1, per se including N-specific chaperon function and modulation of N endocytosis, could be essential for N signaling in *Drosophila*.

In this study, we showed that monomeric *O*-fucose modification of N was essential for Delta-N but not Serrate-N signaling activity in the signal receiving cells of imaginal organs in Drosophila. This novel function of monomeric signaling in a subset of organs. In agreement with this finding, we found that lack of monomeric *O*-fucose modification of N caused a temperature-sensitive neurogenic phenotype in embryos. In addition, disruption of N signaling associated with the lack of *O*-fucose modification was partly rescued by upregulation of the unfolded protein response.These results suggest that monomeric *O*-fucose modification of N has a novel role for the ligand-dependent activity of N, which collaborates with the proper folding of N.

234C

Regulation of *broad* expression by Notch signaling during the mitotic/endocycle switch in *Drosophila* follicle cells. Dongyu Jia, Yoichiro Tamori, Wu-Min Deng. Department of Biological Science, Florida State University, Tallahassee, FL.

During *Drosophila* oogenesis, mediated by multiple signaling pathways, the follicle cells sequentially undergo three distinct cell-cycle programs: the mitotic cycle (stages1-6), the endocycle (stages 7-10a), and gene amplification (stages10b-14). Activation of Notch signaling at stages 6/7 and inactivation of it at around stage 10b in follicle cells are essential for the proper two cell-cycle program switches. *broad (br)*, encoding a family of zinc-finger transcription factors, has been known as an early ecdysone response gene that is pivotal for metamorphosis. During oogenesis, Br is first expressed uniformly in follicle cells at stages 6/7,then expressed at high levels in two patches at the anterior dorsal region from stage 10b. To determine how the early pattern of Br in follicle cells is established, we examined the effect of Notch signaling on Br expression. We found that loss of key components of Notch signaling, such as *Notch, Presenilin, Nicastrin, Suppressor of Hairless* in somatic follicle cells, or *Delta* in germline cells, fails to upregulate Br expression. Recently, Shvartsman's Lab at Princeton University identified two non-overlapping enhancers regulate Br expression in response to EGFR signaling in follicle cells, which are named *Early Enhancer (brEE)* and *Late Enhancer (brLE)*. Expression of brEE reporters is similar to the early pattern of Br, uniform in follicle cells starting at stages 6/7. Consistently, we found that the *brEE* pattern is disrupted when key Notch signaling components are mutated. The facts together suggest that Br is regulated by Notch signaling via *brEE* during the mitotic cycle-endocycle (M/E) switch. Clonal analysis and RNAi studies revealed that follicle cells with loss of *br* function have a mild defect in entering the endocycle at stage 7. In contrast, misexpression of Br isoforms Z1, Z2, or Z4, but not Z3, prompts follicle cells to exit the mitotic cycle earlier. Currently, we are characterizing the potential function of *br* in mediating different Notch regulated cellular processes during the M/E transition.

235A

Xylose: A Novel Modulator of Notch Signaling. Tom V. Lee[1], Maya Sethi[2], Jessica Leonardi[1,4], Nadia Rana[3], Robert Haltiwanger[3], Hans Bakker[2], Hamed Jafar-Nejad[1,4]. 1) University of Texas Health Science Center, Houston, TX; 2) Hannover Medical School, Hannover, Germany; 3) Stony Brook University, Stony Brook, NY; 4) Baylor College of Medicine, Houston, TX.

An important post-translational modification identified on the Notch receptors is the addition of *O*-glucose to Epidermal Growth Factor-like (EGF) repeats by the protein *O*-glucosyltransferase Rumi, a temperature-sensitive activator of Notch signaling. *O*-glucose on EGF repeats can be extended by the addition of two xylose residues, but the functional role of xylose residues in the regulation of Notch signaling is not known. We have recently identified the mammalian glucoside xylosyltransferases (GxylT1 and GxylT2) and xyloside xylosyltransferase (XxylT), which add the first and the second xylose residues to *O*-glucosylated EGF repeats of human Notch, respectively. Here, we show that addition of xylose to *O*-glucose residues negatively regulates Notch signaling in *Drosophila*. Mutations in the sole *Drosophila* homolog of GxylT1/2, *shams*, result in temperature-sensitive mutant phenotypes compatible with increased Notch signaling. Genetic interaction studies show that *shams* mutations suppress both *Notch* and *Delta* haploinsufficient phenotypes, but enhance the *Abruptex* gain-of-function phenotype. Overexpression of xylosyltransferases in *Drosophila* results in Notch-related mutant phenotypes that are dependent on their catalytic activity. Biochemical and *in vivo* analyses indicate that functionally relevant sites of xylosylation are localized to a specific region of the *Drosophila* Notch. Finally, mass spectral analysis of Notch EGF repeats indicates that endogenous levels of the *Drosophila* GxylT (Shams) and XxylT enzymes can only partially xylosylate the *O*-glucosylated Notch EGF repeats. Taken together, these data indicate that altering the level of xylose occupancy on a specific group of Notch EGF repeats can modulate the strength of the *Drosophila* Notch signaling over a broad range and potentially provide a novel tool to fine-tune the strength of Notch signaling.

236B

Investigating the role of the NHR2 domain of Neuralized in Notch Signaling. Sili Liu[1,2], Julia Maeve Bonner[1,2], Gabrielle Boulianne[1,2]. 1) Stem Cell & Developmental Biology, Hospital For Sick Children, Toronto, ON, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.

In the Notch pathway, one critical step in initiating Notch activation is the endocytosis of the Notch ligands, Delta and Serrate in the signal-sending cell. Ligand endocytosis is regulated by one of two E3 ubiquitin ligases, Neuralized or Mind bomb. Neuralized is comprised of a C-terminal RING domain, which is required for Delta ubiquitination, and two Neuralized Homology repeat (NHR) domains. We have previously shown that a conserved glycine residue in the NHR1 domain is required for Delta trafficking. Here we show that this mutation also affects binding and internalization of Serrate. Furthermore, we show that the NHR2 domain is required for Neuralized function and that a point mutation in the corresponding glycine residue in the NHR2 domain (Gly430) abolishes its ligase activity and affects ligand internalization. Lastly, we provide evidence that Neuralized may form oligomers or intramolecular loops through the two NHR domains. These results demonstrate that the NHR1 domain regulates the interaction of Neuralized with both

Notch ligands and suggests that the NHR1 and NHR2 domains together may regulate Neuralized activity and, by extension, Notch signal transduction.

237C

Importin-α3 mediates nuclear import of Notch and it displays synergistic effect with Notch activation on cell proliferation. Nalani Sachan, Abhinava Mishra, Mousumi Mutsuddi, Ashim Mukherjee. Department of Molecular & Human Genetics, Banaras Hindu University, Varanasi, India.

The Notch pathway is an evolutionarily conserved signaling system which has been shown to play major role in cell fate determination, differentiation, proliferation and apoptotic events as well as self-renewal processes of different tissues. The same pathway can be deployed in numerous cellular contexts to play varied and critical roles for the development of an organism. The versatility of this pathway to influence different aspects of development comes from its multiple levels of regulation. In an effort to identify novel components involved in Notch signaling and its regulation, a yeast two-hybrid screen was carried out using intracellular domain of Notch receptor (Notch-ICD) as bait and we identified Drosophila Importin-α3 as binding partner of Notch. Further, GST-pull down experiments confirmed the interaction between Notch and Importin-α3. Immunocytochemical analysis revealed that Importin-α3 and Notch-ICD indeed co-localized in cell nuclei. Different alleles of importin-α3 also showed strong genetic interactions with Notch pathway components in transheterozygous combinations. Somatic clonal analysis of importin-α3 using FLP-FRT system showed that loss of importin-α3 function results in cytoplasmic accumulation of the Notch receptor. Using MARCM (Mosaic Analysis with a Repressible Cell Marker) technique, we demonstrate that Importin-α3 is required for nuclear localization of Notch receptor. These results clearly show that the nuclear transport of Notch-ICD is mediated by the canonical Importin-α3/Importin-β transport pathway. In addition, co-expression of both Notch-ICD and Importin-α3 displays synergistic effects on cell proliferation. Taken together, our results suggest that nuclear import of Notch-ICD may play important role in regulation of Notch signaling.

238A

Mutual bi-directional Notch activation represses fusion competence in swarming adult Drosophila myoblasts. Eyal D. Schejter, Boaz Gildor, Ben-Zion Shilo. Dept. Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

Establishment of the adult Drosophila flight musculature involves hundreds of cell-cell fusions between nascent myotubes, and a migrating “swarm” of wing-disc derived myoblasts. Our analysis suggests that these fusion events require functional contributions from the Ig super-family cell adhesion proteins Dumbfounded/Kirre, which is expressed on the myotubes, and the myoblast specific element Sticks-and-stones (Sns). This molecular paradigm resembles the program of myoblast fusion underlying embryonic muscle fiber formation. However, Sns expression by adult myoblasts is tightly regulated, and is restricted to myoblasts in the immediate vicinity of the myotubes, just prior to fusion. We find that during their migratory phase, myoblasts are maintained in a semi-differentiated state by continuous activation of Notch signaling, where each myoblast provides the source of ligand, Delta, to its neighbors. This unique form of bi-directional Notch activation is achieved by finely tuning the levels of the ligand and the Notch receptor. Activation of Notch signaling in myoblasts represses expression of key fusion elements such as Sns. Only upon reaching the vicinity of the myotubes does Notch signaling decay, leading to terminal differentiation of the myoblasts. The ensuing induction of fusion-related proteins enables formation of actin-rich attachment sites between myoblasts and myotubes, leading to cell-cell fusion and muscle fiber growth.

239B

Function of a neurogenic gene, *pecanex* in Notch signaling. Tomoko Yamakawa[1], Kenta Yamada[1], Takeshi Sasamura[1], Naotaka Nakazawa[1], Maiko Kanai[1], Emiko Suzuki[2], Mark E. Fortini[3], Kenji Matsuno[1]. 1) Dept of Biol Sci/Tec, Tokyo Univ of Sci, Chiba, Japan; 2) Gene Network Lab, National Institute of Genetics, Mishima, Japan; 3) Dept of Biochem and Mol Biol, Kimmel Cancer Center, Thomas Jefferson Univ, Philadelphia, PA, 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 a *N*-like neurogenic phenotype, suggesting that Pcx is 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 protein was specifically localized to the endoplasmic reticulum (ER). 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 in embryos homozygous for *Presenilin* and lacking its maternal contribution. 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 in 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.

240C

Dynamics of the 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.

Rho GTPases are important regulators of cytoskeleton dynamics in a variety of biological events including cell division, cell migration, vesicle trafficking and gene expression. Rho GTPases are highly regulated in space and time, key features for their function as molecular switches that transmit environmental cues to intracellular signaling pathways. Moreover, Rho GTPases are themselves regulated by input from the cytoskeleton, coordinating the multiple dynamic responses required by the cell. One biological process that requires precise spatial and temporal coordination of membrane and cytoskeletal components is cell wound repair. Single cell wounds heal by rapidly plugging the plasma membrane disruption with a vesicle patch, and requires the assembly of contractile actomyosin ring and microtubules reorganization. To assess the role of Rho GTPases as regulators of the cell wound repair response, we laser wounded embryos carrying fluorescently-tagged GTPases, then followed their repair in vivo by 3D microscopy. In our single cell model, Rho, Rac (Rac1 and Rac2) and Cdc42 rapidly accumulate around the wound, and segregate into dynamic zones that move inward (basally) as healing progress. 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 in this response. Surprisingly, we find that Rho GTPases utilize specific effectors to mediate their signals: our data shows that different Rho GTPases and their effectors are locally recruited in response to wounding. This complex spatial-temporal array may also involve crosstalk among the different GTPases and their signaling modules. Importantly, by genetic and pharmacological assays we also find that Rho, Rac and Cdc42 are required for proper wound repair, and each of them make specific contributions to the assembly and organization of the actomyosin array.

241A

Septate junctions play an unexpected role in the cell division of polarized epithelia. Vanessa J. Auld, Kristi Charish. Dept Zoology, Life Sciences Institute, Univ British Columbia, Vancouver, BC, Canada.

Septate junctions (SJs) are found basal to adherens junctions and form permeability barriers in Drosophila epithelia similar to tight junctions in vertebrates. SJs are generated by a large complex of highly conserved proteins, which have recently been shown to function beyond the formation of permeability barriers. Along these lines, in the columnar epithelia of the Drosophila imaginal disc, the plane of cell division occurs exclusively at the SJ. Null mutations in SJ genes are cell lethal when somatic clones are generated in the imaginal disc of Drosophila and thus we wanted to test if the loss of SJ proteins resulted in a disruption of cell division. Using RNAi approaches, we specifically knocked-down SJ components in the wing imaginal disc and then assayed for changes in cell division. In addition using an array of cellular markers we characterized each step of mitosis in the context of the SJ domain. We show that SJs play a critical role in determining the plane of cell division. Loss of the SJ domain results in the basal spread of the plane of cell division away from the apical domain. This spreads correlates with the displacement of the SJ associated protein Gliotactin and can be phenocopied by loss of Gliotactin. In addition we show that the final stage of cell division, cytokinesis, occurs exclusively within the SJ domain. As the cleavage and ingression furrows develop, a SJ tunnel is created that encases the contractile ring and midbody during cytokinesis. Knock down of SJ proteins leads to basal mislocalization of the plane of cell division, dislodgement of the contractile ring and a failure to form the ingression furrow. We propose a model for cytokinesis within differentiated epithelia that requires the presence of the SJs to successfully complete cell division perhaps as a means to maintain the paracellular barrier across epithelia.

242B

Distinct subcellular distributions of three CTP synthase isoforms in *Drosophila*. Ghows Azzam, Ji-Long Liu. MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, Oxfordshire, United Kingdom.

The cytoophidium is a novel filamentous organelle that contains CTP synthase, a critical metabolic enzyme. Cytoophidia have been observed in a wide range of organisms and different cell types. However, the function of the cytoophidium and how it forms is still elusive. *Drosophila* CTP synthase, one of the main components of cytoophidia, has three isoforms. We have made transgenic flies expressing Venus fused with each individual isoform. To our surprise, these three isoforms exhibit distinct intracellular localizations. It appears that only isoform C assembles into the cytoophidium, while isoform B is dispersed in the cytoplasm and isoform A forms punctate in the nucleus. The aim of this study is to understand which region is important to form filaments and whether the non-filament forming isoforms are functional. Dissecting isoform C and studying CTP synthase mutants could shed light on understanding how the cytoophidia is formed and how the cytoophidium functions.

243C

Dissection of the NR box-dependent interaction between the bHLH-PAS paralogs MET and GCE and the FTZ-F1 nuclear receptor. Travis J. Bernardo, Edward B. Dubrovsky. Fordham University, Bronx, NY.

Juvenile hormone (JH) has been implicated in many developmental processes in holometabolous insects, but its mechanism of signaling remains controversial. We previously found that in *Drosophila* S2 cells the orphan nuclear receptor FTZ-F1 is required for the activation of *E75A* by JH. It binds to enhancers upstream of the *E75A* promoter and interacts with two JH receptor candidates, the bHLH-PAS paralogs MET and GCE. Here, we investigated the molecular basis of FTZ-F1 involvement in JH signaling. In quantitative two-hybrid assays we observed that FTZ-F1 interacts with MET and GCE in a JH-dependent manner. These interactions are severely reduced when helix 12 of the FTZ-F1 LBD is removed, implicating AF2 as a potential interacting site. To gain insight into the physical interaction between these proteins we used a homology-modeling approach and found that MET and GCE possess an α-helix C-terminal to the second PAS fold that contains the conserved motif LIxxL. This motif resembles an NR box and is essential for the interactions with FTZ-F1. Docking simulations supported by two-hybrid experiments reveal that the FTZ-F1 AF2 provides a distinct interacting surface for MET and GCE, which resembles that of a typical NR box/AF2 interaction but does not require charge clamp residues. Our findings suggest that a novel NR box enables MET and GCE to interact JH-dependently with the AF2 of FTZ-F1.

244A

Regulation of autophagy by the Atg1/Ulk family of protein kinases in *Drosophila melanogaster*. Christopher R Braden, Thomas P Neufeld. University of Minnesota, Minneapolis, MN.

Macroautophagy (hereafter “autophagy”) is a process involving the sequestration of cytoplasm for lysosomal degradation. The level of autophagic activity varies under the control of the Target of Rapamycin (TOR) pathway. In D. melanogaster models TOR exerts its influence through a complex involving the Ser/Thr protein kinase Atg1. Defects in these regulatory pathways have been implicated in human afflictions such as cancer and neurodegenerative diseases, perhaps due to the role of autophagy in cellular response to nutritional, oxidative, or ER stress. Atg1 is a member of the Unc51-like kinase (Ulk) family. While several Ulk family proteins are described in mammals, only two exist in D. melanogaster: Atg1 and Ulk3 (CG8866). Ulk3 induces autophagy by overexpression in mammalian cell culture, but remains largely unstudied. We show that overexpression of Ulk3 induces autophagy in the fat body, and that Ulk3 suppresses Atg1 activity in overexpression studies. Generation of a large deletion in Ulk3 does not obviously inhibit starvation-induced autophagy, but apparently reduces oxidative stress survival in a manner dependent on Atg1 gene dosage. Together, our data suggest that autophagy is regulated by distinct Ulk-family kinases in response to different upstream cues, with Atg1 required for nutrient responses and Atg1 and Ulk3 behaving antagonistically in oxidative stress signaling.

245B

The effects of PKA in Drosophila fat body. Yu-Yun Chang, Thomas Neufeld. Gen & Cell Development, Univ Minnesota, Minneapolis, MN.

The Drosophila cAMP-dependent protein kinase (PKA) has been identified as one of the key proteins that regulate Hedgehog signaling, which is involved in multiple developmental decisions including segment formation during embryogenesis, and the growth/patterning of imaginal discs. However, little is known about the role of PKA in the larval fat body, a nutrient storage organ that functions equivalently to mammalian liver. By manipulating the level of the PKA-C1 catalytic subunit in individual fat body cells, we have observed an increased cell size in PKA loss-of-function clones and severe size reduction in PKA-overexpressing cells, indicating that PKA negatively regulates cell growth in a cell-autonomous manner. Previous studies have revealed that autophagy, a catabolic process that degrades cellular components to recycle materials and to provide energy, is quickly triggered in the fat body in response to nutrient deprivation. We find that loss of PKA activity impairs the induction of autophagy in starved larvae, and that overexpression of PKA induces autophagy in well-fed animals, indicating that PKA has an essential and sufficient role in autophagy regulation. Genetic analyses further show that PKA is involved in insulin and target of rapamycin (TOR) signaling pathways, which demonstrates a new model in which PKA coordinates cell growth and autophagy in Drosophila.

246C

Characterization of a novel testis-specific mitochondrial protein in sperm formation. Jieyan Chen, Timothy Megraw. Biomedical Sciences, Florida State University, Tallahassee, FL.

Mitochondria are the energy centers in the cell. In testes, the normal functions of mitochondria are required for sperm formation. The Nebenkern is a remarkable mitochondrial form in the Drosophila sperm. Right after meiosis is completed, the mitochondria of the spermatid collect on one side of the haploid pronucleus and fuse together into two giant aggregates which then wrap around one another to produce the spherical Nebenkern. Failure of mitochondrion fusion may affect the sperm tail elongation and motility and lead to the male sterility. Previous studies have shown that mitochondria play a role in sperm tail elongation by providing a structural platform for microtubule reorganization to support the elongation at the tip of sperm tail. We have identified a new protein (CG14128) containing a Ran binding protein 1 (RanBP1) domain, that localizes to the sperm mitochondria, especially the Nebenkerns. RT-PCR and Western Blot results show that CG14128 protein is testis-specific. Previously published yeast two hybrid data suggests that CG14128 interacts with centrosomin (Cnn), indicating a possible role of CG14128 with Cnn in the testis. We will test the genetic interaction between CG14128 and fuzzy onion (fzo), mutation of which results in failure of mitochondrion fusion that leads to male sterility. We will also assay the CG14128 and Cnn interaction during sperm formation, and test the role of CG14128 in sperm tail elongation with a mutant allele.

247A

Natural variation provides a rich source of new genes for ER stress response. Clement Y. Chow, Mariana F. Wolfner, Andrew G. Clark. Dept Mol Bio & Gen, Cornell Uiv, Ithaca, NY.

The endoplasmic reticulum (ER) is responsible for synthesis and maturation of many proteins. ER stress occurs when misfolded proteins accumulate in the ER. Cells respond by increasing transcription of ER chaperones, attenuating translation and degrading misfolded proteins. ER stress is a primary cause or a secondary exacerbating effect of many diseases. To understand its effect on cells and organisms, we measured the extent of natural variation in ER stress response. We compared the survival of 120 wild-derived lines from the DGRP (Drosophila Genetic Reference Panel) on food containing tunicamycin (TM), a drug that causes ER stress. Mortality rates varied across lines by >100 fold. Thus, extensive genetic variation in ER stress response is present in a single population. To understand how ER stress response is buffered by natural variation, we searched for genes whose expression varied with survival to ER stress. Using Agilent microarrays, we compared gene expression during TM exposure in 4 lines that showed high ER stress resistance to 4 that showed low resistance. Genes involved in the cellular response to ER stress, such as chaperones, showed a strong response across all 8 lines; thus the survival differences did not reflect variation in basic ER stress transcriptional responses. However, we found that transcriptome responses between lines with high or low sensitivity to ER stress differed qualitatively and quantitatively. For example, before the strong common response is fully initiated, genes associated with the proteasome and immune response are upregulated in resistant lines, but not in susceptible lines. Many genes showing different responses in high vs. low sensitivity lines had not previously been implicated in ER stress, yet our preliminary RNAi data show their importance to this fundamental cellular response. We demonstrate that there is a large amount of natural variation in ER stress response. This variation is a powerful tool for uncovering new expression patterns and genes involved in ER stress and understanding how natural variation can impact disease processes.

248B

Cell signalling mechanisms in epithelial stress and immune responses. Shireen A Davies, Gayle Overend, Sujith Sebastian, Pablo Cabrero, Selim Terhzaz. Institute of Molecular Cell and Systems Biology, University of Glasgow, Glasgow, United Kingdom.

The Drosophila Malpighian tubule is an epithelial model for cell-specific, organotypic ion transport and cell signalling studies, functional genomics and gene discovery. The tubule is critical for fluid homeostasis and and detoxification in the fly (Beyenbach, Skaer et al. 2010). Fluid transport is modulated by neuroendocrine control and second messengers (Dow and Davies, 2003; Nassel and Winther 2010). As barriers between the external and internal environment, epithelia play key roles in stress defence; and the Drosophila tubule is critical for organismal defence against salt and oxidative stress (Overend, Cabrero et al., 2011; Terhzaz, Finlayson et al., 2010; Stergiopoulos, Cabrero et al., 2009). Tubules are also immune tissues (Tzou, Ohresser et al., 2000; McGettigan, McLennan et al., 2005; Kaneko, Yano et al., 2006), expressing anti-microbial peptides via the IMD and Toll pathways. We now show that a tubule principal cell-specific cGMP-kinase (cGK) 'switch' modulates NF-kB orthologue (Relish) and IMD pathway activation. This cGK 'switch' modulates the survival of immune-challenged whole flies, the first evidence for cyclic nucleotide modulation of innate immunity. The tubule cGK switch also influences the response of the gut to bacteria; thus, response to infection may be dependent on tubule/gut communication. The high rates of metabolic activity in tubules and the associated production of reactive oxygen species results in specific adaptations to counter this, including the enriched expression of 'antioxidant' genes. We show that the manipulation of specific genes in only tubule principal cells is sufficient to modulate organismal stress and immune responses. Thus, the tubule is a key stress-sensing tissue for the whole organism.

249C

Multiple screening approaches suggest novel interaction partners for Eyes absent in the nucleus and cytoplasm. Trevor L. Davis[1,2], Ilaria Rebay[1,2]. 1) Ben May Department for Cancer Research, University of Chicago, Chicago, IL; 2) Committee on Development, Regeneration, and Stem Cell Biology,

University of Chicago, Chicago, IL.

Eyes absent (Eya) is a conserved member of the retinal determination gene network (RDGN) and functions in multiple contexts throughout development. In the nucleus, this protein acts as a transcriptional coactivator with its DNA-binding partner Sine oculis (So). Eya is also present in the cytoplasm, where it functions as a protein tyrosine phosphatase. Few cytosolic Eya substrates have been discovered and little is known about its developmental importance as a phosphatase. To identify new contexts in which Eya might function, we used multiple screening methods to search for novel interactions between it and other proteins. Yeast two-hybrid screening uncovered many putative Eya interactors. We refined these data by evaluating the ability of RNAi-mediated knockdown of each candidate in the eye to modify the Eya^{RNAi} phenotype of adult flies. Lines that strongly enhanced or suppressed the Eya knockdown phenotype were selected for further study. These and other previous screening efforts were hindered by the strong physical interaction between Eya and So; little Eya is thought to exist in the cytosol in cells also expressing So (e.g. in the eye). To increase the probability of detecting interactions that occur outside the nucleus, we simultaneously expressed Eya and knocked down candidate genes in the wing imaginal disc, which lacks significant levels of So. The disruption of each candidate was evaluated for its ability to alter Eya levels, quantified by immunofluorescence, and to impact Eya activity, which we inferred by measuring the size and intensity of Eya-induced ectopic Dachsund expression. This approach identified several novel interactions between Eya and cytoplasmic proteins.

250A

Akt is Negatively Regulated by Hippo Signaling for Growth Inhibition in Drosophila. Yaoting Deng[1], Xin Ye[2], Zhi-Chun Lai[1,2,3]. 1) Biochemistry and Molecular Biology; 2) Graduate Degree Program in Genetics; 3) Department of Biology, Penn State University, University Park, PA.

Growth control of individual cells and the organs is a fundamental question in developmental biology. Hippo (Hpo) pathway functions to restrict cell proliferation and promote cell apoptosis. However, how Hpo pathway regulates cell growth is still unclear. In our study, we found that Hpo signaling regulates cellular growth by inhibiting akt expression through Yki inactivation. Loss of Hpo induces both Akt expression and its activity. When Hpo is overexpressed, the outcome is opposite. We also show that Yki is sufficient to induce Akt expression. Our result suggests that Hippo signaling pathway regulates cellular growth by repressing the Akt pathway activity.

251B

Characterization of cytoplasmic Eyes absent function in *Drosophila* eye development. Charlene Hoi, Fangfang Jiang, Wenjun Xiong, Ilaria Rebay. Ben May Department of Cancer Research, University of Chicago, Chicago, IL.

Understanding how information from multiple signaling pathways coordinates specific developmental programs in context-specific manners is of particular interest. To address this, we study the dual-function transcription factor and protein tyrosine phosphatase, Eyes absent (Eya), which lies at the center of a network of transcription factors known as the retinal determination gene network (RDGN) that is important in Drosophila eye formation. Eya's two functions appear to be spatially separated via a mechanism in which the non-receptor tyrosine kinase, Abelson (Abl), phosphorylates Eya to relocalize it from the nucleus, where it regulates eye specification at the level of transcription, to the cytoplasm, where it directs morphogenetic events as a phosphatase. We hypothesize that Abl-mediated phosphorylation of Eya facilitates interactions with cytoplasmic phosphotyrosine signaling pathways by providing docking sites for proteins containing Src Homology 2 (SH2) and/or Phosphotyrosine binding (PTB) domains. Genetic screens of SH2/PTB domain-containing genes identified several putative Eya interactors, which we are currently analyzing with further genetic tests and biochemistry. By elucidating the bases of these interactions, we hope to further our understanding of Eya's cytoplasmic function.

252C

Ligand-binding properties of the juvenile hormone receptor, Methoprene-tolerant. Marek Jindra[1], Jean-Philippe Charles[2], Thomas Iwema[3], V. Chandana Epa[4], Keiko Takaki[1], Jan Rynes[1]. 1) Biology Center ASCR, Ceske Budejovice, Czech Republic; 2) Université de Bourgogne, Dijon, France; 3) University of La Réunion, Ste Clotilde, Réunion, France; 4) CSIRO, Parkville, Victoria, Australia.

Juvenile hormone (JH) is a sesquiterpenoid of vital importance for insect metamorphosis and reproduction. The molecular basis of JH signaling remains obscure, as a bona fide JH receptor has not yet been identified. Mounting evidence points to the bHLH-PAS protein, Methoprene-tolerant (Met), as the best JH receptor candidate. Met was discovered in *Drosophila* through a genetic screen for resistance to the JH mimic methoprene, and was shown to bind JH. In *Drosophila*, Met acts redundantly with its paralog, encoded by the *germ-cell expressed* (*gce*) gene. Flies lacking either Met or Gce are viable and fertile; only the double-mutants die as pupae. We have previously shown that loss of the single *Met* gene in the beetle *Tribolium* causes larvae to metamorphose precociously, i.e. before reaching their final instar. Such a phenotype is typical for loss of JH itself, therefore supporting the idea that Met could indeed be the elusive JH receptor. However, details of how Met transduces the hormonal signal have been missing. Here, we demonstrate that Met specifically binds juvenile hormone (JH III) and its biologically active mimics, methoprene and pyriproxyfen, through its carboxy-terminal PAS domain. Substitutions of individual amino acids, predicted to form a hydrophobic ligand-binding pocket, with residues possessing bulkier side chains reduce JH III binding, likely due to steric hindrance. While a mutation that completely abolishes JH III binding does not affect a Met-Met complex that forms in the absence of methoprene, it prevents both the ligand-dependent dissociation of the Met-Met dimer and the ligand-dependent interaction of Met with its partner bHLH-PAS protein Taiman. These results show that Met can sense the JH signal through direct, specific binding of the hormone, thus establishing a new class of intracellular hormone receptors.

253A

PKA upstream factors regulating autophagy in *Drosophila melanogaster*. JUNG KIM, THOMAS NEUFELD. GENETICS, CELL BIOLOGY AND DEVELOPMENT, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MN.

Autophagy is a self-eating process which degrades cytoplasmic organelles or proteins to produce an alternative source of nutrients under starved conditions. Recent studies showed that cAMP-dependant protein kinase (PKA) plays a significant role in autophagy. Here, we tested G proteins, G protein coupled receptors (GPCRs) and adenylate cyclases which are upstream factors of the PKA signaling pathway, and 12 RNAi lines of these factors affected autophagic phenotype in Drosophila melanogaster. One of screened candidate is adipokinetic hormone receptor (AKHR), which regulates lipid and carbohydrate homeostasis, and we hypothesize that down regulation of AKHR under starved conditions induces autophagy via PKA in D. melanogaster.

254B

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

One of the key characteristics of neural progenitors is their ability to produce multiple neuron types in the course of an individual's development. Specialized neurons derive at specific times, suggesting that temporal codes act together with lineage neuronal cell fate determinants. Hormones are great candidates for this type of regulation, since they are systemic factors that regulate all major developmental steps. Intriguingly, neuroblasts in mushroom bodies continue to divide after all other neuroblasts have ceased their division and similar to mammalian neural progenitors have the capacity to generate many closely related neurons. We show that in the post-embryonic Drosophila brain, steroids act as temporal cues, which specify cell fate determination of mushroom body neuroblast progeny. Chronological regulation of neurogenesis additionally is refined by miRNA let-7, absence of which causes morphological and physiological defects, leading to learning and memory impediment. let-7 is expressed in response to developmentally regulated steroid pulses to modulate levels of cell adhesion molecules in differentiating neurons via the transcription factor Abrupt and possibly JAK/STAT cytokine signaling. The differential adhesion hypothesis explains how neurons that express different levels of cell adhesion proteins cluster together and form complex internal brain structures. Taken together, our data show that miRNA let-7 is a steroid hormone-dependent cell fate determinant acting as a temporal code along with spatially controlled lineage cues to specify neuronal cell fate via regulation of cell adhesion. Identifying the modulators of temporal codes and the mechanisms of their actions will help to understand how neuronal multiplicity takes place and aid in overcoming age-related obstacles of regenerative therapies that attempt directed neurogenesis.

255C

ULTImate Yeast Two-Hybrid: From High Quality Protein Interaction Mapping to Single Chain Antibody Analysis. Philippe le Clerc[1], Stephanie Miserey-Lenkei[2], Ole Vielemeyer[2], Petra Tafelmeyer[1], Franck Perez[2], Arnaud Echard[2,3], Bruno Goud[2], Jacques Camonis[2], Etienne Formstecher[1], Jean-Christophe Rain[1]. 1) Hybrigenics Services SAS, Paris, France; 2) Institut Curie, Paris, France; 3) Institut Pasteur, Paris, France.

Protein interaction mapping has proven instrumental for the understanding of signaling pathways in Homo sapiens, Drosophila melanogaster and other model organisms. We have published a Drosophila protein interaction map centered on cancer-related and signaling proteins (http://pim.hybrigenics.com/). These data were obtained using a yeast two-hybrid (Y2H)-based technology and a highly complex D. melanogaster embryo cDNA library. We have now applied our domain-based strategy to construct four new high-complexity, random-primed D. melanogaster cDNA libraries from adult head, ovaries, 3rd instar larvae and larvae brain. These libraries are accessible on a fee-for-service basis and will allow deepening the understanding of molecular pathways in Drosophila. Here we illustrate our approach with results obtained in an exhaustive Y2H screen using active Drosophila Rab6 (Q71L) as bait against our Drosophila embryo cDNA library. We identified zipper, the Drosophila non-muscle myosin II heavy chain gene as prey and show evidence for the role of this interaction in vesicle biogenesis and transport. We further characterized using the Y2H technique a conformation-specific antibody selected against human Rab6 (scFv AA2) that exclusively binds the activated form of the protein. For this purpose, we screened our cDNA libraries from human placenta and Drosophila embryos with AA2 as bait. In both screens Rab6 was identified, indicating that the antibody selected against the human protein can also recognize the fly Rab6. Only fragments mostly spanning the entire ORF of Rab6 were found, very likely because the entire core region of Rab6 had to be expressed to allow the binding of this conformation specific antibody. Interestingly, AA2 was also able to act as intrabody, as shown by labeling of Golgi stacks in living Drosophila S2 cells.

256A

Patterning the Drosophila eggshell along two axes by the glypican Dally. David J. Lemon[1], Nir Yakoby[1,2]. 1) Biology Department, Rutgers University, Camden, NJ; 2) Center for Computational and Integrative Biology, Rutgers University, Camden, NJ.

Heparin sulfate proteoglycans (HSPGs) participate in the regulation of numerous cell signaling pathways in tissues throughout animal development. In Drosophila melanogaster, the HSPG Division abnormally delayed (Dally) acts as a co-receptor in several signaling pathways, including bone morphogenetic protein (BMP) signaling, during imaginal wing disc development. During oogenesis, we found that *dally* is patterned in the follicle cells (FCs), a mono-layer of epithelial cells which surround the oocyte. This pattern is evolutionary conserved across species, and spatially overlaps the BMP signaling domain, which was monitored by phosphorylated-MAD (P-MAD). Using genetic perturbations, we determined that, in the FCs, *dally* is a downstream target of BMP signaling. Furthermore, in clones of cells null for *dally*, P-MAD was lost cell autonomously. Another critical regulator of egg development is the epidermal growth factor receptor (EGFR) signaling pathway. It was reported that ectopic expression of a similar HSPG, Dally-like protein (Dlp), modified the EGFR activation by changing the TGF-α like ligand Gurken (GRK) distribution. Overexpression of *dally* had no effect on eggshell patterning; however, depletion of *dally* gives rise to deformed eggshell structures due to the disruption of EGFR activation gradient. Notably, the two dorsal appendages (DAs), the embryo's respirators, were fused. This phenotype is consistent with reduction in EGFR activation. Furthermore, in these perturbations, we found a reduced operculum size, which reflects reduction in levels of BMP signaling. Based upon our results, we propose a model by which Dally contributes to eggshell patterning along the anterior-posterior and dorsal-ventral axes by regulating the BMP and EGFR signaling pathways, respectively.

257B

New roles for the Elmo-Moleskin complex in muscle-tendon attachment. Ze (Cindy) Liu, Erika R. Geisbrecht. School of Biological Science , University of Missouri-Kansas City, Kansas City , MO.

The Mbc-Elmo signaling pathway is highly conserved from C. elegans to vertebrates and is essential for many developmental processes, including phagocytosis and cell migration. Flies that possess mutations in the elmo/ced12 locus are lethal and exhibit defects in myoblast fusion, thorax closure, and border cell migration. Herein, using mass spectrometry approaches to identify new players in the Elmo signaling pathway, we uncovered Drosophila Importin-7 (Dim-7), or Moleskin (Msk) as a potential Elmo-interacting protein. While the canonical role of Msk is in nuclear import, we recently uncovered a new function for Msk in late myogenesis. We showed that Msk is enriched at muscle attachment sites and msk mutant embryos exhibit muscle attachment defects. Many known components of the muscle-tendon attachment sites are properly localized in msk mutant embryos, including the integrins, integrin associated proteins, and extracellular matrix proteins. However, the tendon cell differentiation factor Stripe and activated MAPK, are missing from the

tendon cells. Rescue experiments demonstrate that Msk is required in the muscle cells to influence tendon cell markers. Furthermore, we can rescue the muscle detachment phenotype in msk mutants upon reintroduction of the muscle-secreted tendon signaling factor Vein or by expressing an activated form of MAPK in the tendon cells. The above data support a model whereby Msk functions in the muscle cells to modulate the activity of the Vein-EGFR signaling pathway essential for tendon cell differentiation and subsequent MTJ formation. We will present studies focused on establishing how the Elmo-Msk complex functions in the formation and maintenance of the embryonic muscle-tendon attachment sites. Our preliminary data suggests that the msk muscle detachment phenotype is enhanced by mutations in elmo.

258C

Characterizing the role of a novel gene in the regulation Fat signaling in *Drosophila*. Robyn Rosenfeld[1,2], Helen McNeill[1,2]. 1) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; 2) Molecular Genetics, University of Toronto, Toronto, ON, Canada.

Fat is a large cadherin that plays a role in both tissue growth and planar cell polarity (PCP). How Fat regulates these cellular processes is not fully understood. Fat's involvement in tissue growth is through the Hippo (Hpo) kinase pathway, which has an important role in proliferation, apoptosis and the control of organ size in both *Drosophila* and mammals. A yeast two-hybrid screen conducted in the laboratory identified proteins that can bind the cytoplasmic domain of Fat, providing candidates for mediators of Fat-dependent growth and PCP. Using the available genome-wide RNAi libraries of transgenic flies, we knocked down the function of each gene identified through the screen in the *Drosophila* eye. Through analysis of adult eye size and shape and photoreceptor organization, a gene has been identified that when disrupted, phenocopies the specific overgrowth effects that are indicative of Hpo pathway mutations in the eye. This gene is uncharacterized and is conceptually translated into a multi-pass transmembrane protein of 450 amino acids with a conserved domain of unknown function. These findings suggest that this protein is working with Fat to regulate the Hpo pathway. We have generated a null allele through ends out gene targeting and homozygous mutant flies display male sterility and lethality phenotypes. We are currently examining the potential role this novel gene plays in Fat signaling.

259A

Dissection of an ecdysone-inducible type II transmembrane serine protease-signaling pathway in imaginal discs. Sienna M. Sartori, Cynthia Bayer, Laurence von Kalm. Department of Biology and Biomolecular Sciences Center, University of Central Florida, Orlando, FL.

The ecdysone-inducible Stubble-stubbloid (Sb-sbd) locus encodes a type II transmembrane serine protease (TTSP) required for imaginal disc and bristle morphogenesis. Extracellular Sb-sbd proteolytic activity modulates intracellular Rho1 signaling in leg and wing imaginal discs via an unknown outside-in signaling mechanism. Understanding this mechanism is of interest because signaling by vertebrate TTSPs is linked to a number of human pathologies. We have adopted multiple strategies to identify genes involved in this signaling process. A genetic screen identified the Notopleural (Np) locus as an enhancer of Sb-sbd with respect to imaginal disc morphogenesis. Np encodes a serine protease and is induced by ecdysone in leg imaginal discs. Our data indicate that Np acts downstream of Sb-sbd, raising the possibility of a proteolytic cascade/network leading to activation of Rho1. Additional strategies to identify components of the signaling pathway include analysis of deficiencies that interact with Sb-sbd and Np, and testing genes that are co-expressed with Sb-sbd and Np in imaginal discs, such as candidate cell-surface receptors that might be targets of the Sb-sbd or Np proteases.

260B

MAP3K molecular chimeras for the study of jun kinase pathway signaling specificity. Beth Stronach. Dept Micro & Mol Genetics, Univ Pittsburgh Sch Medicine, Pittsburgh, PA.

A highly diverse set of protein kinases function as early responders in the mitogen- and stress-activated protein kinase (MAPK/SAPK) signaling pathways. For instance, humans possess at least fourteen MAP3K protein kinases that activate Jun Kinase (JNK). To develop specific therapeutic interventions for human diseases linked to dysfunctional JNK signaling, a major challenge is to decipher the selective functions of these upstream kinases. In Drosophila, there are seven MAP3K family members, a few of which have been implicated, singly or in combination, in JNK-dependent stress response and development. To test whether MAP3Ks might substitute for each other and to identify protein domains that could instill selective function, we generated molecular chimeras between two MAP3K family members, Slpr and Tak1, which share 32% identity (53% sim) across the kinase domain, but otherwise do not resemble each other in domain structure or sequence. They have at least one substrate in common, the JNK kinase Hep, and activate JNK signaling in various contexts. If one key function of these proteins is to phosphorylate and activate Hep, then it follows that their kinase domains may functionally compensate for one another. We found that although the Tak1 kinase domain replacement in Slpr was sufficient to activate JNK signaling during dorsal closure, it did not compensate for wildtype Slpr in promoting adult viability, suggesting that there is intrinsic specificity of the catalytic domains, despite having the same substrate. Also, though the C-terminus of Slpr is not essential for viability, it promotes cortical enrichment of the protein. Swapping the C-terminus of Tak1 for that of Slpr did not restore proper localization, but it did not impede Slpr-dependent JNK signaling during dorsal closure. Moreover, the Tak1 C-terminus allowed the integration of chimeric proteins into a Tak1-dependent pathway downstream of Egr, a property not shared with Slpr. These results suggest that the selective deployment of a particular MAP3K can in part be attributed to their intrinsic sequence differences.

261C

Regulation of Hippo signaling by Jun kinase signaling during Drosophila wing discs regeneration and in neoplastic tumors. Gongping Sun, Kenneth Irvine. Waksman Institute of Microbiology, Rutgers, the State University of New Jersey, Piscataway, NJ.

When cells undergo apoptosis, they can stimulate the proliferation of nearby cells, a process referred to as compensatory cell proliferation. The stimulation of proliferation in response to tissue damage or removal is also central to epimorphic regeneration. The Hippo signaling pathway has emerged as an important regulator of growth during normal development and oncogenesis from Drosophila to humans. Our study focused on the role and regulation of Hippo pathway in compensatory proliferation and regeneration in Drosophila wing imaginal discs after disruption of epithelia. We found that induction of apoptosis in the Drosophila wing imaginal disc stimulates activation of the Hippo pathway transcription factor Yorkie in surviving and nearby cells, and that Yorkie is required for the ability of the wing to regenerate after genetic ablation of the wing primordia. Induction of apoptosis activates Yorkie through Jun kinase pathway, an important regulator in apoptosis and regeneration, and direct activation of Jun kinase signaling also promotes Yorkie activation in the wing disc. Our results also showed that depletion of neoplastic tumor suppressor genes, including lethal giant larvae and discs large, or activation of aPKC,

activates Yorkie through Jun kinase signaling, and that Jun kinase activation is necessary, but not sufficient, for the disruption of apical-basal polarity associated with loss of lethal giant larvae. Our observations identify Jnk signaling as a modulator of Hippo pathway activity in wing imaginal discs, and implicate Yorkie activation in compensatory cell proliferation and disc regeneration.

262A

Using *Drosophila* to Understand the Biology of Deubiquitinating Enzymes. Sokol V. Todi, Wei-ling Tsou, Kelly M. McGregor. Departments of Pharmacology & Neurology, Wayne State University School of Medicine, Detroit, MI USA.

Post-translational modification of proteins by ubiquitin regulates most cellular pathways and processes. Ubiquitin - an evolutionarily conserved 8.5 kDa protein expressed in all eukaryotic cells - modulates the interaction properties, functions, or fate of proteins to which it is conjugated. Like other types of post-translational modification, ubiquitination is reversible. Indeed, the process of deubiquitination is vital for normal cellular functions. Deubiquitination is accomplished by deubiquitinating enzymes (DUBs). While a significant body of work has elucidated the function of several DUBs, little is known about the biology of the majority of these enzymes, particularly in intact animals. Here, we describe targeted genetic screens to examine the importance of DUBs in Drosophila development and function. Through amino acid sequence analyses we identified nearly 30 fly DUBs with human orthologues that align at catalytic and non-catalytic domains. RNAi-mediated knockdown of these DUBs ubiquitously, in the nervous system, or only in fly eyes that express toxic proteins isolated several DUBs important for fly development, motility, flight and survival. Most DUB studies thus far have been conducted in yeast or mammalian cell lines. Our work establishes *Drosophila* as a versatile model organism to study the biology DUBs in an intact animal with real physiological and morphological readouts.

263B

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, 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 against Atro followed by microarray in the developing Drosophila eye discs and embryos. I have made a transgenic fly line carrying a UAS 3xFLAG Atro construct. I have shown the fly is able to express the protein in eye discs by immunoprecipitation. I also have preliminary results showing enrichment of a potential target of Atrophin in the eye discs using end point PCR.

264C

Activating transcription factor-3 regulates stem cell homeostasis in the Drosophila intestine. Jun Zhou, Anna-Lisa Boettcher, Michael Boutros. German Cancer Research Center (DKFZ), Div. Signaling and Functional Genomics and Heidelberg University, D-69120 Heidelberg.

Activating transcription factor 3 (ATF3) is a member of the CREB/ATF family of transcription factors, and its exact role in cancer progression is discussed controversially because both tumor suppressive and oncogenic effects have been described. Tissue homeostasis is controlled through stem cell renewal and differentiation of progenitor cells. The Drosophila midgut contains intestinal stem cells that could self renew and produce differentiated cells during life time and different stresses. A number of molecular pathways involved in intestinal stem cell (ISC) proliferation and differentiation has been identified in Drosophila, which are often remarkable conserved in the mammalian intestine. We have previously shown that Ras signaling regulates innate immune responses and ISC proliferation(1). Here, we have analyzed the contribution of putative downstream transcription factors. We found that RNAi knockdown of dATF3 in ISCs led to a dramatic increase in the number of esg-positive cells and promoted stem cell differentiation. Delta and Phospho-histone H3 staining confirmed the ISCs hyperproliferation phenotype. Gene regulating cell-cycle (Cyclin E) and apoptosis (Caspase 3) were highly induced in fly gut after depletion of dATF3. We are currently investigating whether dATF3 is transcriptional control of gene involved in ISCs proliferation and which pathway dATF3 interact with to maintain intestine homeostasis. (1) Ragab, A., Buechling, T., Gesellchen, V., Spirohn, K., Boettcher, A.-L., and Boutros, M. (2011). Drosophila Ras/MAPK signalling regulates innate immune responses in immune and intestinal stem cells. EMBO J 30, 1123-1136.

265A

Characterization of novel epidermal growth factor receptor target genes implicated in *Drosophila* egg and wing development. Jacquelyn Gallo, Luke Dombert, Bethany Guarilia, David Marr, Erica Naperkowski, Nicholas Sweeney, Lisa Kadlec. Dept. 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. Among the target genes currently under investigation are several genes implicated in eggshell formation and/or as part of chorion amplicons (such as *Defective Chorion-1*, CG18419 and yellow-G2), and a number of genes of unknown function (including CG11381, CG13083 and CG14309). RT-PCR has confirmed the up-regulation of several targets, as originally seen by microarray. A number of putative targets demonstrate 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 and/or wing morphogenesis. We are also using *in situ* hybridization to investigate gene expression in wing imaginal discs and to evaluate the effectiveness of our targeted RNA interference, and a neutral red uptake assay to assess vitelline membrane integrity in compromised eggshells.

266B

Identifying novel nuclear targets for MAPK/Erk2. Rona Grossman[1], Tatyana Shestkin[1], David Engelberg[2], Gerardo Jiménez[3], Ze'ev Paroush[1]. 1) Developmental Biology and Cancer Research, IMRIC, Faculty of Medicine, The Hebrew University, Jerusalem, Israel; 2) Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University, Jerusalem, Israel; 3) Institut de Biologia Molecular de Barcelona-CSIC and Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.

Receptor Tyrosine Kinase (RTK) signaling pathways control key cellular processes such as proliferation, migration and cell fate specification, in normal development and in disease. RTK pathways signal through an intracellular cascade of kinases, culminating in the phosphorylation and activation of the downstream effector kinase, MAPK/Erk2. Once active, MAPK/Erk2 enters the nucleus where it phosphorylates transcriptional regulators, thereby modifying their function. This brings about coordinated changes in gene expression profiles that are imperative for subsequent cellular decisions. Despite the critical roles played by RTK pathways in various developmental processes, to date only a few confirmed transcription factors that are directly targeted by *Drosophila* MAPK/Erk2 are known. We have, therefore, established a genome-wide proteomics screen in order to uncover new direct nuclear MAPK/Erk2 substrates. So far, our assay has identified 35 putative targets for MAPK/Erk2, some of which have been previously reported (e.g., Bicoid). For several newly selected MAPK/Erk2 targets, we have generated transgenic flies expressing unphosphorylatable as well as phosphomimetic derivatives. This approach will help validate bona fide MAPK/Erk2-regulated targets, since expression of these variants is expected to exert differential outcomes in vivo. Recognizing novel MAPK/Erk2 substrates should ultimately enhance our understanding of how RTK signaling pathways induce specific cellular responses during development.

267C

***In vivo* analysis of the Midkine/Pleiotrophin fly homologues Miple1 and Miple2.** Fredrik Hugosson[1], Camilla Sjögren[1], Ludmilla Hedlund[1], Anna Birvé[2], Ruth H Palmer[1]. 1) Department of Molecular Biology, Umeå University, Umeå, Sweden; 2) Department of Medical Bioscience, Umeå University, Umeå, Sweden.

The growth factors Midkine (MDK) and Pleiotrophin (PTN) form a family of Heparin binding proteins that have anti-apoptotic, angiogenic, mitogenic, chemoctactic and transforming activity. Midkine and Pleiotrophin have separately been reported as candidate ligands for the Receptor Tyrosine Kinase (RTK) Anaplastic Lymphoma Kinase (ALK) *in vitro* and with this in mind we set out analyse the *Drosophila* MDK/PTN homologues, named *miple1* and *miple2* and their possible role as functional ligands for the fly ALK receptor *in vivo*. *In situ* analysis show that the two genes have a interesting complementary expression to ALK during embryogenesis, with *miple1* in developing CNS and *miple2* in the endoderm. Enhancer analysis using the UAS/GAL4 system show a CNS specific expression of *miple1* in larvae and adults and a broad expression of *miple2* in mouth region,trachea ,gut and brain in larvae and adults. To analyse the function of *miple1* and *miple2 in vivo*, we have generated single deletion mutants, they are homozygous viable and lack obvious developmental defects. There is a possibility of a redundant function so we in next step generated *miple* double mutants. Unexpectedly, they also are viable. Interestingly, in over-expression experiments can Miple1 and Miple2 rescue the gut phenotype seen in mutants for the bonafide ALK ligand Jelly Belly (Jeb), and this result is dependent on ALK activity, suggesting that in some biological context can they function as ligands for ALK. We are currently analysing developmental defects seen during embryogenesis and also the effect on lack of Miple proteins in the adult fly, with focus on gut and brain function.

268A

Dynamic regulation of the transcriptional repressor Capicua by localized receptor tyrosine kinase signaling. Victoria M. Sanchez[1], Oliver Grimm[2], Yoosik Kim[1], Jordi Casanova[3], Eric Wieschaus[2], Stas Shvartsman[1]. 1) Chemical and Biological Engineering, Princeton University, Princeton, NJ; 2) Department of Molecular Biology, Princeton University,Princeton, NJ; 3) Institut de Biologia Molecular de Barcelona, Parc Cientific de Barcelona, Spain.

Receptor tyrosine kinases (RTKs), signaling through MAPK, control a wide range of biological processes, in many cases through regulation of transcription. In one mode of regulation, nuclear levels of the HMG box transcriptional repressor Capicua are reduced by RTK signaling. However, the mechanism of Capicua down-regulation by MAPK is not well understood. Making use of photoswitchable and fluorescently tagged Capicua constructs, we characterize the temporal dynamics of the nuclear Capicua gradient, as well as the effect of RTK signaling on its nucleocytoplasmic shuttling properties in the *Drosophila* embryo. Based on these results, we propose a biophysical model which contains a mobile and an immobile pool of Capicua. In this model, RTK activation regulates Capicua by primarily affecting the nuclear import and export rates of the mobile pool. Furthermore, this effect alone is sufficient to explain the formation and temporal evolution of the observed Capicua nuclear gradient in the terminal patterning of the embryo.

269B

Regulation of midgut metamorphosis via coordinated action between receptor tyrosine phosphatase Ptp52F and TER94/VCP. Abirami Santhanam[1,2,3], Guang-Chao Chen[1,2,3], Tzu-Ching Meng[1,2,3]. 1) Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan; 2) Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan; 3) Taiwan International Graduate Program, Academia Sinica, Taipei, Taiwan.

In *Drosophila*, a number of cellular processes including proliferation and differentiation are regulated by protein tyrosine phosphatases (PTPs).However, to date the mechanisms by which PTPs regulate the developmental processes remains elusive especially in case of receptor PTPs (RPTPs) which are majorly attributed to the regulation of axon guidance and synaptogenesis decisions in *Drosophila* embryos and larvae. To reveal the other potential functions we utilized systematic data mining approaches focusing on RPTP expression profiles during critical stages of development. This lead to the identification of a highly midgut enriched RPTP-the PTP52F especially in the larva-pupa transition during which the ecdysone action kicks in. Results from real-time PCR and cell based experiments confirmed RPTP52F as an ecdysone response gene. Genetic studies showed a critical role of PTP52F in midgut metamorphosis during larva pupa transition. Using a substrate-trapping strategy we identified, transitional endoplasmic reticulum ATPase94 (TER94), ortholog of human Valosin Containing Protein (VCP) as a bonafide substrate of PTP52F. Interestingly, tyrosine 800 of TER94 which is phosphorylated by Src kinase is targeted and dephosphorylated by PTP52F. We showed that PTP52F mediated dephosphorylation of TER94 could facilitate the ubiquitin mediated degradation of various proteins including *Drosophila* inhibitor of apoptosis1 (DIAP1) a key regulator controlling midgut cell death. In vivo evidences demonstrated that the forced expression of TER94 rescued the defect of midgut metamorphosis induced by knockdown of PTP52F, suggesting the

importance of coordinated action between PTP52F and TER94. Our studies for the first time reveal a novel regulatory role of a RPTP that contributes to proper tissue organization of midgut formation in *Drosophila* metamorphosis.

270C

FGF and EGF signaling pathway act together to regulate Drosophila adult muscle patterning. Kumar Vishal, Carli Calderon, Zachary Jump, Brian Gallagher, Joyce Fernandes. Dept Zoology, Maimi Univ, Oxford, OH.

The long term goal of our lab is to understand the hierarchy of interaction among motor neurons, founder cells and myoblasts that leads to fiber patterning of Indirect flight muscles of the thorax. Our preliminary studies indicate that MAP kinase is activated in the nascent fibers and myoblasts during a period of active IFM myogenesis (16-20hrs APF). Since, FGF and EGF act through canonical MAP kinase pathway; we are exploring the involvement of corresponding signaling pathway. We have expressed dominant negative receptors of FGF and EGF pathways in the founder cell and monitored fiber number. We observed a reduction in number of one groups of IFM fibers, the DLM fibers in both cases. 80% of animals with dominant negative EGFR expression have 3 or 4 fibers n=8, whereas, 50 % of animals with dominant negative FGF expression have 5 fibers, n=8) as compared to the control (100% of animals have 6 fibers, n=8). Extra fibers were observed in the IFM groups, the DVMs, 35% of the experimental animals have 4 fibers, whereas 100% of controls have 3 fibers, n= 6. The results suggest that FGF and EGF regulate adult fiber number and have different effects on DLMs and DVMs, which develop using distinct modes of myogenesis. We are currently examining muscle patterning during the 0 to 24 hrs APF 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. Additionally we will also examine MAP kinase levels under these manipulated conditions.

271A

RhoGAP68F inhibits endocytic recycling to promote epithelial elongation during metamorphosis. Beatriz Hernandez de Madrid, Lina Greenberg, Victor Hatini. Anatomy and Cell Biology, Tufts University, Boston, MA.

Epithelial elongation is a conserved morphogenetic process that contributes to the axial lengthening of embryonic and adult structures. It is mediated by polarized changes in cell shape, cell proliferation, cell death and rearrangements of cell-cell contacts. All these processes are associated with extensive remodeling of the cell cortex but the mechanisms involved remain poorly understood. The Drosophila leg imaginal disc elongates dramatically during metamorphic development to form the leg shaft from a flattened epithelial disc. We investigate the role of RhoGTPases and their regulators the RhoGEFs and RhoGAP in leg elongation owing to their role in regulating cell adhesion, vesicle transport and cytoskeletal dynamics. We have previously found that the tarsal region of adult legs depleted for RhoGAP68F are shorter than wild type and have fused joints. To understand the role of RhoGAP68F in epithelial elongation, we characterized the cell biological roles of RhoGAP68F in vivo and in S2 cells. We found that RhoGAP68F localized to Rab4 recycling endosomes and formed a complex with Rab4 and constitutively active Rab4 but not with dominant negative Rab4. Over expression of RhoGAP68F led to enlargement and clustering of Rab4 endosomes, while depletion of RhoGAP68F led to the accumulation of Rab4 endosomes near the apical surface. These phenotypes suggest that RhoGAP68F inhibits the scission and movement of Rab4 endosomes to the cell cortex. Rab4 localized preferentially with Fasciclin III (FasIII), a component of septate junctions. Depletion of RhoGAP68F blocked epithelial elongation in part by blocking the rearrangement of the thick pseudostratified epithelium to a thin simple epithelium. Our data suggest a role for Rho signaling cascades in regulating key trafficking itineraries of junctional proteins. We propose that RhoGAP68F attenuates the recycling of cargo back to septate junction to decrease these adhesive cell-cell contacts to facilitate epithelial flattening.

272B

***crinkled* reveals a new role for Wingless signaling in Drosophila denticle formation.** Amy Bejsovec, Anna T. Chao. Dept of Biology, Duke University, Durham, NC.

The specification of the body plan in vertebrates and invertebrates is controlled by a variety of cell signaling pathways, but how signaling output is translated into morphogenesis is an ongoing question. We have discovered that genetic interactions between the Wingless (Wg) signaling pathway and a nonmuscle myosin heavy chain, encoded by the *crinkled (ck)* locus play an important role in this process. In a screen for mutations that modify *wg* loss of function phenotypes, we isolated multiple independent alleles of *ck*. These *ck* mutations dramatically alter the morphology of the hook-shaped denticles that decorate the ventral surface of the *wg* mutant larval cuticle. In an otherwise wild-type background, *ck* mutations do not substantially alter denticle morphology, suggesting a specific interaction with Wg-mediated aspects of epidermal patterning. Manipulating the level of Wg pathway activity changes the structure of actin bundles during denticle formation in *ck* mutants. This Ck-dependent process is modulated by the activities of the Wg target gene, *shaven-baby (svb)*, and of its transcriptional targets, *miniature (m)* and *forked (f)*. Using a temperature sensitive *wg* allele, we find that continued Wg activity is required in *ck* mutants beyond 10 hours after egg-laying, the point at which Wg ceases to be required for patterning in a wild-type background. This suggests that the *ck* mutant background reveals a late activity of Wg signaling in controlling *svb* expression levels. We propose that the the activity of the Ck cytoplasmic myosin can somehow buffer the effects of misregulated Svb target gene products. We conclude that Ck acts in concert with Wg targets to orchestrate the proper shaping of denticles in the Drosophila embryonic epidermis.

273C

Polarized secretion of Wnt/Wg in *Drosophila* wing imaginal discs. Varun Chaudhary, Julia Gross, Michael Boutros. German Cancer Research Center (DKFZ), Division Signaling and Functional Genomics and University of Heidelberg, Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany.

Wnt/Wingless (Wg) are secreted glycoproteins that are required for variety of developmental processes, which are highly conserved from fly to human. In *Drosophila* wing imaginal discs Wg is secreted form the apical side of cells marking the dorso-ventral boundary. It is believed that Wg is then internalized from the apical side, however a broader Wg gradient is also seen on the basolateral side of the disc. Whether this Wg gradient is generated through apical to basolateral transcytosis and whether the apical secretion of Wg followed by its endocytosis is required for proper signaling has been unclear until now.

We are interested in understanding the mechanism of Wnt/Wg secretion and signaling. Through cell based RNAi screening, we have previously identified proteins for example, Evenness interrupted and Opossum (p24), which are required for proper secretion of Wg. We are now interested in finding other

factors that are required for Wg secretion in polarized cells of *Drosophila* wing imaginal discs. We are addressing this using *Drosophila in vivo* RNAi screening approaches. A focused RNAi library subset was chosen, containing genes with functions in trafficking, such as Rab-protein family member, Exocyst complex genes, retromer and others. Approximately 451 genes from this subset were knocked down in Wg producing polarized cells of the wing imaginal disc. From this subset screen we have identified various candidates, which show reduced secretion of Wg. We will discuss the current findings and their implication in polarized Wg/Wnt transport.

274A

Pebble RhoGEF acts as a negative regulator of Wg/Wnt signaling. Elisabeth R Greer, Kieran R Hendricksen, Anna T Chao, Amy Bejsovec. Dept of Biology, Duke University, Durham, NC.

Wingless/Wnt (Wg/Wnt) signaling directs cell fate decisions in developing flies and other animal species. In humans, deregulated Wnt signaling is associated with cancer; this highlights the importance of understanding how this developmental pathway is regulated. In previous work, we found that the cytokinesis proteins Tumbleweed/RacGAP50C (Tum) and Pavarotti (Pav) act as negative regulators of Wg/Wnt signaling in Drosophila embryos and in mammalian cell lines. Because Tum binds to the RhoGEF Pebble (Pbl) to organize the contractile ring during cell division, we tested whether Pbl also interacts with Tum in regulating Wg/Wnt signaling. We find that Pbl does play a role in the negative regulation of Wg/Wnt signaling, but it does so independently of its interaction with Tum. *pbl* loss of function mutant embryos show expanded expression of the Wg target gene, *engrailed*, and produce an epidermal pattern with excess naked cuticle, which correlates with Wg signaling activity. Conversely, *pbl* overexpression in the embryonic epidermis diminishes the specification of naked cuticle. Thus the *pbl* gene functions to restrict the activity of the Wg pathway. Indeed, overexpressing *pbl* or its human homolog, *Ect2*, represses Wg/Wnt target gene expression in either Drosophila or mammalian cells, indicating that this property is highly conserved. We show that the guanine nucleotide exchange factor (GEF) activity of Pbl or Ect2 is required for this regulation, while other protein domains important for cytokinesis, such as the Tum binding domain, are not. In contrast to Tum and Pav regulation of the Wnt pathway, Pbl and Ect2 do not require nuclear localization to exert their effect. Both Pbl and Ect2 function downstream of Armadillo/beta-catenin stabilization to control Wnt target gene expression, and we find evidence that Pbl acts through a Rho family GTPase to modulate Wnt signaling. These results provide new insight into possible connections between G protein regulation and Wnt pathway modulation.

275B

A developmental function of dWNK kinase in the regulation of canonical Wnt/β-catenin signaling. Andreas Jenny[1], Ekatherina Serysheva[2], Hebist Berhane[1], Kubilay Demir[3], Michael Boutros[3], Marek Mlodzik[2]. 1) Dept Molec & Dev Biol, Albert Einstein Col Med, New York, NY; 2) Dept. of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY; 3) Signaling and Functional Genomics, German Cancer Research Center, Heidelberg, Germany.

During vertebrate development major inductive and morphogenetic events pattern and shape the embryo. For example, canonical Wnt signaling is necessary to specify the embryonic dorsoventral axis. Since D/V axis formation is intrinsically linked to establishment of the anteroposterior axis, Wnt signaling is essential for the three-dimensional vertebrate body plan including the formation and positioning of organs. Furthermore, aberrant Wnt signaling can lead to severe developmental disabilities such as heart abnormalities, ablation of the forebrain, and a variety of cancers. The canonical Wnt/β-Catenin pathway is highly conserved from invertebrates to humans. In *Drosophila*, canonical Wnt signaling is required for segmentation and for the formation of adult structures such as wings. A conserved and relatively upstream readout for Wnt pathway activation is phosphorylation of Dishevelled (Dsh), the major adapter protein of Wnt signaling. We performed a systematic RNAi screen to knock-down all *Drosophila* kinases in cell culture and identified previously known and novel kinases affecting Dsh phosphorylation. In particular, our data show that the single fly ortholog of the conserved Wnk (With No Lysine [K]) kinase family, dWNK (CG7177) modulates peak levels of canonical Wnt/β-catenin signaling. A reduction of *dwnk* activity suppresses overactivation of Wnt signaling and dwnk mutations cause wing margin defects due to a requirement for dWnk for the activation of high threshold targets of canonical Wnt signaling *in vivo*. Human Wnks regulate ion transport and thus cell volume in the kidney and brain, and mutations of Wnk1/4 are associated with Gordon's syndrome (PHAII). Our studies identified an unappreciated and novel role for Wnk in early development and suggest that Wnks potentially have functions beyond the regulation of ion homeostasis.

276C

Role of Wingless in pigment rim formation. Sudha R Kumar, Hinaben Patel, Andrew Tomlinson. Department of Genetics & Development, Columbia University, NY.

The eye of the fly is an ideal tissue to understand how a morphogen gradient can direct cells to various fates. Here, Wingless (Wg) diffusing into the developing eye from the circumscribing head capsule directs the formation of three peripheral retinal specializations, each with a specific threshold response to Wg concentration. The lowest threshold response denudes the ommatidia by removing the bristles. The intermediate response directs the formation of the dorsal rim ommatidia (plane polarized light detectors). The highest threshold response directs the formation of the pigment rim, a thick band of pigment cells that circumscribes the eye and optically insulates it from extraneous light rays. We are focused on how the pigment rim is formed. The outermost ommatidial row dies, leaving the surrounding pigment cells to coalesce to form the pigment rim. In the moribund ommatidia the cone cells respond to the high levels of Wg signaling by activating expression of Wg itself, along with Snail family transcription factors. Shortly afterwards, these ommatidia collapse and undergo apoptosis. Here, we ask whether the expression of these proteins in the cone cells is sufficient for the collapse and death of the ommatidia, and the nature of the death signal that directs this apoptosis. Activation of high levels of Wg throughout the developing eye converts the whole eye into pigment rim. Timed developmental analysis showed that the death of the ommatidia occurred throughout the eye in a similar manner to that which occurs at the periphery. Thus we can use normal ommatidia found throughout the eye as models for testing which gene expressions, in which cells, can mimic high levels of Wg signaling and induce the collapse and apoptosis. We are conducting experiments that express Snail family transcription factors, with and without the activation of the Wg pathway, in the cone cells to determine which aspects of pigment rim formation are regulated by these expressions. We are also investigating other signaling pathways to gain a better insight into the death cascade triggered in response to Wg.

277A

Characterisation of the trafficking route taken by Wingless in secreting cells. Lucy Palmer, Cyrille Alexandre, Karen Beckett, Jean-Paul Vincent. Developmental Biology, NIMR, London, United Kingdom.

Wingless (Wg) is the major *Drosophila* Wnt and is important for patterning, growth and cell survival during development. Wg is produced in a stripe of cells in the *Drosophila* wing imaginal disc and spreads from these secreting cells to form a gradient. Total staining (intra and extracellular) shows that Wingless is localized at the apical surface of secreting cells, suggesting that Wingless could be secreted on the apical surface. However, extracellular staining reveals that the Wingless gradient forms at the basal surface of receiving cell. There is as yet no direct proof that Wingless is indeed secreted on the apical surface and if it is, as expected, the mechanism underlying apical to basal transfer remains unknown. It is also not known how Wingless spreads along the disc surface, despite carrying lipid modifications. We are focusing on Wingless trafficking within secreting cells to address these questions. Within secreting cells Wg associates with Evi/Wntless which is required for its secretion. It is thought that Wg joins Evi in the Golgi and that Evi is required to transport Wg to the plasma membrane. The route Wg and Evi take in Wg-producing cells is not fully understood and we plan to characterize by high-resolution microscopy. To this end we have constructed Bacterial Artificial Chromosomes (BACs) that express tagged forms of Wingless and Evi at endogenous levels. These BACs rescue wg or evi mutants. In addition, we have designed a method to produce a step of Wg within secreting cells. By fixing at defined time points we will be able to visualize its movement through the secretory pathway, and determine where and when it joins and separates from Evi. Taken together these approaches will allow us to define the secretory route that Wg takes out of the cell to form the gradient in the wing imaginal discs.

278B

The microRNA-310/13 cluster antagonizes β-catenin function in *Drosophila*. Felix Peng[1], Raluca Pancratov[1], Peter Smibert[2], Jr-Shiuan Yang[2], Emily R Olson[1], Ciaran Guha-Gilford[1], Eric C Lai[2], Ramanuj DasGupta[1]. 1) New York University School of Medicine, Department of Pharmacology and the NYU Cancer Institute; 522 First Ave., SRB #1211, New York NY 10016; 2) Sloan-Kettering Institute, Department of Developmental Biology, 1275 York Ave Box 252, New York NY 10065.

microRNAs (miRs) are important regulators of global gene expression, and function in regulating a broad range of biological processes. We identified the miR-310/13 cluster as part of a comprehensive cell-based screen for Drosophila microRNAs involved in regulating the activity of the evolutionarily conserved Wnt/wingless (wg) signalling pathway. We demonstrate that this evolutionarily conserved cluster can directly target the 3'UTR of β-catenin/Armadillo (arm) and dTCF in Drosophila cells. Overexpression of miR-310/13 phenocopies a loss of Wg signalling in Drosophila imaginal discs. We observed reduced fertility among males lacking miR310/13. Subsequent examination of mutant testes revealed an abnormal clustering of germ cells. This mutant phenotype can be rescued by reducing Arm activity in both the germ and somatic lineage of the Drosophila testis, thus implicating a previously unrecognised function for Wg/Arm in stem cell regulation in the Drosophila testis.

279C

Screening the *Drosophila* kinome and phosphatome *in vivo* to identify novel regulators of the Wnt/Wg signaling pathway. Tirthadipa Pradhan, Sharan Swarup, Esther Verheyen. SSB7152,MBB, Simon Fraser Unversity, Burnaby, BC.

The Wnt/Wg is an evolutionary conserved signalling pathway in metazoans, which regulates 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, 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 pathway. The key components such as Armadillo, Dishevelled, Arrow, APC, Axin and TCF are phosphorylated in the pathway. The ubiquitous kinases GSK-3β and CKIα regulate multiple steps of the pathway by distinct and opposing mechanisms. Other kinases such as Nemo, Hipk and lipid kinases (PI4KIIα, PIP5K1β) and phosphatases such as PP1 and PP2 were also found to regulate different aspects of this pathway. Although the Wnt pathway is regulated by numerous phosphorylation events, the significance of most of these events is not well understood. To fill the gap in our knowledge we are in the process of performing an *in vivo* kinome and phosphatome RNAi screen in the *Drosophila* third instar wing disc to identify novel regulators of the Wnt pathway. Our primary screen has yielded a number of potential novel regulators of the Wnt pathway. Preliminary characterization of the one of the phosphatases by loss of function and O/A analysis revealed its role in Wnt secretion. We are in the process of performing further genetic interaction studies with the members of Wnt secretion machinery. Further characterization of the hits fond in our screen will help us to understand the Wnt/Wg pathway as a whole and how this pathway regulates different aspects of development and diseases in different organisms.

280A

The destruction complex in the Wnt pathway: APC's mechanism of action in βcatenin degradation. Mira I. Pronobis[1], David M. Roberts[2], John S. Poulton[1], Mark Peifer[1]. 1) Biology, UNC, Chapel Hill, NC; 2) Biology, F&M, Lancaster, PA.

The canonical Wnt signaling pathway controls cell proliferation and cell fate choices, and is regulated by the protein levels of βcatenin, a transcriptional co-activator. The tumor suppressor APC acts in the destruction complex together with Axin, GSK3 and casein kinase to target βcatenin for degradation in the proteasome. However, APC's role in the destruction complex remains unclear. We tested APC's mechanism of action using both Drosophila embryos and colon cancer cells. Current models suggest that APC's high-affinity βcatenin-binding sites are essential for βcatenin degradation. However, our findings show that high-affinity βcatenin-binding sites are fully dispensable for down regulation of βcatenin in both colon cancer cells and Drosophila, and that multiple binding sites act additively to fine tune Wnt signaling via βcatenin sequestration. The SAMP motifs of APC were defined as the binding sites for Axin. However, our study identified a novel SAMP-independent Axin interaction site, which we mapped to APC's Armadillo repeats (Arm rpts). We also found 2 additional sites in APC, 20-amino-acid repeat 2 (20R2) and conserved region B, which are essential for βcatenin degradation in colon cancer cells and Drosophila. Our data suggest they help to release APC's Arm rpts from Axin and that this mechanism of disassembly is critical to target βcatenin for degradation, as part of an essential catalytic cycle. We hypothesize that the Arm rpts and 20R2/conserved region B of APC act as binding sites for yet unknown proteins. Using mass spec analysis, we are screening for candidates that are involved in this catalytic cycle of the destruction complex. Since colon tumors invariably express truncated APC proteins that retain the Arm rpts, we hypothesize that truncated APCs can influence the destruction complex by acting on Axin. To test this, we are performing live cell imaging and FRAP analysis to elucidate dynamic features of the destruction complex. Our study will

provide new insights into how the destruction complex down regulates Wnt signaling in development and disease.

281B

Frizzled 2 is critical for the regulation of vitellogenesis in the mosquito Aedes aegypti. Shin-Hong Shiao. Department of Parasitology, National Taiwan University, Taipei, Taipei, Taiwan.

Mosquito-borne diseases are the most devastating agents for human being, due to its high diversity of transmissible pathogens like protozoan and viruses. Despite the efforts from government agencies that have contributed the eradication of the mosquito-borne diseases for several decades, the goal has not been achieved yet. Therefore, many research institutes turn their attentions toward the mosquito life cycle and immune system to halt the disease transmission. Previous studies have already demonstrated that Target of Rapamycin (TOR) pathway plays an important role in mosquito vitellogenesis, whereas WNT pathway participates in the embryonic development and cell polarity. However, the interactions between these pathways are poorly understood. In this study, we propose a hypothesis that factors of TOR and WNT signaling pathway play synergistically in the mosquito vitelloginesis. We attempt to characterize WNT signaling components in the mosquito, Aedes aegypti. Our results showed that silencing of Frizzled2 (FZ2), a transmembrane receptor of WNT signaling pathway, and TOR resulted in the decrease of Aedes aegypti survival fitness against S. aureus and E. coli infection. Interestingly, the oviposition ability has been altered in the absence of FZ2. Also, we demonstrated that FZ2 is highly expressed in the mosquito fat body at 6 hours post blood meal in turns of transcriptional and translational level, suggesting the amino acid-stimulated feature of FZ2. The transcriptional expression of TOR is reduced in the absence of key components in the WNT pathway. Our results showed that Frizzled 2 is critical in the regulation of mosquito vitellogenesis.

282C

A screen for mutations that affect Drosophila eye development identifies new regulators of signaling pathways. Annabelle Y.T. Suisse[1], Josepha Steinhauer[1,2], Jessica E. Treisman[1]. 1) Developmental Genetics, Skirball Institute of Biomolecular Medicine, New York, NY; 2) Department of Biology, Yeshiva University, New York, NY.

The *Drosophila* eye provides a remarkable system to study the processes of cell growth, differentiation, and death that drive tissue specification and morphogenesis. All these processes are governed by cell signaling pathways that are highly conserved among vertebrates and invertebrates. We have carried out a mosaic genetic screen to discover genes required for the normal pattern of photoreceptor differentiation. The genes characterized so far include components of the Hedgehog (Hh), Wingless (Wg), Epidermal Growth Factor Receptor (EGFR) and Notch signaling pathways. We are currently characterizing additional mutations that appear to affect these signaling pathways. One such mutation, *7D9*, completely prevents differentiation of photoreceptors. Target genes of both the Wg and Hh pathways are ectopically activated in the mutant clones. Moreover, the mutant cells are enlarged. Another complementation group consisting of two alleles is required for normal expression levels of the neuronal nuclear protein Elav and the Hh target gene *decapentaplegic*, but does not affect *patched*, another Hh target gene. Cells homozygous for these mutations also strongly upregulate the EGFR target gene *argos*. Identifying the genes affected by these mutations and determining their molecular mechanisms of action will improve our understanding of the functions of signaling pathways that act in eye disc patterning, and the interactions between them.

283A

Role of kinesin II - Armadillo interaction in Wingless signaling pathway. Linh Thuong Vuong, Kwang Wook Choi. Department of Biological Sciences, KAIST, Graduate School of Nanoscience and Technology, Daejeon, Korea.

The *Drosophila* kinesin II motor subunit encoded by Klp64D is involved in the axonal transport of choline acetyltransferase and the formation of chordotonal sensory cilia (*Sarpal et al., 2003*). Our previous study revealed a new role for Klp64D in the localization of adherens junction (AJ) proteins, Armadillo (Arm) and Bazooka (Baz), during photoreceptor morphogenesis in the eye (*Mukhopadhyay et al., 2010*). Since Arm is an important component in Wingless (Wg) signaling pathway, we tested whether Klp64D is involved in Wg signaling. Here we show that Arm forms a protein complex with Klp64D by binding to the motor's cargo domain. To study the function of this interaction, we tested whether Klp64D is required for Wg - Arm signaling in the wing imaginal disc that is critical for the wing outgrowth. We show that clonal loss of Klp64D in wing discs causes a reduced Wg expression at the dorsoventral border. Further, loss of Klp64D function in the wing resulted in defective wing margin bristles and notching of the wing. We also provide evidence for strong genetic interaction between Klp64D and Arm in the wing and eye. Taken together, our data suggests that kinesin II - dependent transport Arm is required for Wg signaling.

284B

The Role of Cyclin B3 in Drosophila Female Meiosis. Mohammed R Bourouh, Rajdeep Dhaliwal, Andrew Swan. Biological Sciences, University of Windsor, Windsor, Ontario, Canada.

The meiotic cell cycle is a highly regulated cell division yielding four genetically different gametes. As in mitosis, the meiotic cell cycle is regulated by Cyclin Dependent Kinases (CDKs). CDKs are activated when bound to their cyclin partners. The type of cyclin bound confers the substrate specificity of the CDK, but some redundancies exist within cyclin families. In Drosophila, there are three major mitotic cyclins, Cyclin A, B, and B3, whose role in female meiosis has yet to be characterized. Our research focuses on the characterization of Cyclin B3 in female meiosis. By studying the loss of function phenotype of Cyclin B3, we found that mutants arrest in anaphase of meiosis I or II. Cyclin B3 null mutants also arrest with elevated levels of Cyclin A and B. Interestingly, expressing a stable form of Cyclin B3 causes reduced levels of the mitotic cyclins. These results suggest that cyclin B3 could play a role in APC activation. Furthermore, expressing the stable form of Cyclin B3 causes dramatic microtubule polymerization near the meiotic spindle, as well as near the cortex of the oocyte, though interestingly not near the male pronucleus. In wild type Drosophila, the mature oocyte arrests in metaphase of meiosis I, with microtubules forming a network along the cortex of the oocyte. Following egg activation through ovulation, the APC is activated allowing progression of meiosis, and the cortical microtubule network is broken down. Given our results, we hypothesize that Cyclin B3 functions after egg activation as both an APC activator, and a regulator of microtubule dynamics.

285C

Checkpoint defects reveal specific requirements for T14 and Y15-mediated Cdk1 inhibitory phosphorylation during Drosophila development. Joseph O Ayeni[1], Oindrila Mukherjee[1], Ramya Varadarajan[1], David T Stuart[2], Frank Sprenger[3], Shelagh Campbell[1]. 1) Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada; 2) Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada; 3) Institute for Biochemistry, Genetic and Microbiology, NF III, University of Regensburg, 93049 Regensburg, Germany.

During animal development, cell division is dynamically regulated by mechanisms that spatially and temporally control the mitotic regulatory kinase Cdk1. The activity of Cdk1 is controlled by inhibitory kinases (Wee1 and Myt1) and Cdc25 phosphatases that regulate the phosphorylation state of two Cdk1 residues: Y15 and T14. Both Wee1 and Myt1 target Y15 whereas only Myt1 can phosphorylate both residues, producing three distinct inhibitory isoforms. The relevance of different phospho-isoforms catalyzed by the two Cdk1 inhibitory kinases remains unclear, however. To address this question we examined the developmental significance of Y15 and/or T14 phosphorylation of Cdk1 by expressing Cdk1-VFP phospho-inhibition mutants in wing imaginal discs and neuroblasts. Expression of completely non-inhibitable Cdk1 caused chromosomal aberrations and cellular defects, resulting in severe developmental defects. Despite T14 and Y15 phosphorylation having similar inhibitory effects on Cdk1 catalytic activity, mutants affecting these residues produced distinct cellular effects when expressed. Notably, we found that Y15 phosphorylation of Cdk1 is necessary and sufficient for checkpoint-mediated G2 phase arrest. Although Myt1-mediated T14 phosphorylation of Cdk1 was neither necessary nor sufficient for checkpoint arrest, this modification could prevent genome instability associated with completely non-inhibitable Cdk1. This role may underlie some of the unique developmental functions ascribed to Myt1 kinases in Drosophila, C. elegans, X. laevis and M. muluscus. Our study has therefore provided new insights into conserved Cdk1 regulatory mechanisms that coordinate cell cycle progression with development processes.

286A

Examination of Arf1 GTPase activity on mitotic events in early *Drosophila* embryos. Rabab Khodary, Blake Riggs. San Francisco State University ,1600 Holloway Avenue, San Francisco , CA, 94132.

An important aspect of cellular life is the process of cell division and the equal segregation of the genetic material. Crucial to this process, is the assembly of a microtubule based structure known as the mitotic spindle, which attaches to and segregates the chromosomes to the newly formed daughter cells. There has been evidence suggesting that Golgi-associated factors including the small GTPase Arf1 , may play a role in mitotic spindle function. However, the molecular mechanism involved in Golgi membrane factors and mitotic spindle function is poorly understood. We propose that Arf1 GTPase activity plays a role in mitotic spindle function. To examine Arf1 activity, we injected the small molecule inhibitor of Arf1 activity, Brefeldin A (BFA) into the early syncytial blastoderm of *Drosophila melanogaster* embryos. Using live fluorescent analysis, BFA was injected into embryos expressing GFP-tubulin / RFP-Histone prior to entry into mitosis and examined for effects on mitotic spindle assembly and chromosome segregation. We observed defects in spindle sizing and positioning and a delay in mitotic progression. In addition, injections of BFA into embryos containing a fluorescent marker for the spindle assembly checkpoint (SAC) showed that this delay is not due to activation of the SAC. Taken together, this suggests that Arf1 activity plays a specific role in proper spindle positioning. In addition, we plan to examine Arf1 activity by double stranded RNA inhibition of Arf1 in the early embryo, followed by injection of recombinant Arf1 mutants. These experiments will allow for a detailed examination of changes in Arf1 activity on the effects of mitotic spindle function.

287B

Role of SCF^{Skp2} in Maintaining Genome Stability. Biju Vasavan, Nilanjana Das, Andrew Swan. Biological Sciences, University of Windsor, Windsor, Ontario.

Polyploidy and genetic instability is characteristic of cancer. Overexpression of Skp2, an F box protein for the SCF-type E3 ligase has been observed in many types of cancer. Skp2 negatively regulates the cell cycle by ubiquitilating key cell cycle regulators and recognizes its substrates with the help of a Cdk (cyclin dependent kinase) interacting protein Cks. We have generated null alleles of Skp2 and Cks1 (Cks85A) in Drosophila and have noticed that loss of either gene results in polyploidy and genetic instability. This indicates that Skp2 has a potential role as a tumour suppressor. Additionally, we see abnormal accumulation of cells in prometaphase and metapahase, which could be a result of mitotic arrest or delay due to activation of the spindle assembly checkpoint. We are trying to determine if this is the cause or consequence of polyploidy. Previous studies in human cell lines have shown that Skp2 protects Cyclin A/Cdk from possible inhibition by p27, an inhibitor of Cdk. We are currently exploring the genetic and physical interaction between Skp2 and Cyclin A in preventing polyploidy in Drosophila. Deciphering the mechanisms by which SCF^{Skp2} prevents genetic instability and tumoriogenesis will be helpful in future therapeutic research targeting cancer.

288C

Characterization of Caprin Phosphorylation at the mid-blastula transition. Xi Chen, Ophelia Papoulas. The Section of Molecular Cell and Developmental Biology, The University of Texas at Austin, Austin, TX.

The molecular signals driving the developmental shift referred to as the mid-blastula transition (MBT) remain mysterious. In the syncytial *Drosophila* embryo, rapid synchronous nuclear divisions cease at the MBT to permit membrane invagination and formation of a cellular blastoderm. We have reported that the RNA-binding translational regulators Fragile X mental retardation protein (FMRP) and Cytoplasmic activation/proliferation-associated protein (Caprin) act at the MBT to modulate levels of cell cycle regulators necessary for this prolonged interphase. FMRP has been most extensively studied in the nervous system because loss of FMRP causes Fragile X Syndrome, the most common form of heritable human mental retardation and autism. However both FMRP and Caprin (CAPR) are believed to mediate rapid local changes in translation in response to synaptic signals in neuronal dendrites. We find that both proteins are present throughout early embryogenesis but act specifically at the MBT suggesting they may be responding to specific signaling at that time. The nucleo-cytoplasmic ratio has long been viewed as a key signal triggering events of the MBT, but the molecular nature of the signal is unknown. Through immunoblotting of precisely staged embryos we have found that CAPR becomes phosphorylated specifically at the MBT. We are characterizing the timing and sites of phosphorylation using GST-fusions comprising thirds of the CAPR protein and a staged embryo cell extract-based in vitro kinase assay. Preliminary data suggest that the middle portion of CAPR, containing the conserved G3BP/Rasputin binding domain, is specifically phosphorylated. Current work is aimed at characterizing the functional significance of this modification, and identifying the specific residues modified and the kinase responsible. Through these studies we hope to better understand signal-responsive control of translation and the signaling mechanisms underlying the MBT.

289A

***mu2* affects mitosis and meiosis by regulating BubR1 expression in *Drosophila melanogaster*.** James M. Mason[1], Raghuvar Dronamraju[1,2]. 1) Laboratory of Molecular Genetics, NIH/NIEHS, Research Triangle Park, NC; 2) Department of Biochemistry and Biophysics, UNC, Chapel Hill, NC.

The molecular components that decide the development of an oocyte are largely uncharacterized. The *mu2* gene of *Drosophila melanogaster* encodes a chromatin protein found in the oocyte nucleus that acts as a scaffold during DNA repair to elicit a DNA damage response and meiotic recombination in oocytes. $mu2^a$ mutant females delay the repair of radiation induced chromosome breaks in oocytes. Accurate segregation of chromosomes during cell division requires organized centromeres and telomeres, which when defective activate the spindle assembly checkpoint. Using immunohistochemistry we show that $mu2^a$ mutants exhibit defective inner and outer kinetochore components, such as CIN and BubR1, during oocyte development. These defects may lead to other observed phenotypes, such as delayed maturation of the pro-oocyte produced by a mutant mother, aneuploidy in the resulting zygote, asynchronous mitosis in the early embryo, and an increase in the number of pole cells later in embryogenesis. MU2 is likely a non-essential downstream effecter protein in cell cycle control, checkpoint activation and DNA repair processes.

290B

Distinct roles for multiple translesion polymerases during DNA double-strand break repair. Mitch McVey[1], Daniel P Kane[1], Michael Shusterman[1], Kelly Beagan[1], Yikang Rong[2]. 1) Biology, Tufts University, Medford, MA; 2) Laboratory of Biochemistry and Molecular Biology, National Cancer Institute, Bethesda, MD.

DNA double-strand breaks, when repaired inaccurately, can promote mutagenesis in the form of point mutations, deletions, and genome rearrangements. Historically, DNA translesion polymerases have been associated with mutagenesis during lesion bypass and postreplication repair. However, their role(s) during DNA double-strand break repair are poorly defined, particularly in metazoans. To address this, we carried out a systematic genetic analysis of DNA polymerase mutants in *Drosophila melanogaster*. We generated stocks with null mutations in genes encoding polymerases eta, zeta, theta, Rev1, and the nonessential Pol32 subunit of polymerase delta. Using mutagen sensitivity analysis and two independent site-specific break repair assays, we showed that translesion DNA polymerases eta and zeta are both involved in homologous recombination repair of DNA breaks. Furthermore, Pol32 is required for extensive DNA synthesis during double-strand gap repair. Flies lacking both Pol32 and polymerase zeta have extreme defects in repair synthesis, indicating that these polymerases may operate in separate stages of gap repair. Interestingly, rev1 mutants display an enhanced ability to carry out gap repair and have increased repair synthesis tract lengths. In cases where homologous recombination aborts prematurely, polymerase theta functions in an alternative end joining repair mechanism, independent of DNA ligase 4.

Based on these findings, we propose a model in which replicative and translesion polymerases compete for access to D-loop intermediates during homologous recombination repair. Rev1 appears to be an important mediator during gap repair and may promote the recruitment of translesion polymerases during the early stages of repair synthesis. Together, our results suggest surprising complexity in the enzymology of DNA synthesis during double-strand break repair.

291C

Establishing linkage between GINS complex sub-unit Sld5 and checkpoint protein Chk2*(loki)* using *Drosophila melanogaster* as the model organism. Divya Devadasan, Tim Christensen. East Carolina University, Greenville, NC.

Eukaryotic DNA replication is controlled by a number of proteins that ensures the process takes place accurately. GINS, a hetero-tetrameric protein complex is known to be essential for the initiation and progression of eukaryotic DNA replication. The GINS complex constitutes four subunits; Sld5, Psf1, Psf2, Psf3. The Sld5 subunit of GINS is an evolutionarily conserved protein. Previous research from our lab shows that Sld5 is required for normal cell cycle progression and the maintenance of genomic integrity. In addition, the depletion of other GINS sub-units Psf1 and Psf2 by siRNA in human fibroblasts lead to genomic instability and activation of Chk2. Preliminary results in Drosophila show that there are mitotic phase delays in the Sld5 mutant lines compared to wild-type. To further investigate the role of Sld5 in checkpoint signaling, a multifaceted approach is being used. First, Sld5-Chk2 double mutants were generated to check for replication defects and cell cycle progression. The mitotic delay observed in Sld5 mutants were found to be mediated through Chk2. Interestingly enough, the S-phase delay observed in Sld5 mutants did not appear to be mediated through Chk2, though there was an S-phase delay observed endogenously in the loki/loki;Sld5/+ mutants. Evidence of endo-replication defects and differences in the packaging ratio of DNA between wild-type and mutants was investigated in salivary glands. Sld5 mutants showed significantly larger amounts of DNA per nuclei although the nuclei were packaged similar to wild-type. Levels of apoptotic cells in single and double mutants is also being investigated to determine if cell death caused due to Sld5 depletion is regulated by Chk2. Errors due to under-replication or over-replication can lead to disastrous consequences leading to several genetic diseases like cancer,

developmental abnormalities etc. Therefore, analyzing the role of Sld5 in checkpoint regulation is essential for understanding its contribution in the maintenance of genomic stability.

292A

Regulation of Replication Initiation and Fork Progression during *Drosophila* Follicle Cell Gene Amplification. Brian Hua, Jessica L Alexander*, Terry Orr-Weaver. Whitehead Institute, Cambridge, MA.

During follicle cell differentiation six genomic regions undergo repeated origin firing and bidirectional replication fork movement to increase gene copy number. These *Drosophila Amplicons in Follicle Cells* (*DAFCs*) have specific replication origins that utilize the same machinery as normal DNA replication, including the Origin Recognition Complex (ORC), DUP/Cdt1, and the DNA helicase MCM2-7. The six *DAFCs* amplify to differing extents because they undergo distinct numbers of rounds of initiation, but the mechanisms controlling firing of amplification origins are unknown. We found that cis-acting position effects acting over at least five kb influence whether ORC is bound and amplification occurs at *DAFC-22B*. Transposons with *DAFC* origins inserted at ectopic sites are subject to position effects influencing the level of amplification. We will present experiments testing whether these position effects also impact replication fork progression and the extent of the amplified domain. We have generated *DAFC* transposon insertions that depend on insulator function for amplification to occur. These are being exploited to define the effects of insulators on ORC binding and origin activation.

*First two authors are co-presenters.

293B

Mutation of the lethal(2)denticless gene results in larval lethality and sterility. S. Catherine S. Key[1], Roketa Sloan[1], Christina Swanson[2], Maryonne Snow-Smith[1], Kristen Smith[1]. 1) Department of Biology, North Carolina Central University, Durham, NC; 2) Department of Biology, University of North Carolina-Chapel Hill, Chapel Hill, NC.

The lethal(2)denticleless (l(2)dtl) gene was originally reported as essential for embryogenesis and formation of the tiny rows of hairs known as the denticle belt in Drosophila. It is now well-established that l(2)dtl/cdt2 produces an E3 ubiquitin ligase protein which is a key regulator of the cell cycle targeting a number of essential cell cycle factors including p21, Cdt1, E2F1 and Set8. To investigate the role of l(2)dtl/cdt2 during development, we characterized existing disruption mutants and generated new deletion strains. We found that heterozygous disruption of the l(2)dtl/cdt2 gene results in a male sterility phenotype that is corrected by restoration of the gene. All homozygous mutant embryos, selected by negative GFP fluorescence, had intact denticle bands. The mutant embryos progressed through embryogenesis and died during larval development. New mutant strains generated by P-element mobilization resulted in deletion strains that are also larval lethal with intact denticle bands. Although mutation of cdt2 in yeast and mice embryos results in replication defects, based on BrdU assays, there is no detectable replication phenotype during embryogenesis in mutant embryos. However, indirect immunofluorscence with anti-Cdt2 antibody suggests that L2DTL/Cdt2 is maternally deposited. We conclude that the name l(2)dtl is a misnomer, that lethality occurs during larval rather than embryonic development, that maternally deposited Cdt2/L2DTL allows progression through embryogenesis, and that l(2)dtl/cdt2 is important for male fertility.

294C

Integrins are required for proper cell cycle progression and differentiation. Maria J. Gomez-Lamarca, Laura Cobreros, Maria D. Martin-Bermudo. Centro Andaluz de Biologia del Desarrollo (CABD), Univ. Pablo Olavide-CSIC, SEVILLA, Spain.

Coordinating differentiation with exit from the cell cycle is critical for proper organogenesis, yet how this is achieved remains largely unknown. The development of the follicular epithelium of the *Drosophila* ovary represents an ideal system to study the mechanisms controlling the transition from cell cycle exit to differentiation. The ovary of the adult *Drosophila* female is composed of various tubular structures called ovarioles that contains a line of egg chambers at different developmental stages. Each egg chamber begins as a 16-cell germline cyst surrounded by a monolayer of somatic follicle cells (FCs) precursors. During the early stages (up to stage 6), FCs undergo a mitotic division program giving rise to approximately 1000 FCs, which will form a monolayer known as the follicular epithelium. After stage 6, FCs differentiate and switch from normal mitotic cycle to undergo three rounds of endoreplication. Later in oogenesis, four different loci synchronously initiate a gene amplification event. By clonal and FACS analysis, we show that integrins are required for proper proliferation-to-differentiation switch. Interestingly, although integrin mutant cells exit mitosis they remain in an undifferentiated state and do not enter endocycle. In addition, integrin mutant follicle cells do not initiate the amplification event. At present we are investigating the molecular mechanisms by which integrins regulate the cell cycle exit to differentiation switch. Our results suggest that integrin mediated signalling controls this transition by regulating key cell cycle regulator proteins, such as Cyclin B and Dacapo.

295A

Tissue Growth Coordination in the Drosophila Brian via Glia Polyploidization. Yingdee Unhavaithaya, Terry Orr-Weaver. Whitehead Institute and Dept. of Biology, Massachusetts Institute of Technology, Cambridge MA 02142.

Proper development requires coordination in growth of the tissue layers comprising an organ. Although there are many examples of large polyploid cells, little is known about how these polyploid tissues contribute to organ growth. Through examination of nuclear DNA content in situ, we found the Drosophila subperineurial glia (SPG) to be polyploid in the brain, ventral nerve cord and peripheral nervous system, with ploidy levels ranging up to 22C. Subperineurial glia cells polyploidize either through endoreplication or endomitosis, producing cells with a single polyploidy or multiple nuclei, respectively. Inhibition of SPG polyploidy resulted in blood-brain barrier defects, revealing that the increased DNA content and resultant cell size is required to accomodate the growing brain to maintain the blood-brain barrier. We could rescue these blood-brain barrier defects with dmyc overexpression in the SPG or by attenuating neuroblast proliferation, indicating that polyploidy is essential for the maintenance of the blood-brain barrier. The increased ploidy of the SPG defines a new mechanism to coordinate growth of the glial and neuronal tissue layers during brain development; failure of this coordination ruptures the septate junctions of the SPG envelope around the brain. This mechanism is likely conserved, with potential vertebrates examples in megakaryocytes and giant trophoblasts.

296B

Role of p8 during spermatogenesis and the early embryonic development of *Drosophila melanogaster*. Grisel L. Cruz, Enrique A. Reynaud, Mario E. Zurita. Department of Developmental Genetics and Molecular Physiology, Institute of Biotechnology, Cuernavaca, Morelos, Mexico.

The DNA repair and transcription factor IIH (TFIIH) consists of ten polypeptides (p8, p34, p44, p52, p62, XPB, XPD, cdk7, cycH y MAT1) conserved from yeast to human. Mutations in some of these proteins have been implicated in three syndromes: xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). In TTD cells, it was demonstrated that p8 subunit is responsible of maintaining the normal TFIIH concentration. However, the role of this protein during development has not yet been elucidated. In this work, we are characterizing p8 by using Drosophila as a model. By using a specific antibody against p8 in western blot assay, we observed that this protein is expressed at all developmental stages in Drosophila. During early embryonic development, p8 co-localizes with DNA at interphase and mitotic nucleus in syncytial blastoderm, however, at cellular blastoderm and gastrulation p8 is nuclear only in the polar cells of the embryo. We found that p8 is necessary to maintain the synchrony and spindle stability during mitosis, as p8 null embryos show defects in shape and spindle symmetry, as well as lost of DNA condensation in some nucleus. Likewise, we observed that p8 could have a role during spermatogenesis, as p8 mutant males are sterile (even though it express other TFIIH subunits at wild type levels) with phenotypes similar to meiotic arrest mutants, in which sperm differentiation is arrested at primary spermatocyte stage. Interestingly, in transgenic flies that express p8-CFP fusion protein, p8 is located at nucleus and nucleolus of primary spermatocytes and it seems to co-localize with bivalent chromosomes during meiosis in this cells. All together, these data suggest a possible role of p8 in mitosis and meiosis during development. By using biochemistry and molecular and cellular biology, at this moment we continue analyzing these possibilities.

297C

Mitotic Reorganization of the Endoplasmic Reticulum is dependent on the Microtubule Network. Justin D Mclaurin, Blake Riggs PhD. San Francisco State University, 1600 Holloway ave. San Francisco, CA. 94132.

The endoplasmic reticulum (ER) is a perinuclear organelle that is congruent with the nuclear envelope and performs a variety of functions for the cell, including protein folding, calcium sequestration and drug detoxification. Defects in ER structure and function are implicated in a host of chronic diseases such as Hereditary Spastic Paraplegia and Diabetes. During mitosis, the ER undergoes a dramatic reorganization necessary for proper partitioning and nuclear membrane reformation, however little is known on how mitotic ER changes occur and how they are regulated. Our central aim is to study the mechanisms by which this occurs. To do this, we examined mitotic ER reorganization using live fluorescent analysis in early Drosophila melanogaster syncytial embryos containing the ER marker, PDI-GFP. Microinjection of the microtubule inhibitor colchicine in just prior to entry into mitosis perturbs both ER structure and inhibits its segregation. Additionally, microinjection of the microtubule stabilizer, taxol, blocks ER segregation and induces loss of ER membrane around the mitotic spindle and onto condensed chromatin. Lastly, we find that free centrosomes maintain the ability to organize ER membrane in the absence of their associated nuclei upon microinjection of the DNA polymerase inhibitor, aphidicolin. These data point toward a role for centrosomes in mediating ER reorganization during mitosis.

Poster Full Abstracts - Cell Death

Poster board number is above title. The first author is the presenter

298A

A screening for autophagic genes in *Drosophila melanogaster*. Ahrum Jin[1,2], Joonho Choe[1], Thomas Neufeld[2]. 1) Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, South Korea; 2) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

Autophagy is known as a 'self-eating mechanism' which is conserved from yeasts to mammals. When autophagy is induced, cytoplasmic components are sequestered in double-membrane vesicles called autophagosomes and degraded after fusing with lysosomes. Autophagy is involved in various cellular processes such as the starvation response, immune response, development, maintenance of cellular homeostasis (protein turnover and removal of damaged organelles), cell survival and death and the oxidative stress response. During the process of autophagy, the ubiquitin-related protein Atg8/LC3 is cleaved and conjugated with phosphatidylethanolamine. This conversion is reflected in Western blot by the appearance of two bands (LC3-I and LC3-II) that change in intensity as the rate of autophagy increases. We used this LC3 conversion assay in an RNAi screen in S2 cells to identify novel genes required for autophagy regulation. From ~1500 genes screened, we obtained ~100 hits with an altered LC3-I/II ratio. We have performed secondary screening in vivo using Drosophila RNAi fly lines, and identified 17 genes that affect autophagy activity in the larval fat body. We will describe the characterization of these genes in autophagy regulation.

299B

Regulation of neural stem cell fate in Drosophila cell death mutants. RICHA ARYA, Ying Tan, Hsiao-Yu Huang, Megumu Yamada-Mabuchi, Kristin White. CBRC, MGH/HARVARD, CHARLESTOWN, MA.

In mammals and non-mammalian systems, many neural stem cells (neuroblasts or NBs) are eliminated by apoptosis once they generate a stereotyped set of progeny cells. However the mechanism by which specific subset of NBs is selected for death is largely unknown. We are studying the spatial and temporal regulation of developmental apoptosis, using Drosophila NBs as a model. Our genetic studies showed that rpr and grim are required for normal apoptosis of NBs in the abdominal region of the ventral nerve cord. In rpr-grim mutants NBs survive and divide to produce large numbers of neuronal progeny resulting in neural hypertrophy. Furthermore we identified a cis-regulatory region (NBRR) between rpr and grim that controls the expression of these genes in the doomed NBs. Whole embryo chip-chip and chip-seq data and in silico analysis strongly suggest the presence of many transcription factor binding sites within the NBRR. Based on these binding analyses we selected a 5kb region (enhancer 1or enh1) to generate GFP reporter transgenic flies, to ask how this regulatory region activates apoptosis in doomed cells. We analyzed enh1-GFP during development and found that it is expressed in a subset of abdominal NBs in the late embryo, likely to be those that will die. In the larvae, the three abdominal NBs that normally survive in each hemisegment start expressing low levels of enh1-GFP in early L2. This expression peaks at mid-third instar, when these NBs die. Surprisingly, some thoracic NBs that normally do not undergo apoptosis also express enh1-GFP in the larvae, indicating either a missing repressor or the presence of survival signal in these cells. To identify the upstream regulators of the NBRR we performed an RNAi screen for factors that affect the expression of enh1-GFP. In a preliminary screen we identified three transcription factors that regulate the expression of enh1-GFP in abdominal neuroblasts. Further studies will describe how upstream pathways are integrated to regulate the expression of apoptotic effectors to eliminate individual cells during development.

300C

Investigating a role of dHb9-positive motor neurons in eclosion behavior. David S Conway, Soumya Banerjee, Marcus Toral, Alexander Busch, Joyce Fernandes. Zoology, Miami Univ, Oxford, OH.

The nervous system of is remodeling extensively during metamorphosis from a larval framework to develop adult specific neural circuits which drive adult specific behaviors. One of the major changes that takes place is the shift of locomotor control from the entire body (crawling larvae) to the thorax (walking and flying adult). The adult abdominal segment is still essential for carrying out other adult specific behaviors like mating and eclosion. The eclosion behavior is mediated by persistent muscle fibers (PMFs), which are retained from the larval stage into the adult stage, and are degraded shortly after the eclosion behavior is complete. We specifically study PMFs 12 and 13, in segments A1 and A2, which have been implicated in eclosion behavior (Kimura and Truman, 1990). These PMFs are innervated by motor neurons that express the transcription factor dHb9, as shown by previous work in our lab. We intend to test the hypothesis that dHb9+ motor neurons are essential for bringing about the eclosion behavior. Apoptosis will be induced or prevented in dHb9+ motor neurons during the pupal stage using the targeted expression of the genes Reaper and p35 respectively. The onset of the expression of these genes will be manipulated through the use of the Gal4/Gal80 temporal control system. The resulting adults will be examined and classified according to their eclosion behavior, normal eclosion, late eclosion or absence of eclosion. For the targeted reaper expression, we will examine the presence or absence of dHb9 driven GFP expression in the ventral ganglion and the body wall to determine the extent to which dHb9 cells are eliminated. A preliminary trial of this experiment showed that flies in which Reaper was activated failed to eclose 25% of the time, as compared to control flies failing to eclose 14% of the time (n=60 animals). Current work is aimed at completing this study and to examine any co-relations of the three categories of eclosion to the extent of eliminating dHb9-positive neurons.

301A

A Genetic Screen to Identify Cell Death Regulators In the *Drosophila* Ovary. Tatevik Keshishyan, Jeremy Nguyen, Olivia Rudnicki, Michelle Gammill, Jemma Taipan, Sarah Durrin, Luz Ceballos, Aileen Leung, Elizabeth Tanner, Jeanne Peterson, Kim McCall. Boston University, Boston, MA.

Cell death is an essential mechanism for the survival and development of many organisms. Excessive cell death can lead to degenerative disorders such as Parkinson's disease, while too little cell death can result in diseases such as cancer. *Drosophila* oogenesis is an excellent model system for studying programmed cell death (PCD). One type of programmed cell death occurs during mid-stage oogenesis in response to nutrient deprivation. Cell death also occurs during late-stage oogenesis when nurse cells, which contain contents essential for development of an embryo, dump their cytoplasmic contents into a developing oocyte and are then eliminated through PCD. We performed an unbiased mis-expression screen based on EY P element lines to identify regulators of cell death. Over-expression in the germline was accomplished by crossing to nanos-GAL4. Of the 1200 lines we have screened, 27 fly lines consistently showed abnormal cell death phenotypes. These abnormal phenotypes included persisting nurse cell (NC) nuclei in late oogenesis, death-resistant NCs in mid-oogenesis, and excessive degeneration of egg chambers. The affected genes function in processes such as DNA/RNA binding, cytoskeleton arrangement, cell signaling, and mitochondrial events. To further analyze the causes of the abnormal phenotypes, staining has been performed to determine

abnormalities in autophagy and cytoskeletal components. We also looked to see if our target genes interact with the caspase pathway by over-expressing a caspase inhibitor along with our genes of interest. These studies will illuminate which cell biological processes are affected in our mutants. Through this screen we have identified potential new regulators of cell death. Ongoing experiments will reveal the exact role of these specific genes and thus expand on our understanding of cell death mechanisms in the ovary.

302B

Determination of the contributions of caspases and autophagy to cell death in the ovary. Jeanne S. Peterson, Kim McCall. Dept Biol, Boston Univ, Boston, MA.

We are investigating the mechanisms controlling two types of cell death that occur in the fly ovary: developmental death of nurse cells, and starvation-induced death of entire egg chambers at mid-oogenesis. Starvation-induced cell death requires caspases and shows condensation and fragmentation of nurse cell nuclei, and enlargement of follicle cells during engulfment of nurse cell material. In *dcp-1* mutants there is failure of nurse cell nuclei to condense, and egg chambers show premature death of follicle cells. Mid-oogenesis cell death is only partially disrupted in autophagy mutants. In developmental cell death, in late oogenesis, the nurse cells are removed completely when their cytoplasm is dumped into the oocyte and their condensed nuclei are broken down. This process is partly affected in caspase or autophagy mutants and is indicated by the persistence of nurse cell nuclei in mature egg chambers. Here we present our findings on the combined inhibition of caspases and autophagy during both mid and late oogenesis.

303C

An EMS genetic screen to identify mutations that modulate loss of Rb phenotypes. Tianyi Zhang, Zhentao Sheng, Wei Du. Ben May Department for Cancer Research, University of Chicago, Chicago, IL.

Retinoblastoma protein (Rb) is a tumor suppressor gene that is often inactivated in a wide variety of human cancers. Rb functions to regulate cell proliferation, differentiation, as well as apoptosis in both flies and mammalian systems. Interestingly, the effects of Rb loss differ in different regions of the developing eye disc. For example, the Rb mutant cells in the morphogenetic furrow (MF) but not other regions of eye imaginal disc are very sensitive to apoptosis. These observations suggest that consequences of Rb loss are modified by genes/pathways function in those different regions. To better understand the in vivo functions of Rb, a genetic screen was carried out to identify mutated genes which have synergistic effects with Rb loss. We used FLP/FRT to generate mosaic clones in adult eyes, and compared the differences between single and double clones of the Rb mutants and the EMS-induced mutants. From about 18,000 EMS alleles on chromosome 2L and 3R, 50 mutants were identified with synergistic effects on clone size or differentiation.

304A

Screening for genes regulating mitochondrial dynamics in Drosophila apoptosis. Eltyeb Abdelwahid[1], Michael Thomenius[2], Sally Kornbluth[2], Kristin White[1]. 1) Cutaneous Biology Research Center, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA; 2) Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, USA.

Aberrant regulation of mitochondrial dynamics is associated with human diseases, including neurological disorders and cancer. Mitochondrial fission is associated with apoptosis in a wide variety of model systems including Drosophila. The mechanistic connection between mitochondrial dynamics and cell death in flies remains largely uncharacterized. We have recently shown that the conserved mitofusin (dMFN/MARF) mediates mitochondrial fusion and promotes Drosophila cell survival. MARF physically interacts with the apoptosis regulator Reaper. We also showed that MARF knockdown causes tissue loss and significant cell death in Drosophila. Using these findings, we are performing an RNAi screen for genes that modify the MARF RNAi phenotype. Our screen identified a number of candidates that suppress the MARF RNAi mitochondrial phenotype, and those that suppress MARF RNAi induced tissue loss independently of the mitochondria, possibly acting downstream of mitochondrial fission. We are currently characterizing these candidates to dissect the pathways by which MARF blocks cell death. This approach will allow us to understand how mitochondrial dynamics modulates Drosophila apoptosis and to identify new components of the mitochondrial fission/fusion machinery.

305B

The *corp* gene regulates cell fate following DNA damage. Riddhita Chakraborty, Kent Golic. Department of Biology, University of Utah, Salt Lake City, UT.

In *Drosophila*, when somatic cells experience telomere loss, a form of DNA damage that cannot be repaired by conventional mechanisms, most cells die. However, a few do survive. We used the BARTL (Bar and Telomere Loss) assay, which provides a rapid assessment of cell fate after telomere loss, to screen for EP elements that modify cell fate.

The BARTL assay relies on FLP-mediated recombination to induce dicentric chromosome formation on a *Y* chromosome, just proximal to the *Bar of Stone* (B^S) mutation. When the cell divides, the dicentric chromosome typically breaks, delivering a chromosome lacking a telomere to each daughter cell. The B^S mutation, now present on an acentric chromosome, is lost from at least some of the daughter cells. In wildtype flies, when FLP is expressed from the eyeless promoter, the eyes of the adults are larger than B^S eyes (though still smaller than wildtype) owing to survival, proliferation and differentiation of some cells carrying a chromosome that has lost a telomere. We identified *corp* (*companion of reaper*) as a gene whose overexpression makes eyes much larger than the control in the BARTL assay, resulting in essentially wildtype eye size. Knockdown of *corp* gene function, via RNAi, produced the opposite effect, eliminating the eye entirely. Furthermore, we found that when corp is over-expressed in the developing eye by the *GMR* driver, it suppresses the small eye phenotype resulting from expression of pro-apoptotic genes *hid* or *reaper*. Finally, ubiquitous *corp* overexpression increases organismal viability following irradiation.

The *corp* gene is one of many transcriptional targets of the *p53* tumor suppressor. Other targets include genes that mediate apoptosis and DNA repair. The specific function of *corp* is unknown, but it appears to be predominantly associated with components of the proteasome (DPIM, https://interfly.med.harvard.edu/). We propose that *corp* targets specific cellular proteins for degradation, resulting in reduced apoptosis. Possible targets include caspases and p53.

306C

Loss of TBP function causes developmental arrest and apoptosis in Drosophila melanogaster. Tun-Chieh Hsu, Chin Sern Yong, Ming-Tsan Su. Department of Life Science-National Taiwan Normal University, Taipei, Taiwan.

TBP is a general transcription factor that is required for the transcription of virtually all genes in cells. Previous studies reported that inactivation of the murine TBP gene by homologous recombination results in growth arrest and apoptosis at the embryonic blastocyst stages. Moreover, inhibition of TBP expression by injecting antisense morpholino oligos causes developmental arrest and block of epiboly movements in zebrafish embryos at midblastula stages. How TBP regulates animal development and its involvement in apoptosis are elusive. The main objective of my research is to investigate the role of TBP in development and apoptosis. Additionally, it was reported that p53, a major pro-apoptotic regulator, interacted with TBP physically. We suspect that TBP may suppress function of p53. Using genetics approach, we dissect the epistatic relationship of TBP and various apoptotic components, including diap1 and p53. In the future, we will also take biochemistry approach to exam if TBP modulates function of p53 directly.

307A

Genetic characterization of a Drosophila DUB involved in apoptosis. Levente Kovács[1], Olga Nagy[1], Margit Pál[1], Octavian Popescu[2], Péter Deák[1]. 1) Institute of Biochemistry, Hungarian Academy of Sciences Biological Research Centre, Szeged, Hungary; 2) Molecular Biology Center, Interdisciplinary Research Institute on Bio-Nano-Sciences, Cluj-Napoca, Romania.

Removal of ubiquitin from poly-ubiquitylated proteins is performed by deubiquitylating enzymes (DUBs).Although the study of DUBs intensified in the last few years, understanding of their functions remains considerably limited. Genetic analysis of mutant phenotypes in Drosophila melanogaster can provide important information to elucidate the function of DUBs. From a genome-wide search using bioinformatics techniques, we identified 40 Drosophila genes sharing high sequence homology with known human and yeast DUBs. Analyses of P element insertion mutants and/or transgenic RNA interference (RNAi) knockdown lines suggest that the function of 24 of them is essential. These results can stimulate further functional studies of DUB genes in this model organism. Ubiquitous knockdown of the CG12082 gene by RNAi causes early pupal lethality, accompanied with an increase in the number apoptotic cells in the larval brain and imaginal discs. Eye specific induction of RNAi causes rough eye phenotype underlining apoptosis. Null alleles of CG12082 were established by P element remobilization. The development of the homozygous null animals stops in L3 and they die in this stage after a 5 day long stagnation. Acridine orange staining of L3 larval brains and wing discs revealed a very high incidence of apoptosis in these animals. In addition to this, the expressions of reaper and hid, but not grim pro-apoptotic genes have been elevated in the CG12082 null mutant larval brains and imaginal discs. A heterologous complementation experiment confirmed functional homology between CG12082 gene and yeast Ubp14. In addition to this, we also show that free polyubiquitin chains accumulated in CG12082 mutants similarly to the yeast Ubp14 mutants that further support functional conservancy. Based on these observations we conclude that the CG12082 gene encodes the Drosophila ortholog of the human Usp5 and yeast Ubp14 DUB enzymes, and it appears to be involved in regulating apoptosis.

308B

Fzy/Cdc20 promotes neural stem cell survival. Chaoyuan Kuang[1], Cheng-Yu Lee[1,2,3,4]. 1) Cellular and Molecular Biology Graduate Program; 2) Department of Cell and Developmental Biology; 3) Division of Molecular Medicine and Genetics, Department of Internal Medicine; 4) Center for Stem Cell Biology, Life Sciences Institute, University of Michigan Medical School, Ann Arbor, MI 48109.

Premature loss of stem cells would perturb development and tissue homeostasis. However, cell survival is generally perceived as a passive process rather than an integral component of the genetic program that maintains stem cell identity. Hence, specific mechanisms that maintain the viability of stem cells *in vivo* remain completely unknown. Using neural stem cells (neuroblasts) in *Drosophila* larval brains as a genetic model system, we identified a novel mutation in the *fizzy* (*fzy*) gene which results in premature loss of neuroblasts. This mutation (fzy^{G291R}) acts as a genetic null mutant allele of *fzy*, and leads to the substitution of glycine 291 with arginine in the WD40 repeats of the Fzy protein. Neuroblasts in fzy^{G291R} mutant brains never express neuronal and glial markers, and mosaic clones derived from single fzy^{G291R} mutant neuroblasts contain few post-mitotic cells but frequently lack the parental neuroblast. Furthermore, distinct from neuroblasts experiencing mitotic catastrophe due to defective cytokinesis, neuroblasts in fzy^{G291R} mutant brains show loss of DNA content, absence of activated Dronc caspase activity, and displayed morphological characteristics of necrosis. Fzy expression in the fzy^{G291R} mutant is indistinguishable from the control and a *fzy* genomic rescue transgene restores neuroblasts in fzy^{G291R} mutant brains. Interestingly, in contrast to *fzy* null intestinal stem cell and imaginal disc mosaic clones which are unable to proliferate, fzy^{G291R} clones show expansion and proliferation similar to wild type. Together, these results indicate that fzy^{G291R} is capable of promoting normal cell cycle progression but is unable to maintain neuroblast survival. Additionally, this work suggests that *fzy* functions to prevent neuroblast death specifically via a novel caspase-independent mechanism.

309C

Induction of IAP-antagonist and apoptosis in Drosophila and mosquito larvae following virus infection. BO LIU[1], JAMES BECNEL[2], ROLLIE CLEM[3], LEI ZHOU[1]. 1) Dept. Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL; 2) United States Department of Agriculture, ARS, Gainesville, FL; 3) Division of Biology, Kansas State University, Manhattan, KS.

Many vertebrate and insect viruses possess antiapoptotic genes that are required for their infectivity. This led to the hypothesis that apoptosis is an innate immunoresponse important for limiting virus infections. The role of apoptosis may be especially important in insect antiviral defense because of the lack of adaptive immunity. However, the cellular mechanism that elicits apoptosis in response to viral infection in insects was poorly understood. Our previous work has demonstrated that the rapid induction of IAP-antagonists and apoptosis correlates with the resistance of mosquito larvae to CuniNPV infection (Liu et al, Cell Death Differentiation. 2011). Using an in vivo infection system with the fall armyworm baculovirus AcMNPV (Autographa californica nuclear polyhedrosis virus), we demonstrated that pro-apoptotic gene hid, not reaper, is induced in Drosophila larvae following injection of virus. This induction happens within 1hr post virus injection (p.v.i.). By 2hr p.v.i., the level of hid mRNA had returned to the same level as control. Further characterization of the cellular identity of viral infection-induced apoptotic response will be reported. We believe that establishing of this AcMNPV infection system in Drosophila will allow us to use the genetic resources that are uniquely available in Drosophila to study the signaling pathways responsible for viral infection-induced apoptosis. Moreover, this experimental system will allow us to test the role of apoptosis as an innate immunoresponse to limit viral infection in insects.

310A

NMDA receptor and protein tyrosine phosphatase Ptpmeg implicate calcium signaling in the control of developmental cell death in *Drosophila*.
Brandy C. Ree, Yanling Liu, Michael Lehmann. University of Arkansas, Fayetteville, AR.

Excessive activation of the NMDA receptor (NMDAR) is a major cause of excitatory cell death in the nervous system. This type of cell death is triggered by neurotoxins (e.g. alcohol) or brain injury, and is involved in neurodegenerative diseases such as Parkinson's and Alzheimer's disease. Surprisingly, we found that loss of function of the *Drosophila NMDAR1* gene leads to defects in developmentally programmed cell death (PCD). We identified the protein tyrosine phosphatase Ptpmeg as a potential interaction partner of NMDAR1 in PCD control. Both NMDAR1 and Ptpmeg are required for normal functioning of the mushroom body, the center for olfactory learning and memory in the *Drosophila* brain. Control of developmental cell death is a novel role for these two proteins. Our results show that both NMDAR1 and Ptpmeg are required for the accurately timed removal of the larval salivary glands during metamorphosis. Loss of either *NMDAR1* or *Ptpmeg* leads to salivary gland persistence with an enhanced penetrance of this phenotype in double-knockdown animals. NMDAR1 is a phospho-tyrosine protein that forms a calcium channel in the cell membrane. Importantly, in *Ptpmeg* mutants we found decreased levels of cellular Ca++ in the salivary glands. These data support a model in which dephosphorylation of NMDAR1 by the tyrosine phosphatase activity of Ptpmeg is required for normal Ca++ influx and cell death. Interestingly, whereas levels of active caspase are reduced in *Ptpmeg* mutants, transcriptional activation of the death genes *hid* and *reaper* does not seem to be affected. This suggests that Ptpmeg and NMDAR1 define a novel calcium-dependent pathway that controls PCD independent of these death genes or at the level of hid and reaper protein activity. The larval salivary glands of *Drosophila* provide an excellent experimental system to further test this model.

311B

Translational repression by reaper is mediated by targeted degradation of a translation factor.
ROLANDO RIVERA-POMAR[1,2], CARLOS BERTONCINI[3], M. PAULA VAZQUEZ-PIANZOLA[4], DIEGO VAISMAN[1,5], PAOLA FERRERO[1,5]. 1) Centro de Bioinvestigaciones,, UNNOBA, Pergamino, Buenos Aires, Argentina; 2) Centro Regional de Estudios Genómicos, UNLP, Florencio Varela, Buenos Aires, Argentina; 3) University of Cambridge, Cambridge, UK; 4) University of Bern, Bern, Switzerland; 5) Departamento de Ciencias Básicas y Experimentales, UNNOBA, Pergamino, Argentina.

Inhibition of protein synthesis is a key process during apoptosis. We have previously demonstrated that the pro-apoptotic genes reaper, hid and grim are translated in a cap-independent manner and escape cap-dependent translational repression during the early apoptosis phase (Hernandez et al., 2005; Vazquez-Pianzola et al., 2007). However, all translational mechanisms are shut off in later steps. It has been demonstrated that the ribosome is a primary target of RPR to repress translation (Colon-Ramos et al., 2006). Here we propose an additional, redundant mechanism for translational repression. We show that recombinant RPR represses translation in vitro in Drosophila and mammalian cells extracts. By co-purification we have identified RPR-interactig proteins in the translation extracts. We demonstrate that RPR interacts with the eukaryotic elongation 2 (eEF2) in vitro in both, mammalian and Drosophila cells. Upon addition of recombinant RPR eEF2 is degraded in translation extracts, thus, translation is impaired. This effect was not observed in other translation factors. The degradation of eEF2 is temperature-dependent and can be blocked by proteasome inhibitors. We propose a model in which RPR acts at different levels of the translational machinery for a complete shut off of protein synthesis. This work was supported by ANPCyT and MPG grants to RRP, and CONICET and UNNOBA grants to PVF.

312C

An epigenetically regulated enhancer region mediates cell competition -induced cell death.
Can Zhang[1], Sergio Casas Tintó[2], Michelle Chang[1], Eduardo Moreno[3], Lei Zhou[1]. 1) Dept of Molecular Genetics and Microbiology, Univ of Florida, Gainesville, FL; 2) Cajal Institute, CSIC, Madrid, Spain; 3) Molecular Oncology Program, CNIO, Madrid, Spain.

Previous work from our lab has identified that an epigenetically regulated, irradiation responsive enhancer region (IRER) is required for radiation-induced expression of pro-apoptotic genes in early stage embryos. Here we demonstrate that IRER is also required for cell competition-induced cell death. Cell competition has been implicated in growth control and early steps of tumorigenesis, which occurs between cells with different metabolic properties or growth rates and results in growth of the stronger population at the expense of the weaker. It has been well documented that expression of the *Drosophila* growth regulator dMyc transforms the cell into supercompetitors and induces apoptosis from neighboring wild-type cells in developing wings. However, the mechanism controlling the induction of cell death genes in response to cell competition remains largely unknown. In this study, we observed that cell competition-induced cell death was attenuated in IRER-deficient background. In the absence of IRER, the induction of pro-apoptotic gene *hid* upon cell competition was significantly blocked. To monitor the epigenetic status of IRER in individual cells, a reporter strain was generated by introducing an ubiquitin-DsRed transgene into IRER through homologous recombination. Interestingly, we noticed that cells with higher level of DsRed (open IRER) were preferentially eliminated from wing discs upon dMyc-induced cell competition, indicating that the epigenetic status of IRER determines sensitivity to dMyc supercompetitor-induced apoptosis. Moreover, IRER-deficient flies displayed an overgrown phenotype from multiple organs such as the wing and the central nervous system. Our work demonstrated that modulating cellular sensitivity to stress-induced cell death through epigenetic regulation may be an important mechanism of preventing tumorigenesis and achieving homeostasis.

313A

Identification of CDK7 as a protein required for IAP-antagonist-induced apoptosis.
Jun Morishita Funabiki, Min-Ji Kang, Kevin Fidelin, Hyung Don Ryoo. Cell Biology, New York Univ Sch Medicine, New York, NY.

It is now established that certain cellular genes help cells die in response to injury and stress. However, the underlying mechanisms that contribute to this process are not well understood. To investigate how misfolded proteins trigger apoptosis, we developed a system where we overexpress a mutant rhodopsin-1 protein that fails to fold properly in the developing fly eye. This generates a malformed eye, in part, due to excessive cell death. Through an RNAi screen for suppressors of this phenotype, we identified CDK7 and MAT1. CDK7, MAT1 and cyclin H are known to form the Cyclin-Dependent kinase (CDK) Activating Kinase (CAK) complex. CAK phosphorylates other CDKs to be activated during cell cycle progression. It is also involved in general transcription by the phosphorylating the RNA polymerase II. To independently validate the role of CDK7, we used a hypomorphic allele, that has its own T-loop phospho-acceptor sites (S164 and T170) mutated to alanines which acts a temperature-sensitive allele. In semi-permissive temperature, we found no defects in developmental gene expression or cell cycle progression, but the mutant rhodopsin-1 overexpression phenotype was suppressed. Furthermore, it

suppressed the apoptosis phenotype caused by IAP-antagonist (Hid) overexpression through the eye-specific GMR promoter. We also examined the degree of IAP-antagonist induction in response to gamma irradiation. This treatment induced Hid expression in cdk7+ imaginal discs, but inactivation of cdk7 almost completely blocked radiation-induced Hid expression. These results indicate that CDK7 has a previously unrecognized role in regulating apoptosis, independent of its role in cell cycle and general transcription.

314B

The Role of JNK in Cell Competition. John F Fullard, Wei Li, Nicholas E Baker. Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

JNK signalling has been implicated in cell death in cell competition. We describe the non-autonomous requirement for JNK signalling, not in the M/+ cells that die, but in the nearby wild type cells that kill them. Draper and Shark, components of the ced-1,6,7 pathway in Drosophila, activate the JNK pathway near to M/+ cells and activate transcription of Pvr, which acts through the ced-2,5,10,12 pathway to eliminate M/+ cells. Part of this pathway of M/+ cell removal is common to the elimination of oncogenic scribbled mutant cells (Ohsawa et al 2011). In that context, JNK activity depends on eiger, the Drosophila ortholog of TNF, rather than on Draper and Shark. Thus, two examples of cell competition converge on a common pathway of cell removal and replacement that is also used in dorsal closure and wound healing. In addition, we have carried out a genetic screen to identify novel components of the Draper pathway.

315C

Cell competition during adult gut homeostasis. Golnar Kolahgar, Enzo Poirier, Sarah Mansour, Eugenia Piddini. The Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.

When a tissue is made of cells of different fitness levels, fitter cells induce death of less fit cells, in a sort of Darwinian selection process. This cellular behavior, called cell competition, directly regulates tissue colonization during development and in some adult tissues both in the Drosophila and mouse systems. We want to understand if cell competition regulates tissue homeostasis and cellular turnover in the adult fly intestine. In other words, are the cells that begin to fail the ones that are replaced first to allow optimal gut function? By taking advantage of different mutations in the Minute genes, we investigate how cell competition impacts on tissue homeostasis both in young and in ageing adult tissues. Specifically, we investigate how proliferation, survival and cell fate decisions of stem and differentiated cells are affected when tissues are composed of cells of different fitness, and how that in turns affects tissue homeostasis and organ physiology. We find that progenitor and stem cells renewal are locally affected but that the overall cell composition of the tissue is not changed, suggesting that tissue turnover is locally responding to a phenomenon akin to cell competition.

316A

A model to study the influence of Hippo signaling on local cell-cell interactions. Indrayani Waghmare[1], Shilpi Verghese[1], Katelin Hanes[1], Alyssa Lesko[2], Amit Singh[1,3,4], Madhuri Kango-Singh[1,3,4]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) Department of Chemistry, University of Dayton, Dayton OH; 3) Pre Medical Programs, University of Dayton, Dayton OH; 4) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton OH.

The Hippo pathway regulates organ size from flies to mammals. Molecular genetic approaches in Drosophila established the crucial foundation of the mechanisms of signal transduction and function of the Hippo pathway. Recent studies uncover a role for Hippo pathway in phenomena involving local cell-cell interactions like cell competition or compensatory proliferation. Hyperactivation of Yorkie (Yki) is crucial for compensatory proliferation, cell competition, and regenerative growth. We have studied the micro-environment of *scribble* (*scrib*) mutant cells to gain insights into the competitive ability of *scrib* mutant cells in the context of changes in Yki activity. *scrib* is a neoplastic tumor suppressor gene and loss of *scrib* in homozygous larvae causes massive tumors to form. However, somatic clones comprising *scrib* mutant cells are slow growing and are competed out by the surrounding wild-type cells by activation of JNK mediated apoptosis. We note that *scrib* mutant cells survive and proliferate when additional mutations in *scrib* mutant cells either accelerate their proliferation or favor reduced cell competition (e.g., in *Minute* heterozygous background). We found that JNK and Hippo signaling both play an important role in the growth and survival of scrib mutant cells. Furthermore, activation of JNK can suppress the over-growth of Yki over-expressing cells. We decided to compare the clone size and Hippo pathway read-outs (*ex-lacZ, diap1-lacz or diap-GFP*) in five conditions viz., *scrib*$^{-/-}$, *scrib*$^{-/-}$ *M/+; scrib*$^{-/-}$ + *P35, wts*$^{-/-}$ *scrib*$^{-/-}$ and *RasV12 scrib*$^{-/-}$. Our preliminary data shows that the interaction of *scrib* mutant cells with the surrounding wild type cells differ for each genotype. Here we present our analysis of Yki activity in *scrib* mutant cells challenged with different cell competitive environments.

317B

Regulation of Drosophila glial cell proliferation by Merlin-Hippo signaling. venu bommireddy venkata, Ken Irvine. Waksman Institute, Piscataway, NJ.

Glia perform diverse and essential roles in the nervous system, but the mechanisms that regulate glial cell numbers are not well understood. Here, we identify and characterize a requirement for the Hippo pathway and its transcriptional co-activator Yorkie in controlling Drosophila glial proliferation. We find that Yorkie is both necessary for normal glial cell numbers, and, when activated, sufficient to drive glial over-proliferation. Yorkie activity in glial cells is controlled by a Merlin-Hippo signaling pathway, whereas the upstream Hippo pathway regulators Fat, Expanded, Crumbs, and Lethal giant larvae have no role. We extend functional characterization of Merlin-Hippo signaling by showing that Merlin and Hippo can be physically linked by the Salvador tumor suppressor. Yorkie promotes expression of the microRNA gene bantam in glia, and bantam promotes expression of Myc, which is required for Yorkie and bantam-induced glial proliferation. Our results provide new insights into the control of glial growth, and establish glia as a model for Merlin-specific Hippo signaling. Moreover, as several of the genes we studied have been linked to human gliomas, our results suggest that this linkage could reflect their organization into a conserved pathway for the control of glial cell proliferation.

318C

Hippo signaling controls Dronc activity to regulate organ size in Drosophila. Shilpi Verghese[1], Shimpi Bedi[1], Madhuri Kango-Singh[1,2,3]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) Pre-Med Programs, University of Dayton, Dayton, OH, USA;; 3) Centre for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, 300 College Park Dayton, OH 45469 USA.

The Hippo signaling pathway regulates organ size by simultaneously inhibiting cell proliferation and promoting apoptosis. The Hippo pathway is composed of a highly conserved core kinase cascade that is regulated by multiple upstream inputs and has multiple transcriptional outputs. Hippo signaling is required for cells to stop proliferation when organs have reached their proper size and hippo mutant animals produce severely overgrown structures. In contrast, over-expression of Hippo (or loss of yki) results in formation of smaller organs due to induction of apoptosis. Hippo pathway regulates apoptosis through its apoptotic target genes e.g., Hid, DIAP1 and the microRNA bantam. We found that cell death induced by Hippo over-expression cannot be

rescued by co-expression of pan caspase inhibitor p35 or DIAP1. Hence, we investigated the role of Dronc in Hippo mediated cell death, as Dronc activity is resistant to p35 and not affected by changes in DIAP1 levels. We found that Hippo genetically interacts with Dronc and requires dronc to induce cell death. Our data suggests Hippo pathway can regulate cell death through the RHG proteins and via Dronc. Dronc along with its binding partner Dark can suppress the over-growth induced by over-expression of Yki, suggesting that normally Hippo pathway needs to restrict Dronc activity to maintain tissue homeostasis. Consistent with this idea, we found the Hippo pathway transcriptionally regulates dronc. Loss of dronc results in cell survival and dronc mutant cells proliferate faster than their wild-type twin spots. Here we present dronc (a gene required for cell competition and for caspase-mediated cell death) as a novel target of Hippo signaling.

319A

A ciliopathy model to test the regenerative capacity of primary cilia and to screen small molecule therapies. Jieyan Chen, Timothy Megraw. Biomedical Sciences, Florida State University, Tallahassee, FL.

The primary cilium is found on nearly all cells and it plays critical roles in development, cell signaling, and environmental sensing. There are no treatments for ciliopathies in humans. A treatment, if possible, will require that cilia have the potential to regenerate. It is not known if centrioles or cilia can regenerate in differentiated cells. Flies have primary cilia only on mechanosensory, chemosensory and visual system neurons. Orthologs for most ciliopathy genes exist in Drosophila. Loss of function for some of these genes results in an uncoordinated phenotype due to loss of mechanosensation. Inducible expression of hairpin constructs that elicit RNA interference (RNAi) using the "GAL4 system" with a temperature-sensitive GAL80 inhibitor of GAL4 permits induction and termination of RNAi during development or in adults. We have performed an initial RNAi screen of candidate proteins represent three classes of cilium assembly or function: 1) centriole biogenesis proteins, 2) cilium assembly proteins, and 3) ciliary membrane ion channels. Among 35 genes screened, 11 show a severe "uncoordinated" phenotype upon RNAi, 4 of which are orthologs of genes associated with ciliopathies in humans. We are now testing regenerative capacity of cilia upon restoration of gene expression in adult by analyzing cilium functions with quantitative locomotive assay, combined with immunofluorescence and electron microscopy ultrastructural imaging of cilia and basal bodies in adult neurons. We will also complement the RNAi approach with inducing transgenes in null mutant backgrounds to assay cilium regeneration in adult neurons. Meanwhile, we select two candidate ciliopathy gene models to score cilium function recovery by assaying locomotive behavior "rescue" in RNAi knockdown flies, following treatment with a library of chemicals, to screen for potential drugs that relieve the developmental and/or adult-onset ciliopathies. With this ciliopathy model, we will define the regenerative plasticity of centrioles and cilia in vivo, and discover potential drugs for the treatment of ciliopathies.

320B

Heterochromatin-mediated pairing and segregation of achiasmate chromosomes depends on HP1. Christopher C. Giauque, Justin J. Gaudet, Sharon E. Bickel. Department of Biological Sciences, Dartmouth College, Hanover, NH.

Meiotic nondisjunction is one of the leading causes of human infertility and birth defects, including Down Syndrome, but little is known about its underlying causes. Accurate segregation of meiotic homologous chromosomes is usually dependent on the establishment of chiasmata between homologues during recombination. However, in Drosophila oocytes, although *4th* chromosomes are always achiasmate and *X* chromosomes fail to recombine 6-12% of the time, these chromosomes still segregate with high fidelity. Work from a number of labs has demonstrated that proper segregation of achiasmate homologues depends on the physical association of their centromere-proximal heterochromatin, but the role of heterochromatin proteins in this process has not been investigated. Our lab has previously reported that weak mutations in the meiotic cohesion protein ORD disrupt heterochromatin-mediated pairing of achiasmate chromosomes. Here, we explore the role of the heterochromatin protein HP1 (encoded by the *Su(var)205* gene) in the pairing and segregation of achiasmate chromosomes. HP1 is a highly conserved essential gene product required for normal heterochromatin formation. In addition, HP1 has been implicated in recruiting the cohesin complex to pericentric heterochromatin. Using a Gal4/UAS RNAi strategy, we reduced HP1 in the female germ line and used FISH to monitor heterochromatin pairing of the obligate achiasmate *FM7/X* chromosome pair. We find that reduction of HP1 during meiotic prophase disrupts heterochromatin mediated pairing of the *FM7/X* homologues. In addition, in *Su(var)205* heterozygous oocytes, we observe a small but significant increase in nondisjunction of the *FM7/X* chromosome pair. These data indicate that wild-type levels of HP1 protein are required for normal pairing and segregation of achiasmate chromosomes. We are currently exploring the possibility that reduction of HP1 disrupts heterochromatin-mediated pairing of homologues because recruitment of cohesion proteins to pericentric regions is decreased.

321C

Nondisjunctional segregation in *Drosophila* female meiosis I is preceded by homolog malorientation at metaphase arrest. William Gilliland, Shane Gillies, Khateriaa Pyrtel, Wonbeom Paik, Nneka Wallace. Department of Biological Sciences, DePaul University, Chicago, IL.

The recent discovery that chromosome congression does occur in the first meiotic division in *Drosophila* female meiosis requires reexamination of what goes wrong to cause nondisjunctional segregation. We have found that several mutants that cause high rates of nondisjunction are still competent to complete congression. One possibility is that congression errors result in metaphase arrested oocytes with maloriented homologs in the congressed karyosome. If this is the case, then chromosome malorientation rates should be equal to nondisjunction rates. To test this hypothesis we assayed an *ald* allelic series for both genetic nondisjunction rates as well as homolog coorientation rates using combined immunofluorescence plus chromosome-specific FISH. These two rates are highly correlated, indicating that events during congression are responsible for chromosome nondisjunction during meiosis I.

322A

Chromosome axis proteins regulate synapsis initiation in Drosophila oocytes. Kathryn Landy, Mercedes Gyruicza, Kim McKim. Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ.

Accurate chromosome segregation is crucial for the proper completion of meiosis and involves multiple intricate processes, including synapsis, which tethers homologous chromosomes, and cohesion, which tethers sister chromatids. Synapsis is the process of assembling the synaptonemal complex (SC), a proteinaceous structure that joins homologs along the chromosome axis. The axis runs along the length of each chromosome and is composed of several proteins, including the SC protein C(2)M, ORD and cohesion proteins. We have found that these chromosome axis proteins regulate three distinct stages of synapsis initiation: first, at the centromeres, second at 6-8 ORD-dependent euchromatic sites, and third at 15 to 18 additional C(2)M-dependent euchromatic

sites. Double mutant analysis shows that the two types of euchromatic SC initiation can occur independently, however both pathways are dependent on cohesion proteins SMC1 and SMC3. In addition, we have genetic evidence that suggests synapsis initiates at many locations along the chromosomes, which may correspond to crossover sites. This data represents a novel model for SC initiation, which we hope to expand upon in order to gain more insight into the mechanism behind synapsis initiation. To do this, we investigated synapsis in additional cohesion defective backgrounds. When the cohesion proteins Stromalin (SCC3) and Nipped-B (SCC2) are knocked-down by RNAi we observed incomplete SC formation, similar to *c(2)M* mutants. Surprisingly, they were not needed for centromere synapsis as SMC1 and SMC3 are. We are working to determine how these two proteins and others including RAD21 (SCC1), WAPL, ECO, and PDS5 contribute to the synapsis initiation pathway. This analysis of synapsis initiation begins to unweave the intricate details and relationships that occur during meiotic prophase and gives insight into how proper chromosome segregation is accomplished.

323B

Heterologous segregation during *Drosophila* female meiosis I is preceded by heterologous coorientation at metaphase arrest. Ashley Snouffer, Wonbeom Paik, William Gilliland. Biological Science Department, DePaul University, Chicago, IL.

Heterologous Segregation is observed in flies with certain rearranged chromosomes, where non-homologous chromosomes are able to segregate away from each other at high frequency, even though they do not pair. While the first observation of Heterologous Segregation was made over 70 years ago, it has never been clear how chromosomes are able to segregate from each other without pairing. The recent discovery that female meiosis undergoes chromosome congression prior to metaphase arrest provides a new step in meiosis where the coorientation of heterologous chromosomes could take place. If heterologous segregation is a consequence of congression, then the coorientation of heterologous chromosomes at metaphase arrest should predict their eventual pattern of segregation. We have examined metaphase-arrested oocytes in flies carrying multiple compound chromosomes that undergo heterologous segregation, using chromosome-specific FISH. This demonstrated that heterologs are cooriented at the same high rates that they segregate. This demonstrates that congression provides a mechanism for establishing heterologous segregation patterns.

324C

Genome-wide functional analysis of cyclic transcription in the developing *Drosophila* wing. Liang Liang[1,2], Matthew Gibson[1]. 1) Stowers Institute for Medical Research, Kansas City, MO, USA; 2) OU program, UK.

The development of multicellular organisms relies on the coordinated control of cell division, growth and morphogenesis. Although previous studies have analyzed global cell cycle-dependent transcription using single celled systems, precisely how cell cycle-dependent processes are integrated in the more complex context of multicellular development remains unclear. Here, we report the global cell cycle-associated transcriptional profiles of *Drosophila* wing disc epithelial cells (multicellular system) and cultured S2 cells (unicellular system). With an integrative FACS-microarray technique, we identified over 600 genes with cyclic expression profiles in each context. Intriguingly, despite the common cyclic genes identified, we identified 200 genes cyclically expressed only in wing disc cells, including many core cell cycle components. Next, we explored the function of these wing disc cyclic genes by tissue-specific RNAi knockdown. Combining flow cytometry and confocal imaging, we defined 95 cyclic genes that control wing growth, wherein 16 novel cyclic genes controlled cell cycle progression in the developing wing but not in S2 cells (compared with results from S2 RNAi screens). In addition to several novel regulators of mitotic cell size and chromosome segregation, we also identified two novel cyclic genes that control Interkinetic Nuclear Migration (IKNM), a conserved process by which mitotic nuclei translocate to the apical epithelial surface during mitosis. Taken together, our studies reveal a surprising degree of plasticity of cell cycle regulation at the level of cyclic transcription, and identify several novel genes that control growth and cell proliferation in tissue development.

325A

Cytokinesis-deficient binucleation in *Drosophila* accessory gland for providing plasticity of organ size. Kiichiro Taniguchi[1], Akihiko Kokyuryo[1,2], Takao Imano[1,2], Rumi Sakata[1], Ryunosuke Minami[3], Hideki Nakagoshi[3], Takashi Adachi-Yamada[1,2]. 1) Dept. of Life Sci., Gakushuin Univ., Japan; 2) Dept. of Biol., Grad. Sch. of Sci., Kobe Univ., Japan; 3) Dept. of Biol., Grad. Sch. of Nat. Sci./Tech., Okayama Univ., Japan.

As cytokinesis theoretically follows karyokinesis during the M phase in a cell cycle, most eukaryotic cells contain only a single nucleus. Nonetheless, cytokinesis does not occur in particular kinds of cells, such as myocyte, which results in cells containing two nuclei. However, mechanisms to skip cytokinesis and the significance of binucleation are largely unknown. Here, we examined the binucleation by using the *Drosophila* adult male accessory gland, a reproductive organ producing seminal fluid proteins. All of the accessory gland epithelial cells are binucleated by cytokinesis-skipping at the final mitosis.

First, we examined the mechanisms to skip cytokinesis in binucleation. As a result, both the central spindle and contractile ring did not form during binucleation. Thereby, the activity of Rho GTPase was insufficient to drive cytokinesis completion. Moreover, we found out that Mud, a *Drosophila* NuMA, was a key regulator of binucleation, which impaired the central spindle formation and following cytokinesis. These results suggest that impairment of central spindle formation by NuMA leads to a restriction of Rho activity to skip cytokinesis in binucleation. Second, we elucidated the significance of binucleated state of cells. We compared the apical area in adult accessory gland cells between binucleated, endoreplicated and divided cells. The apical area of binucleated cells became more enlarged than that in endoreplicated mononucleate cells. On the other hand, the apical area of binucleated cells became more shrunken after mating than that of divided mononucleate cells. These results suggest binucleation is an effective strategy to provide a higher plasticity in the apical area size, which turns out that in the organ volume.

326B

Identifying mutations in the chromosomal passenger complex and associated regulators of spindle assembly. Arunika Das[1], Shital Shah[2], Kim McKim[1]. 1) Waksman Institute, Rutgers University, Piscataway, NJ; 2) University of Medicine and Dentistry, New Jersey.

Accurate segregation of chromosomes during cell division is facilitated by the formation of a bipolar array of microtubules known as the spindle. Kinesin motor proteins are known to regulate microtubule dynamics of spindle formation. Kinesin 6 family member Subito regulates bipolar spindle assembly and interacts with the components of the chromosomal passenger complex (CPC), like *Incenp* or *ial*, which encodes for Aurora B. A homozygous mutation in *subito* is synthetic lethal with a heterozygous mutation in a component of the CPC like *Incenp* or *ial*, which led us to hypothesize that *subito* may also

interact with other proteins involved in spindle assembly. We have isolated 18 EMS induced mutations in a screen, which induce synthetic lethality in a homozygous sub mutant background. These mutations may identify new genes involved in spindle assembly and microtubule organization especially in meiotic spindle assembly which is not well understood. The screen yielded mutations in at least 6 different genes, we are still testing the rest by complementation. Of the mutations, 3 were alleles of *Incenp* and 2 of *ial*. There were previously no alleles available for *ial* in Drosophila. Two other mutations *22.64* and *15.173*, both with 2 alleles, map to narrow intervals on the second chromosome containing 10 and 17 genes respectively. Both these mutations are homozygous lethal and hence in essential genes. We will characterize the role of these mutations and their interactions with *subito* in mitotic and meiotic spindle assembly.

327C

The Regulation of Microtubule Dynamics is Essential for Meiotic Spindle Organization in Drosophila Females. Sarah Radford, Andrew Harrison, Kim 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 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 absence of centrosomes, the cues controlling spindle organization remain unknown. In addition, microtubules are inherently dynamic; therefore, we hypothesized that the regulation of microtubule dynamics plays an important role in the organization of the meiotic spindle. Because many of the proteins involved in the regulation of microtubule dynamics are essential for development, much of our work has been made possible by the germline-specific RNAi technology generated by the Transgenic RNAi Project. We have shown that a member of the kinesin-13 family of microtubule-depolymerizing enzymes, KLP10A, is required for the control of meiotic spindle organization, microtubule length, and chromosome orientation. We have also shown that a second kinesin-13, KLP59D, is not required in meiosis and, surprisingly, is not essential for development. Investigation into the third Drosophila kinesin-13, KLP59C, is ongoing. We have also shown that the Drosophila CLASP homolog, Orbit/MAST, partly antagonizes KLP10A activity, suggesting that meiotic spindle organization results from a balance of microtubule dynamics. We are currently investigating several other proteins involved in the regulation of microtubule dynamics, including Patronin and Sentin, to gain further insight into how the regulation of microtubule dynamics contributes to the formation of a functional meiotic spindle. Our results thus far show that the regulation of microtubule dynamics is critical for the generation of a meiotic spindle with the capacity to effect accurate chromosome segregation.

328A

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) Department of Neuroscience, Mount Sinai School of Medicine, New York, NY; 3) Department of Developmental and Molecular 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. However, the mechanisms by which the pathway effects normal tissue homeostasis remain less well-understood. Typically, organ homeostasis engages mechanisms to ensure that variations in proliferation do not alter organ size. Thus, how the pathway integrates restricting proliferation and activating an “organ size checkpoint” remains a major unanswered question. 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”.

329B

Translationally Controlled Tumour Protein (TCTP) regulates 14.3.3s function during *Drosophila* organ development. Phuong Thao Le. KAIST, Daejeon, South Korea.

Translationally controlled tumour protein (TCTP) has drawn interests because of its potential roles in tumorigenesis. Our previous study has shown that *Drosophila* TCTP (dTCTP) is essential for organ growth by acting through Rheb GTPase in TOR signaling. However, evidence suggests that dTCTP interacts with additional signaling components. By genetic modifier screening, we have identified *14-3-3ε* and *14-3-3ζ* as potential candidate genes that interact with *TCTP*. Here we present analysis of these genetic interactions in *Drosophila* organ development. Reduced levels of either form of 14-3-3 proteins led to strong enhancement of the small eye/wing phenotype resulting from TCTP RNA interference (RNAi). In addition, TCTP co-localizes with 14-3-3 in different tissues. We also show that 14-3-3 proteins interact with other TOR signaling components like Rheb and TSC1/2 in controlling the organ growth. 14-3-3 proteins are known to bind to TSC2 at multiple, phosphorylated sites, and negatively regulate TSC1/2 function. In TSC-TOR pathway, TCTP acts antagonistically to TSC1/2. There is possibility that TCTP affects 14-3-3s and restricts the TSC1/2 activity to control organ growth. Our data suggest that TCTP is required for stabilizing 14-3-3s, thereby inhibiting the TSC complex function. This research provides new insights into the function of dTCTP in growth regulation.

330C

***uninflatable* and *Matrix metalloproteinase 1* are required for tissue specific growth in the larval trachea of *Drosophila melanogaster*.** Paulo Leal, Joshua Neff, Robert Ward. Molecular Biosciences, University of Kansas, Lawrence, KS.

Post-embryonic growth in *Drosophila* is tied to nutrition through the Insulin receptor phosphoinositide-3-kinase - Target of Rapamycin (InR/PI3K - TOR) signaling pathway. Yet specific organs show variable growth rates suggesting that organs and tissues can independently regulate growth. To identify mechanisms of tissue specific growth, we are taking a genetic approach to identify mutations that specifically alter trachea size relative to overall body size. The larval trachea system of *Drosophila* is well suited for this study as the trachea is a branched tubular organ required for gas exchange that expands

dramatically in length and diameter as the larva increases in size 1000-fold over the four day larval period. We identified mutations in *uninflatable* (*uif*) that show specific reductions in tracheal length, such that at the end of the third instar the *uif* mutant trachea are roughly half the relative length of trachea in wild type animals. *uif* encodes a large transmembrane protein with carbohydrate-binding and cell signaling motifs in the extracellular domain. It is expressed in ectodermally derived epithelial cells, but is most strongly expressed in the trachea, where it localizes to the apical plasma membrane. To our knowledge, only one other gene has a similar mutant phenotype: *Matrix metalloproteinase 1* (*Mmp1*). *Mmp1* encodes a secreted protease involved in remodeling the extracellular matrix. Mmp1 is strongly expressed in tracheal cells where it localizes to cellular junctions and to the apical surface. To explore the relationship between *uif* and *Mmp1*, we examined the expression and localization of each protein in loss-of-function mutations in each gene. Whereas Uif expression and localization are unaffected in *Mmp1* mutant trachea, Mmp1 localization is altered in *uif* mutant animals. Specifically, a large portion of Mmp1 protein is found in punctate structures in the cytoplasm. We are currently attempting to understand how *uif* regulates Mmp1 localization, and are conducting genetic rescue experiments in order to place *uif* and *Mmp1* within the InR/PI3K - TOR pathway.

331A

The role of the AP-4 transcription factor *cropped* in imaginal disc growth and regeneration. Sutton Matt, Halme Adrian. Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA.

Drosophila imaginal discs have a remarkable capacity to regenerate following damage. To better understand the genetic pathways that mediate this regenerative process, we have begun to examine the role of the AP-4 transcription factor Cropped in imaginal tissue homeostasis. We have shown that *cropped* expression is increased in damaged tissues. Using clonal analysis to examine the growth of *cropped* mutant tissues during normal development and during regenerative growth, we have found that weaker hypomorphic alleles of *cropped* exhibit regeneration-specific growth defects, whereas stronger amorphic alleles of *cropped* display defects in both regenerative growth and growth in undamaged tissues. We have found that the inhibition of amorphic *cropped* clone growth during normal disc development can be attributed to a substantial amount of cell death within these clones. We have found that *cropped* clones can be rescued by introducing a heterozygous *Minute* mutation into the genetic background, suggesting that amorphic *cropped* clones are eliminated by cell-competition. We are currently examining whether *cropped* activity regulates competitive cell interactions and whether cell-competition plays an important role during imaginal disc regeneration.

332B

Non-autonomous tumor progression driven by mitochondrial dysfunction. Shizue Ohsawa[1], Yoshitaka Sato[1], Masato Enomoto[1], Mai Nakamura[1], Aya Betsumiya[1], Tatsushi Igaki[1,2]. 1) Department of Cell Biology, G-COE, Kobe University Graduate School of Medicine, Kobe; 2) PRESTO, Japan Science and Technology Agency, Japan.

Tumor progression is accomplished by both intra- and inter-cellular cooperation of oncogenic alterations. However, the underlying mechanism of how each oncogenic mutation cooperates with other mutations to progress toward malignancy through cell-cell communications remains elusive. We performed a genetic screen in *Drosophila* for identifying genes that drive non-autonomous tumor progression. Clones of cells expressing oncogenic Ras (RasV12) result in the formation of benign tumors in eye imaginal epithelium. We introduced additional mutations in RasV12-tumors and screened for mutants that cause non-autonomous overgrowth of surrounding wild-type tissue. We identified a series of mutations that affect the function of mitochondrial respiratory chain as responsible genes of these mutants. Intriguingly, when RasV12-expressing clones were induced nearby these mutant clones, the RasV12-tumors not only overgrew but acquired metastatic ability. The molecular mechanism by which Ras activation and mitochondrial dysfunction cooperate to drive non-autonomous tumor progression will be discussed.

333C

Identification of the gene disrupted in *fried* mutants. Kimberley Seoane, Henrique Valim, Jason Morris. Dep't of Natural Sciences, Fordham University, New York, NY.

Drosophila *fried* mutants were originally isolated in a clonal screen for oogenesis defects. *fried* mutant egg chambers exhibit reduced endoreplication in nurse cells and abnormal morphology of chromosomes in nurse cells and oocytes. *fried* homozygotes arrest as larvae. Deficiency mapping and complementation testing and sequencing of candidate genes enabled us to identify the gene affected in *fried* mutants. One allele, $fried^{150}$, is disrupted by a 61 bp deletion early in the gene and is a likely candidate for a null allele. The second allele, $fried^{212}$, displays a weaker phenotype and encodes a nonsense mutation later in the protein. Mutations in this gene have not been isolated previously in Drosophila, and the closest homologs of the gene have not been analyzed in other systems. We are currently attempting to generate antibodies against Fried protein and we are carrying out experiments with rescue constructs in order to study the function of Fried in development.

334A

Regulation of the archipelago ubiquitin ligase subunit by a dynein light chain. Daniel Allyn Barron, Kenneth Moberg. Dept Cell Biol, Emory Univ Sch Med, Atlanta, GA.

Carcinogenesis is fueled by the inactivation of tumor suppressor genes, which allows for excessive cell growth and division. FBXW7 (F-box/WD protein 7) and archipelago (ago) are orthologous tumor suppressor genes in humans and Drosophila melanogaster, respectively, that encode a conserved component of an E3 ubiquitin ligase that targets pro-proliferative substrates for proteasomal degradation. FBXW7 is biallelically inactivated in a wide range of human cancers including primary endometrial, colorectal, and prostate tumors. Although several pro-proliferative targets of FBXW7/Ago-mediated degradation have been identified (e.g. Cyclin E and Myc), the upstream regulation of FBXW7/Ago has not been studied extensively. We have used Drosophila as a model to address the role of Cut Up (Ctp; aka LC8 or dlc-1), a cytoplasmic dynein light chain protein, as a potential antagonist of the Ago Ub ligase. Yeast two-hybrid data suggests that Ctp physically interacts with Ago, and loss of Ctp (due to either a hypomorphic allele or inverted repeat knockdown) leads to a reduced growth phenotype that can be dominantly modified by ago null alleles. Furthermore, dMyc, a pro-proliferative target of the Ago Ub ligase, has reduced steady-state levels in cells lacking Ctp, suggesting that Ctp acts to inhibit Ago activity. In sum these genetic and molecular data suggest there is a physiologically relevant interaction between ago and ctp in imaginal disc cells. As Ctp antagonizes the pro-apoptotic activity of the protein Bim by sequestrating Bim to dynein motor complexes (Puthalakath et al, 1999), we hypothesize that Ctp inhibits Ago-mediated turnover of pro-proliferative

substrates by binding to Ago and inhibiting its Ub ligase activity.

335B

Non-Cell Autonomous Regulation of Hippo signaling in Drosophila by the Hedgehog receptor Patched. Jacob Daniel Kagey[1,2], Jordan Brown[2], Kenneth Moberg[2]. 1) Biology, University of Detroit Mercy, Detroit, MI; 2) Cell Biology, Emory University School of Medicine, Atlanta, Ga.

We conducted a Flp/FRT based EMS screens for mutations on chromosome 2R that confer a growth advantage conditional upon a block in cell death. From this screen, we identified an allele of patched, $ptc^{B.2.13}$. Immortalized $ptc^{B.2.13}$ mutant clones lead to dramatic increases in eye and wing size, mainly by expanding wild type tissue, suggesting Ptc normally functions as a non-autonomous growth suppressor. At the molecular level, the $ptc^{B.2.13}$ mutation results in the autonomous increase of the Hedgehog pathway, including the activation of the downstream transcriptional factor, Cubitus Interuptus (Ci). Additionally, we observe an increase of several known Ci transcriptional targets, including Decapentaplegic (Dpp). This aberrant production of Dpp in $ptc^{B.2.13}$ cells creates ectopic gradients of Dpp signaling radiating from mutant clones into surrounding wild type tissue. These gradients of Dpp activate Mothers Against Decapentaplegic (pMad), with the most robust activation observed in wild type cells immediately adjacent to mutant clones. This activation spatially correlated with the increase of several targets of the potent pro-growth transcription factor Yorkie (Yki). Yki can function as a co-activator with pMad, specifically in the transcriptional activation of the pro-growth miRNA, *Bantam*. Domains of pMad activation surrounding $ptc^{B.2.13}$ clones also correspond to regions of increased cellular proliferation, suggesting the ectopic activation of pMad may be driving the overgrowth phenotype. In testing this hypothesis, we find that a single loss of function *Bantam* allele is capable of dominantly suppressing the overgrowth phenotype, suggesting a model where the clonal loss of Ptc leads to a Dpp/Yki dependent non-autonomous increase in proliferation. Given the frequent loss of the human Ptc1 gene in basal cell carcinomas and medulloblastomas, this model could have implications for the pathogenesis of a subset of human cancers.

336C

Identifying novel components of the Fat cadherin pathway. Srdjana Ratkovic, Helen McNeill. Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, Ontario.

fat is a *Drosophila* tumor suppressor gene that encodes a large cadherin involved in regulation of growth and a form of tissue organization called planar cell polarity (PCP). Fat regulates growth through its actions upstream of the Hippo kinase pathway, however the precise mechanism of its action is still unknown and very few proteins that biochemically associate with Fat have been identified. To better understand the biology of Fat signaling, I am using a proteomic screening approach to identify novel Fat interactors using affinity purification coupled with mass spectrometry. I generated flies and KC167 cells that overexpress truncated versions of tagged Fat protein, which are used as bait in these studies. Potential Fat interactors will further be evaluated for effects on growth, and how they genetically and biochemically interact with Fat.

337A

Lipid droplets control histone levels and promote mitotic fidelity in syncytial embryos. Michael A. Welte[1], Zhihuan Li[1], Dipak Manna[1], Katharina Thiel[3], Mathias Beller[2,3]. 1) Dept Biol, Univ Rochester, Rochester, NY; 2) Inst Math Modeling Biol Systems, Heinrich-Heine Univ, Düsseldorf, Germany; 3) Max Planck Inst for Biophys Chem, Göttingen, Germany.

Histones are essential for chromatin packing, yet free histones not incorporated into chromatin are toxic. Early *Drosophila* embryos contain massive amounts of maternally provided histones: What is the biological function of these histone deposits, and why do they not interfere with development? The extra-nuclear stores of histones H2A, H2B, and H2Av are bound to lipid droplets, fat storage organelles abundant in embryos. We now report that histones are anchored to droplets via the novel protein Jabba: Jabba localizes to droplets, co-immunoprecipitates with histones, and is necessary to recruit histones to droplets. *Jabba* mutant embryos lack the maternal H2A, H2B and H2Av deposits altogether; apparently, these deposits are degraded unless sequestered on droplets. In yeast, degradation of free histones prevents damage from histone overexpression. *Jabba* embryos develop grossly normally and, by translating maternal mRNAs, can reach near normal histone levels by cycle 14. Thus, new histone synthesis can be sufficient to sustain life. The maternal protein deposit nevertheless contributes to chromatin assembly: First, histones can be transferred from droplets to nuclei, as demonstrated by transplantation and photo-activation experiments. Second, reducing histone mRNA levels in *Jabba* mutants results in lethality during syncytial stages, with widespread mitotic defects. Histone sequestration on lipid droplets may additionally mitigate the toxicity of supernumerary histones: overexpression of H2Av in *Jabba* embryos results in synthetic lethality and phenotypes indicative of defects in replication and mitosis, including anaphase bridges and massive nuclear fallout. We propose that in wild-type embryos sequestration on lipid droplets buffers the histone supply: droplets provide a source of histones for chromatin assembly by shielding them from premature degradation, yet they limit the pool of free, potentially toxic histones.

338B

Psf2: A Role in Chromosome Condensation. Jeffrey P. Chmielewski[1], Laura Henderson[2], Tim Christensen[1]. 1) Biology, East Carolina University, Greenville, NC; 2) Howard Hughes Medical Institute, Janelia Farm Research Campus, Ashburn, VA.

In *D. melanogaster*, the CMG complex is a group of proteins that function as the DNA helicase during replication. The CMG complex is composed of cdc45, MCM2-7, and the GINS complex. The GINS complex is a heterotetrameric complex composed of the protein subunits Psf1, Psf2, Psf3, and Sld5. Recent research in human dermal fibroblasts shows GINS is essential for the initiation and elongation stages of chromosomal replication. Working with a null mutation, I have designed a series of experiments aimed at elucidating the function of Psf2 *in vivo*. Using phosphoH3 immunostaining and M-phase indices, I have shown that heterozygous mutants exhibit a significant M-phase delay. EdU incorporation assays in third in-star larval brains shows no significant difference in the number of cells in S-phase. However, when compared to wild type, the pattern of EdU incorporation indicates cells take longer to replicate euchromatin; possibly resulting in the improper packaging of euchromatin as heterochromatin. To corroborate the data seen in the pattern of EdU incorporation, we designed a novel technique to establish the packing ratio of salivary gland polytene chromosomes. Using this novel technique, we are able to show that heterozygous mutants exhibit a significant increase in packing ratio compared to WT. Additionally, in the later stages of egg chamber development, nurse cell nuclei display overly condense polytene chromosomes during the psuedo M-phase at the end of endocycle 5. We have evidence that indicates these instances of overly condensed chromosomes results in apoptotic egg chambers, seen later in development. We have also taken a genetic approach to further substantiate our cytological characterizations. Heterozygous Psf2 flies show a significant decrease in viability with complete homozygous lethality. Position effect variegation analysis show Psf2 enhances variegation an indication that chromosomes are overly condensed. This data, when combined, suggests Psf2 has a role during replication that ultimately determines how DNA is packaged.

339C

Multiple interactions between Heterochromatin Protein 1 (HP1) and nucleosomes. Diane E. Cryderman[1], Abd Elhamid M. Azzaz[2], Michael W. Vitalini[1], Andrew H. Thomas[1], Adrian H. Elcock[1], Michael A. Shogren-Knaak[2], Lori L. Wallrath[1]. 1) Dept Biochemistry, Univ Iowa, Iowa City, IA; 2) Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA.

The Heterochromatin Protein 1 (HP1) family is comprised of conserved nonhistone chromosomal proteins that package chromatin, regulate transcription and function in DNA repair. HP1 proteins contain an N-terminal chromo domain (CD) and C-terminal chromo shadow domain (CSD), separated by an unstructured hinge region. The association between HP1 and chromosomes is thought to involve HP1 dimerization and interaction with histone H3 di- and tri-methylated at lysine 9 (H3K9me2/3). It is not known how HP1 interacts with nucleosomes to form heterochromatin. A multi-disciplinary approach using *in silico* modeling, *in vitro* biochemistry and *in vivo* functional assays was undertaken. Molecular dynamic simulations and atomic resolution modeling revealed that human $HP1^{Hs\alpha}$ is a highly flexible protein possessing the ability to bind both adjacent and non-adjacent methylated nucleosomes within an array. Models generated *in silico* guided *in vitro* experiments showing that $HP1^{Hs\alpha}$ fosters chromatin compaction by interacting with nucleosomes within an array and enhancing interactions between nucleosome arrays. These *in vitro* findings supported *in vivo* studies in which Drosophila HP1a was found to promote interactions between distant chromosome sites, akin to inter-array interactions observed *in vitro*. These *in vivo* interactions were reduced upon expression of mutant forms of HP1a that lacked the hinge or the ability to dimerize. The dimerization mutant failed to support viability of an HP1a null. Surprisingly, flies expressing only the hinge deletion were viable and possessed nucleosome arrays characteristic of heterochromatin, suggesting the hinge is dispensable for heterochromatin formation and viability. Taken together, dimerization and the flexibility of HP1 allow for multiple interactions with nucleosomes necessary for heterochromatin formation.

340A

Understanding the Role of *Topoisomerase 2* in Chromosome Associations. Amber M. Hohl[1,2,3], Pamela K. Geyer[2], Ting Wu[3]. 1) Genetics Program, University of Iowa, Iowa City, IA; 2) Department of Biochemistry, University of Iowa, Iowa City, IA; 3) Department of Genetics, Harvard Medical School, Boston, MA.

Homologous chromosomes display associations in many organisms. In Drosophila, chromosomes are paired in all somatic cells throughout development. For many genes, the degree of homolog association influences gene expression. These effects, collectively referred to as transvection, can promote gene activation or silencing. The requirements for transvection are poorly understood. Recent studies implicated a requirement for *Topoisomerase 2* (*Top2*) in chromosome pairing. To expand our understanding of the role of Top2 in chromosome associations, we tested whether mutations in *Top2* disrupted

transvection *in vivo*. Viable heteroallelic combinations of *Top2* mutants were used to examine transvection at three classically studied loci (*yellow, white,* and *Ultrabithorax*). Transvection at *yellow* and *Ultrabithorax* lead to transcriptional activation, while mutant *zeste*[1] causes pairing-dependent gene silencing of the *white* gene. For each gene, homologous interactions between transvecting alleles were analyzed for alterations in pairing-dependent changes in phenotype. An effect was seen at two of three loci assayed. Current studies are examining chromosome interactions in larval tissues using fluorescent *in situ* hybridization. Data obtained from these studies will determine whether *Top2* is required for homolog interactions in somatic cells.

341B

HP1b is a non-essential protein enriched at TSSs that positively affects transcription. Nicole C. Riddle, Artyom A. Alekseyenko, Tingting Gu, Youngsook L. Jung, Aki Minoda, Michael Y. Tolstorukov, Mitzi I. Kuroda, Vincenzo Pirrotta, Peter J. Park, Sarah C. R. Elgin, Gary H. Karpen. Drosophila modENCODE Chromatin Group.

In *Drosophila melanogaster*, the Heterochromatin Protein 1 (HP1) family of proteins is represented by five different proteins, HP1a, HP1b, HP1c, rhino (HP1d), and HP1e. HP1a was first to be identified due to its strong association with the heterochromatic fraction of the Drosophila genome. HP1a is composed of two conserved protein domains, the chromo domain and the chromo-shadow domain, connected by a hinge domain. This basic structure is common to all HP1 proteins; however, the N- and C-terminal "tails" as well as the hinge domain vary in size. While the expression of *rhino* and *HP1e* are restricted mainly to the germline, *Su(var)205[HP1a]*, *HP1b*, and *HP1c* are expressed ubiquitously. However, they differ in their genomic distribution, HP1a associating mainly with heterochromatin, HP1c with euchromatin, and HP1b localizing to both. Here, we report the generation of *HP1b* mutants and a detailed, comparative analysis of the genome-wide localization of HP1a, HP1b, and HP1c. We find that *HP1b* is a non-essential gene, with mutants viable, fertile, and lacking morphological defects. *HP1b* mutations enhance variegation of an *hsp70-white* reporter. HP1b is found primarily associated with transcription start sites, similar to HP1c. In S2 cells, approximately 6000 genes are associated each with HP1b and HP1c. Of these, approximately 5000 genes are enriched for both HP1 family members. Genes targeted by HP1b and HP1c occur both in euchromatin and heterochromatin and are enriched for GO terms related to "development" and "regulation". Interestingly, approximately 1300 of these genes also associate with HP1a, indicating that all three non-germline HP1 family members occur together at a significant number of sites. On-going experiments explore how mutations in HP1 family members influence the localization patterns of the other paralogs and will shed light on the mechanisms regulating the genomic enrichment patterns.

342C

Investigating the impact of an invading B chromosome on nuclear dynamics in N. vitripennis. Megan Swim, Patrick M. Ferree. Keck Science Department, Scripps College, 925 N. Mills Ave. Claremont, CA.

Supernumerary B chromosomes are nonessential extra chromosomes present in numerous plant and animal species. Some B chromosomes are capable of imposing strong positive or negative effects. An extreme example this is the Paternal Sex Ratio (PSR) chromosome in the jewel wasp Nasonia vitripennis. PSR is transmitted through the sperm at a frequency near 100%. PSR is believed to imprint the paternal chromatin during spermatogenesis such that it becomes hyper-condensed and fails to resolve into distinct chromosomes during the first embryonic mitosis. Nasonia, like all hymenopteran insects, exhibits haplodiploid reproduction, in which females develop as diploid individuals from fertilized eggs while males develop as haploids from unfertilized eggs. By destroying the paternal chromosomes, PSR converts fertilized embryos that should become females into haploid males, thus selfishly facilitating its own propagation. Two vital questions regarding PSR transmission are: (i) what is the nature of the epigenetic modification that PSR inflicts upon the paternal chromatin and how does this occur? And (ii) how does PSR evade this lethal effect despite its close association with paternal chromatin during spermatogenesis. To address these questions, we engineered several FISH probes that have allowed us to follow PSR during early development. We found that PSR localizes to the outer periphery of the paternal pronucleus in a position that is often directly adjacent to the maternal pronucleus. Additionally, PSR escapes the doomed paternal chromatin during anaphase of the first mitosis in order to associate with the viable female chromosomes. To investigate the localization of PSR at the periphery of the paternal pronucleus, we used FISH to examine PSR in developing sperm. Intriguingly, we discovered that PSR localizes to the extreme apical tip of the elongated sperm nucleus at near perfect frequencies. PSR also appears to reorder the autosomes within the nucleus. These findings provide new and exciting insights into how selfish genetic elements can utilize host nuclear processes for their survival.

343A

Characterizing Chromosome Territory Formation in Drosophila Primary Spermatocytes. Sheng (Jimmy) Tang[1], Tom Hartl[1,2], Matthew Scott[1,2]. 1) Stanford University, Stanford, CA; 2) Stanford University School of Medicine, Stanford, CA.

In the nuclei of interphase cells, chromosomes occupy distinct, non-overlapping domains called chromosome territories (CTs). Proper territory formation is necessary for the expression and regulation of many genes. Aberrant CTs are often seen in the cells of apoptotic or cancerous tissues. In addition, despite having different numbers of chromosomes, many species ranging from flies to mammals possess cells where CTs can be observed. In the nuclei of primary spermatocytes of Drosophila, the three major chromosomes form three distinct, triametrically opposed CTs. Using nuclear DNA staining and confocal microscopy, we have developed a system to qualitatively and quantitatively characterize CT formation within these cells. We have found that in spermatocytes where the second and third chromosomes are fused at a single centromere, the two fused chromosomes form two distinct territories. This finding suggests that CT formation is chromosome-specific, such that each chromosome recognizes "self" and "non-self" chromatin during territory compaction. Immediate studies will focus on using translocation constructs to determine whether CT formation is specific to chromatin at the sub-chromosomal level. In addition, using a germline-specific GAL4 driver, we have initiated an RNAi screen to search for specific protein factors that are responsible the formation or maintenance of CTs in spermatocytes.

344B

Telomere dynamics and organization in early embryonic development. Natalia Wesolowska[1,2], Yikang Rong[1]. 1) Lab of Biochemistry and Molecular Biology, National Institutes of Health, Bethesda, MD; 2) NIH Graduate Partnership Program with the CMDB Program, Johns Hopkins University, Baltimore, Bethesda.

Telomeres are specialized structures that delineate the ends of linear chromosomes. When their function is compromised, natural ends resemble broken DNA and can be subjected to repair, resulting in chromosomal fusions and genomic instability. We would like to address a major aspect of nuclear dynamics

of telomeres as an important chromosomal landmark - whether telomeres organize in the nucleus based on clustering. The four final cell cycles before the onset of cellularization in the fly development take place at the surface of the embryo. This time-period presents a perfect experimental setting for simultaneous imaging of many synchronized nuclei through consecutive divisions. To follow telomeres in vivo in this system we live-imaged fluorescently-labeled telomere capping proteins. We gathered evidence that telomeres do assemble into 4-6 clusters per nucleus during these early divisions. In light of the long-established findings from S. cerevisiae that yeast interphase telomeres cluster at the nuclear periphery, our results suggest that clustering may be a feature conserved through evolution. We propose that this behavior is mediated through one of the following mechanisms: 1) pairing of the homologous chromosomes, 2) self-affinity of homologous telomeric sequences, 3) pairing of telomeres of p and q arms of each chromosome, 4) interactions of telomeric proteins (with each other or the nuclear envelope). Through fluorescence in situ hybridization (FISH) to a natural telomere sequence as well as to a specifically-marked telomere, we have so far determined that homology does not play a major role in clustering, eliminating possibilities 1 and 2. Through further detailed studies we hope to distinguish between the remaining possibilities and elucidate how clustering occurs in the fly.

345C

Role of Drosophila's HKMTs in the recruitment of HP1. Margarida Figueiredo, Anna-Mia Johansson, Jan Larsson. Department of Molecular Biology, Umeå University, Umeå, Umeå, Sweden.

The fourth chromosome of Drosophila melanogaster is largely heterochromatic being enriched in satellite repeats and in DNA from transposable elements. Despite its heterochromatic properties it has a gene density similar to the major chromosome arms. The expression of almost all chromosome 4 genes is fine-tuned by at least two different proteins: Painting of Fourth (POF) that stimulates transcription and Heterochromatin Protein 1 (HP1) that represses transcription. It has been shown that POF and HP1 bind interdependently to this chromosome and that the binding and spreading of POF depends on heterochromatin. POF and HP1 colocalize in exons of active genes and HP1 binds additionally to promoters of the active genes. HP1 is recruited by H3K9me2 and H3K9mme3, which is performed by the HKMTs SU(VAR)3-9, SETDB1 and G9a. It has been proposed that SU(VAR)3-9 is responsible for H3K9me in the chromocentre whereas SETDB1 acts mainly on the 4th chromosome. To understand the roles of these HKMTs on the recruitment of HP1 we have performed ChIP-on-chip and immunostainings experiments in Su(var)3-9, Setdb1, G9a and Pof mutants and analysed the binding of POF, HP1, H3K9me2 and H3K9me3. We found that POF and SETDB1 are essential for recruiting HP1 to the gene bodies on the 4th chromosome whereas SU(VAR)3-9 is essential for recruiting HP1 to the centromeric regions of all chromosomes. The region-specific recruitment of HP1 by SETDB1 and SU(VAR)3-9 is explained by the finding that both these HKMTs are essential for region-specific production of both H3K9me2 and H3K9me3. G9a doesn't seem to be essential for H3K9me2, H3K9me3 and neither for HP1 recruitment anywhere in the genome. HP1 found in promoters of genes on the 4th and genes on other chromosomes seems to be independent on all the HKMTs and on both H3K9me2 and H3K9me3. Our results also show that in the absence of SETDB1 or SU(VAR)3-9 there is a relocalization of HP1 and of both H3K9me2 and H3K9me3 in other places.

346A

Co-ordinate regulation of heterochromatic genes in *Drosophila melanogaster* males. S.Kiran Koya, Xinxian Deng, Ying Kong, Victoria Meller. Dept of Biological Sciences, Wayne State University, Detroit, MI.

Drosophila dosage compensation equalizes the expression of X-linked genes between males and females. Dosage compensation is mediated by the MSL (Male-Specific Lethal) complex. The MSL complex is composed of five proteins (MSL1, MSL2, MSL3, MOF and MLE) and two non-coding RNAs, *roX1* and *roX2*. The *roX* RNAs are essential for normal targeting of the MSL complex along the length of the single male X chromosome. Chromatin modification by the MSL complex results in a two-fold elevation of X-linked gene expression. Microarray analysis of *roX1 roX2* males revealed decreased expression of X-linked genes, confirming the role of these RNAs in dosage compensation. Surprisingly, expression from the autosomal heterochromatic genes, including the entire 4th chromosome, is also reduced in *roX1 roX2* males. Expression of these genes is unchanged in *roX1 roX2* females. We used microarray and quantitative real time PCR to measure gene expression in male larvae mutated for each of the *msl* genes. Our findings revealed that MSL1, MSL3 and MLE, but not MSL2, are required for heterochromatic gene regulation. This suggests that the MSL proteins participate in two distinct complexes, one that regulates the male X chromosome, and one that is necessary at heterochromatic genes. We postulate that a complex formed of *roX* and a subset of the MSL proteins may be necessary for correct establishment of heterochromatin in male embryos. We are using a genetic assay to establish the critical period for *roX* during development, and are using chromatin immuno-precipitation (ChIP) to measured enrichment of MSL1 and MSL3 proteins at heterochromatic genes during embryogenesis. Our results suggest a fundamental sex-specific difference in heterochromatin in *D. melanogaster*. My future work will focus on the molecular mechanism by which this regulation occurs.

347B

Modifiers of X recognition: exploring the secrets of sex chromosome identity. Debashish Menon, Victoria Meller. Department of Biological Science, Wayne State University, Detroit, MI.

Dosage compensation modulates expression of an entire chromosome to address the potentially fatal imbalance in X-linked gene dose between males and females. In male Drosophila, the X chromosome is up-regulated through the activity of the Male Specific Lethal (MSL) complex, consisting of five proteins and two large, non-coding RNA on the X (*roX*) transcripts (*roX1* and *roX2*). Simultaneous mutation of *roX1* and *roX2* reduces X-localization of the MSL proteins, lowers X chromosome expression and reduces male survival. The Y chromosome is a potent modifier of the *roX1 roX2* phenotype. Examination of this effect led us to postulate a role for small RNA in dosage compensation. A directed screen of small RNA pathways revealed one that affects X chromosome recognition and interacts genetically with *roX1 roX2* mutations. Our findings suggest that small RNA cooperates with the MSL complex to establish X-chromosome binding. Together these processes may underlie X chromosome identity.

348C

The H3K36 Demethylase KDM4A is a Novel Regulator of Heterochromatin Organization and Dynamics. Serafin U. Colmenares[1,2], Sasha Langley[1,2], Cameron Kennedy[1,2], Joel Swenson[1,2], Irene Chiolo[1,2], Gary Karpen[1,2]. 1) Genome Dynamics, Life Sciences Division, Lawrence Berkeley National Lab, Berkeley, CA; 2) Molecular and Cell Biology, University of California, Berkeley, CA.

Heterochromatin comprises 30% of the *Drosophila melanogaster* genome and is characterized by highly-condensed, repetitive DNA enriched for

Heterochromatin Protein 1a (HP1a). Though traditionally associated with gene silencing or position-effect variegation, heterochromatin also contains many genes that are transcriptionally active despite high levels of HP1a. KDM4A, a member of the jumonji family of protein demethylases exhibits specificity for the tri- and di-methylated forms of H3K36 and binds HP1a. In this study, we show that KDM4A is enriched in heterochromatin, particularly over active genes, and functions as a suppressor of variegation of reporter genes. We further show that KDM4A exhibits differential effects in H3K36 methylation levels between euchromatic and heterochromatic genes, and that loss of KDM4A alters organization of the heterochromatin landscape. Surprisingly, we find that KDM4A also plays a role in the clearing of DNA repair foci from heterochromatin, which is thought to proceed through movement of damaged heterochromatic DNA into euchromatin in a HP1a-dependent manner. We propose that KDM4A functions to regulate heterochromatin organization and dynamics of HP1a complexes.

349A

Functions of the RNAi system and heterochromatin components in heterochromatin formation. Tingting Gu, Sarah Elgin. Biology, Washington University, St Louis, MO.

The RNAi system is believed to be involved in post-transcriptional silencing of transposable elements (TEs) both in germline and somatic cells, but whether it also operates through a chromatin-based transcriptional silencing mechanism in Drosophila is not clear. To explore whether and when the RNAi system and other heterochromatin components are essential for silencing by heterochromatin formation, we use the GAL4-UAS system to knock down genes specifically in early embryos, when heterochromatin is first observed, or at later stages, to study their function in initiation and maintenance of heterochromatin during development. We focus on PIWI (piRNA system), HP1a (heterochromatin component), and EGG (histone methyltransferase). Using the germline specific nos-GAL4 driver and the shmiR hairpins produced by the Perrimon lab, we are able to knock down the mRNA levels of these proteins in 1.5-3 hr early embryos (the critical time for heterochromatin initiation); in all three cases this leads to elevated expression of hsp70-lacZ PEV reporters inserted in the Y chromosome or at the pericentric region of 3L in both 3rd instar larvae and adult flies, suggesting that early depletion of PIWI, HP1a or EGG has a long-lasting effect in later stage animals. ChIP-qPCR assays show that the increased expression of hsp70-lacZ is coupled with HP1a depletion on the hsp70 promoter region, arguing that this long-lasting effect is chromatin-based. Knock down of two of these protein products in the developing eye (using the eye lineage-specific ey-GAL4 driver) results in loss of silencing of the wm4 reporter, suggesting that HP1a and EGG, but not PIWI, are also crucial in the maintenance of heterochromatin structure in developing animals. On-going experiments are examining the distribution of HP1a and H3K9me2 in piwi mutant animals, to look in detail at the impact of PIWI on heterochromatin. Our efforts to dissect the roles of RNAi and heterochromatin components in different developmental stages will shed light on how heterochromatin is established and maintained.

350B

dSet1 acts as the main global H3K4 di- and tri-methyltransferase throughout Drosophila development. Graham Hallson, Robert E. Hollebakken, Taosui Li, Monika Syrzycka, Inho Kim, Shawn Cotsworth, Kathleen A. Fitzpatrick, Donald A. R. Sinclair, Barry M. Honda. Dept MBB, Simon Fraser Univ, Burnaby, BC.

In eukaryotes, the post-translational methylation of H3 lysine 4 (H3K4) is catalysed by the Set1 and MLL classes of histone methyltransferases (HMTs), and correlates with active transcription and open chromatin. The MLL classes of HMTs Trithorax (Trx) and Trithorax-related (Trr), were thought to be responsible for this modification in Drosophila, along with possible contributions from the trithorax group protein Ash1. In our efforts to functionally annotate essential genes within the centric heterochromatin of the Drosophila third chromosome, we have linked a genetically defined heterochromatic locus (*lethal 5/l(3L)h5*) to the Drosophila ortholog of *set1* (*dSet1*). Surprisingly, we observe that dSet1 acts as the main H3K4 di- and trimethylase throughout development: levels of di- and trimethyl H3K4 are significantly reduced in *dSet1* mutants but not in animals carrying mutations in or expressing dsRNAs targeting *trr*, *trx*, or *ash1*. We also provide biochemical evidence that dSet1 interacts within an evolutionary conserved protein complex (COMPASS), and demonstrate the functional requirement of other members of this complex for H3K4 methylation. Our results establish a model system for studying the functional roles of and mechanisms underlying H3K4 methylation in animal development.

351C

Loss of heterochromatic repression with age in Drosophila. Nan Jiang, Guyu Du, Ethan Tobias, Stephen Helfand. Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI.

In the nucleus, chromatin structure is an essential player in regulation of gene expression and maintenance of genome stability. It has recently been observed that certain chromatin marks undergo changes in distribution in aged organisms, raising a hypothesized link between the chromatin changes and the age-related dysregulation of genes. To test the possible effect of these chromatin changes on gene regulation in flies, we used an inducible reporter gene residing near heterochromatin to measure its expression in individual cells from different tissues during aging. With age there is a strong increase in reporter gene expression in diverse tissues, suggesting a loss of heterochromatic repression in those genomic regions. Furthermore, this age-related increase in loss of gene silencing is delayed by calorie restriction, an intervention that is shown to extend the life span. These results provide evidence for a change in gene regulation with age resulting directly from the changes of chromatin, highlighting the role of chromatin in the aging process.

352A

Separation of stem cell maintenance and transposon silencing functions of Piwi protein. Mikhail S. Klenov, Olesya A. Sokolova, Evgeny Y. Yakushev, Sergey A. Lavrov, Vladimir A. Gvozdev. Dept Molecular Gen of Cell, Inst Molecular Genetics, Moscow, Russia.

piRNAs and Piwi proteins have the evolutionarily conserved function of silencing of repetitive genetic elements in germlines. The founder of the Piwi subfamily, Drosophila nuclear Piwi protein, was also shown to be required for the maintenance of germline stem cells (GSCs). It remained unknown whether the failure of GSC maintenance is related to transposon derepression or GSC self-renewal and piRNA silencing are two distinct functions of the Piwi protein. We have revealed a new mutation piwiNt removing the nuclear localization signal of the Piwi protein. piwiNt females retain the ability of GSC self-renewal and a near to normal number of egg chambers in the ovarioles but display a drastic transposable element derepression and nuclear accumulation of their transcripts in the germline. piwiNt mutants are sterile most likely due to the disturbance of piRNA-mediated transposon silencing. Analysis of chromatin modifications in the piwiNt ovaries indicated that Piwi causes chromatin silencing only of certain types of transposons, whereas others are repressed in the

nuclei without their chromatin modification.

353B

Functional Characteristics of HP1a. Deanna L Mendez[1], Sepideh Khorasanizadeh[2], Sarah CR Elgin[1]. 1) Department of Biology, Washington University, CB-1137, St. Louis, MO 63130; 2) Sanford-Burnham Medical Research Institute, 6400 Sanger Road, Orlando, FL 32827.

Eukaryotic genomes are roughly partitioned into heterochromatin, gene-poor domains that remain highly packaged throughout the cell cycle, and euchromatin, gene-rich domains that are more accessible for transcription. Heterochromatic silencing is thought to be essential to maintain the integrity of the genome, minimizing transposition of endogenous DNA transposons and retroviruses found in eukaryotic genomes. Heterochromatin Protein 1a (HP1a) plays a key role in establishing and maintaining heterochromatin structure. HP1a binds to a wide array of protein partners among which are HP2, a non-histone chromosomal protein and PIWI, an RNA binding protein. We have found that HP2 binds 66-fold more tightly to HP1a compared to HP2 in a fluorescence polarization assay, consistent with their predicted roles. This differential affinity corresponds to a more extensive binding surface for HP2 compared to PIWI, as shown by mapping the chemical shift perturbations of the HP1a residues in a complex with HP2 or PIWI respectively. In addition, deleting the HP1a C-terminal extension differentially impacts HP2 and PIWI binding. We are continuing to investigate the binding surface of HP1a by systematically mutating hydrophobic patches with the goal of identifying the patch responsible for the preferential binding of HP2. Our results are consistent with a model where heterochromatin is initiated through a transient interaction between HP1a and PIWI, but once initiated, heterochromatin is maintained through the more stable interaction between HP1a and HP2. Thus HP1a has a number of built in mechanisms to discriminate between its binding partners as a means to coordinate its various functions. This research is supported by NIH GM068388 to SCRE and GM 070558 SK.

354C

Identifying Proteins that Interact with Drosophila melanogaster Heterochromatin Protein 2 (HP2) and Characterizing Their Contribution to Heterochromatin Formation. Patrick C. Ng[1], Elizabeth E. Slawson-Tempel[1], Hien P. Nguyen[2], Chris D. Shaffer[1], Sarah C. Elgin[1]. 1) Biology, Washington Univ, St. Louis, MO; 2) Biochemistry and Molecular Biology, Saint Louis Univ, St. Louis, MO.

Heterochromatin Protein 2 (HP2) interacts and co-localizes with Heterochromatin Protein 1 (HP1a) within D. melanogaster chromatin, and is itself involved in heterochromatin formation. Several mutations in the HP2 gene cause suppression of position effect variegation (PEV), a loss of reporter gene silencing. Of the 17 HP2 mutations recovered, three missense mutations, 288, P2763L, and 230, have been identified, one each in exons 6, 8, and 9 of HP2; the latter two have been selected for further study. We postulate that interactions between HP2 and its binding partners at the sites of these mutations impact heterochromatin formation. A Yeast-2-Hybrid (Y2H) mating screen was utilized to find proteins potentially interacting with HP2 exon 9, and identified 37 protein-coding clones from the D. melanogaster library. None of these clones displayed a loss of protein interaction with the mutant form of HP2 exon 9 relative to its wild type counterpart. However, a Y2H screen did identify interacting proteins that distinguish between the wild type and missense mutation in HP2 exon 8. We examined one of these proteins, *cheerio*, for possible Su[var] effects by comparing expression of a *lacZ* reporter in D. melanogaster stocks with wild type or mutant *cheerio*. In these experiments the *lacZ* gene has been juxtaposed with a region of heterochromatin to produce PEV. Differences in the β-galactosidase activity are observed qualitatively in tissue staining and quantitatively in whole fly assays. Differences in wild type and mutant suppression of PEV for *white* activity are observed in another exon 8 interacting protein, *sinuous*. The results indicate that *cheerio* and *sinuous* are associated with gene silencing due to heterochromatin formation. Supported by grant GM068388 to SCRE and a WU/HHMI SURF to PCN.

355A

Studying the functions of the hybrid lethality proteins- LHR and HMR. Satyaki P. Rajavasireddy, Nathan L Clark, Tawny Cuykendall, Shuqing Ji, Hojoong Kwak, Daniel Barbash. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

The Dobzhansky-Muller (DM) model suggests that hybrid incompatibilities are caused by negative epistasis between divergent genes among sister species. However, this model does not suggest a cause for the interspecific divergence between these hybrid incompatibility (HI) genes. Further, it makes no prediction for the molecular mechanism underlying HI. Our lab is utilizing hybrids of *D. melanogaster* mothers and *D. simulans* fathers to address these questions. In this system, hybrid male progeny die as larvae. This lethality is caused in part, by two rapidly evolving genes- *Lethal hybrid rescue* (*Lhr*) and *Hybrid male rescue* (*Hmr*). We seek to understand the functions and interactions of *Lhr* and *Hmr* in *D.melanogaster* in order to answer the questions of what is causing them to rapidly evolve and how they cause lethality in hybrids. *Hmr* encodes a putative nuclear protein necessary for normal levels of female fertility in *D. melanogaster*. LHR is an HP1 interacting protein with extensive localization to pericentric heterochromatin and the telomeres. We have previously shown that both *Lhr* and *Hmr* are required for normal levels of female fertility. We report here that *Lhr* and *Hmr* orthologs show highly correlated rates of evolution along different *Drosophila* lineages suggesting that they may be coevolving. These signatures of co-evolution suggest that LHR and HMR may have similar functions. We make multiple observations that support this model. First, mutations in both genes affect female fertility. Second, we find that LHR and HMR can be co-immunoprecipitated showing that they are members of the same complex. Third, we find that both HMR and LHR, localize to pericentric heterochromatin and the telomeres. However, we find that HMR additionally localizes to the nucleolus. We are now carrying out experiments to test if LHR and HMR affect either structure or function at pericentric chromatin, at the telomeres or the nucleolus.

356B

A dissection of Mcm10's functions in *D. melanogaster*. Michael C. Reubens, Casi Strickland, Tim W. Christensen. Biology Dept, East Carolina University, Greenville, NC.

Highly efficient DNA replication is essential for the accurate transmission of genetic material from cells to their progeny; likewise, the maintenance of epigenetic chromatin states is essential for the faithful reproduction of the transcriptional state of the cell. Improper regulation, and coordination, of these essential processes can result in genomic instability, which can manifest in disease or potentially the death of the organism. It is becoming more apparent that these two processes are linked through interactions between DNA replication proteins and chromatin associated proteins. Recently our lab demonstrated that Mcm10 not only plays a role in DNA replication, but also has a role in heterochromatic silencing and chromosome condensation; thus the *D. melanogaster* homolog Mcm10 provides an excellent subject to study the connections of these two essential processes. Interaction studies in yeast, as well as phenotypic and genetic analyses in Drosophila, imply that the conserved C-terminus is important for the many interactions carried out by this promiscuous

protein. Therefore, our investigation of Mcm10 in *Drosophila* has continued using a collection of three truncation alleles and over 20 missense mutations generated via a Tilling screen; as well as four deletion alleles resulting from imprecise P-element excisions. This collection of mutants will allow for an in-depth dissection of this conserved protein's functions through the analysis of mitotic chromosome phenotypes; EdU incorporation analysis; and evaluation of chromatin dynamics using PEV analysis, polytene chromosomes, and ovarian tissues. Genetic analyses such as complementation testing, unlinked non-complementation screens, and yeast two-hybrid analysis will also allow for the evaluation of changes in interactions resulting from these mutations as well. Throughout this study, we intend to elucidate the domains of the protein responsible for its biological functions, in hopes of better understanding Mcm10's roles in these essential biological processes, as well as replication and chromatin biology in general.

357C

HP1a Mediates the DNA Damage Response in Heterochromatin. Joel Swenson[1,2], Serafin Colmenares[2], Irene Chiolo[2], Cameron Kennedy[2], Sylvain Costes[2], Gary Karpen[2]. 1) Molec & Cell Biol, Univ California-Berkeley, Berkeley, CA; 2) Genome Dynamics, Lawrence Berkeley National Lab, Berkeley, CA.

Heterochromatin is characterized by the presence of Heterochromatin Protein 1a (HP1a) and is enriched for repeated sequences. Improper repair of DNA double-strand breaks (DSBs) in repetitive elements leads to expansion or contraction of sequences, translocations, or aneuploidy. Studies from our lab (Chiolo et al.) have shown that DSBs in heterochromatin are repaired differently from DSBs in euchromatin. We identified several components of this pathway, but more information about the molecular components of this pathway are required to better understand this process. To address this we performed a genome-wide RNAi screen to identify components involved in the relocalization of heterochromatic DSBs to euchromatin using pH2Av and HP1a immuno-fluorescence. Data from our lab and the literature suggest that HP1a plays a key role in regulating the repair of DSBs in heterochromatin via the homologous recombination (HR) pathway. As a functional secondary analysis we purified HP1a before and after damage and showed a change in associated proteins. These proteins were identified by mass spectrometry analysis and several of these proteins are shown to be novel heterochromatin components. By comparing the candidates identified by the biochemical approach and genome-wide RNAi approach we aim to identify components of the HP1a complexes involved in the heterochromatic DNA damage response as well as a larger subset of proteins involved in this response.

358A

Chromatin remodeling during aging and dietary restriction in Drosophila melanogaster. Jason G. Wood[1], Peter V. Kharchenko[2], Sara Hillenmeyer[1], Chengyi Chang[1], Meyrolin Garcia[1], Priyan Wickremesinghe[1], Nan Jiang[1], Peter J. Park[2], Nicola Neretti[1], Stephen L. Helfand[1]. 1) Molecular Bio, Cell Bio, and Biochemistry, Brown University, Providence, RI; 2) Center for Biomedical Informatics, Harvard Medical School and Children's Hospital, Boston, MA.

Epigenetic changes during development have been widely studied, but relatively little is known about changes in chromatin structure that take place during aging. We examined the epigenetic state of chromatin and its effect on gene expression during aging in adult Drosophila at both the whole genome and the cellular level, and found dramatic reorganization of chromosomal regions with age. We observed a robust ChIP-seq enrichment of heterochromatin protein HP1 as well as the H3K9me3 histone modification at the pericentric heterochromatin, the 4th chromosome, and islands of facultative heterochromatin throughout the genome in young adult fly heads. In older animals, there is a decrease in HP1 and H3K9me3 signal in heterochromatic regions as compared to euchromatic regions, resulting in a reduction in enrichment at characteristic heterochromatin loci. However, by Western blot we also observe a significant increase in overall H3K9me3 signal with age, indicating the decreasing enrichment seen with age likely stems from an increase in H3K9me3 marks in euchromatin rather than a reduction in heterochromatin. Interestingly, we also observed a similar decrease in enrichment when flies were reared on a dietary restriction regime, an intervention that extends lifespan, as compared to controls on a richer food source, suggesting that the changes observed in heterochromatin may be an adaptive rather than a deteriorative effect of aging. In addition, we performed whole transcriptome analysis using mRNA-seq and observed a number of age and diet-related changes in gene expression. Preliminary experiments suggest that these changes in expression may be coupled to the observed changes in chromatin structure, especially at heterochromatic loci.

359B

Invadolysin plays a role in the functioning of the SAGA complex. Michal M. Janiszewski[1], Shubha Gururaja Rao[2], Edward Duca[1], Margarete M.S. Heck[1]. 1) University/BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom; 2) Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, California, USA.

In this study, we analyse the role of invadolysin, a novel and essential metalloprotease, in chromosome condensation through its interaction with the SAGA (Spt-Ada-Gcn5-Acetyltransferase) complex. We have previously shown that invadolysin interacts genetically with *non-stop* (*not*), a de-ubiquitinating enzyme that has recently been shown to be a component of the SAGA chromatin-remodelling complex. Both *invadolysin* and *non-stop* mutants exhibit phenotypic similarities in terms of diploid and polyploid chromosome structure abnormalities and accumulation of H2Bub1 and H3K4me3 histone modifications. We also examined the localization of both H2Bub1 and H3K4me3 in greater detail in wild type polytene chromosomes from salivary glands. Staining for H2Bub1 and H3K4me3 was noticeably diminished in the polytene chromosome chromocentre, suggesting that these two modifications are not associated with heterochromatin. In addition, H2Bub1 with H3K4me3 showed a striking lack of co-localization on chromosomes arms, despite their general 'co-appearance' in cells. Intriguingly, whole mount immunostaining of $not^{1}/IX\text{-}14^{1}$ transheterozygous salivary glands revealed that H2Bub1 accumulates in the cytoplasm, rather than the nucleus. As the SAGA complex exhibits both DUB and HAT activity, it is thus significant that mutants in other SAGA subunits (*gcn5*, *ada2b* and *sgf11*) suppress an invadolysin-induced rough eye phenotype. Taken together, our data suggest that invadolysin may act to regulate both the DUB and HAT activities of the SAGA complex.

360C

Investigating the Potential Interaction of SIN3 with Methionine Metabolism. Mengying Liu, Valerie L. Barnes, Lori A. Pile. Department of Biological Sciences, Wayne State University, Detroit, MI.

SIN3 is a master transcriptional scaffold and corepressor capable of transcription repression via associated histone deacetylases (HDACs) (Adrienne et al., 2009). Our laboratory has previously found that a histone H3K4 demethylase named LID also co-immunoprecipitates with SIN3. This indicates that SIN3 may regulate methylation in addition to acetylation. Given that methionine is the major source of the methyl group for methylation (Cellarier et al., 2003),

the interaction between SIN3 and methylation has led us to examine the connection of SIN3 to methionine metabolism. RNA interference (RNAi) induced knockdown (KD) of SIN3 results in a low cell density in *Drosophila* cultured cells (S2 cells) and a curly wing phenotype in adult *Drosophila* wings. The adult wings are also smaller and have fewer cells. These results suggest that reduction of SIN3 results in a reduction in cell proliferation. Moreover, RNAi KD of some methionine pathway genes suppresses the SIN3 KD curly wing phenotype in adult *Drosophila*. These interesting data lead us to investigate the potential changes of cell proliferation in S2 cells resulting from RNAi KD of some methionine metabolic enzymes. Our results show that in S2 cells, RNAi KD of some methionine metabolic enzymes leads to loss of cell proliferation, similar to the effect seen in SIN3 KD cells. But RNAi KD of some methionine pathway genes does not suppress the SIN3 KD phenotype in S2 cells. Given the finding that RNAi KD of some methionine pathway genes suppresses the SIN3 KD phenotype in adult *Drosophila* but not in S2 cells, we are currently examining whether there are some specific genes regulated by both acetylation and methylation during development. In addition, we are determining whether RNAi KD of some methionine pathway genes or SIN3 leads to potential global histone methylation changes in S2 cells. Taken together, these data imply a connection between SIN3 and the regulation of biological methylation and cellular proliferation.

361A

The role of Lid in mediating the cellular response to oxidative stress. Xingyin Liu, Christina Greer, Julie Secombe. Department of Genetics, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY.

Drosophila Lid and its four human orthologs, KDM5A-D, are developmentally and clinically important: Lid is an essential gene in Drosophila, overexpression of human KDM5A and KDM5B are associated with cancer, and loss of KDM5C causes mental retardation. Yet the basic mechanisms by which this family of proteins function in vivo remain unknown. Lid/KDM5 proteins have multiple domains implicated in chromatin-mediated transcriptional regulation, including a Jumonji C (JmjC) lysine demethylase domain and histone binding PHD fingers. Using these different domains, Lid can both read and erase the histone code, suggesting that it regulates transcription by several mechanisms. To investigate the biological role of Lid/KDM5 proteins in vivo, we are using Drosophila to take a whole genome (microarray) approach combined with bioinformatics assays to identify Lid target genes. Of the 18,500 genes represented on the array, a total of 1049 genes were expressed differentially in lid homozygous mutants compared to wildtype, and that 345 genes were expressed differentially in response to Lid overexpression. Gene ontology analysis revealed a significant enrichment of genes involved in the response to oxidative stress and longevity. Consistent with this, we find that modulating Lid levels confers resistance or sensitivity to the oxidative stress agent paraquat when overexpressed or reduced by RNAi, respectively. ChIP assays using larvae with Lid-overexpression demonstrate that Lid is recruited to the promoter regions of the stress response genes Prx2540-2, Hsp22, Hsp67Bb and Glaz. In addition, we have shown a direct physical interaction between Lid and heat shock factor (HSF), a key stress response transcription factor. Our current working model is that Lid is an essential component of the cellular response to oxidative stress and that it is recruited to promoters via HSF to activate transcription.

362B

Epigenetic Regulation of Replication Origins. Neha P. Paranjape, Jun Liu, Brian R. Calvi. Department of Biology, Indiana University, Bloomington, IN.

Origins of DNA replication are bound by a pre-Replicative complex (pre-RC) that is activated to initiate replication during S phase. It is not known, however, how specific genomic loci are specified to be pre-RC binding sites; a DNA consensus sequence has not been found in metazoans. Moreover, the genomic location of pre-RC binding sites and their activation during S phase changes during development. Evidence suggests that chromatin plays a key role in specifying which origins are active in different cells. To investigate the role of chromatin in origin regulation, we are using the developmental gene amplification in *Drosophila* ovary as a model system for origins. We showed that nucleosome acetylation influences the selection and activity of these origins in follicle cells late in oogenesis. The mechanism by which nucleosome modification regulates origins remains unclear. Here, we present our new high-resolution ChIP data, which reveals new aspects of the chromatin landscape at origins and its relationship to the pre-RC and origin activity. Histones are highly acetylated on multiple lysines specifically when the origin is active, suggesting that multiple HATs regulate the origins. At the 3rd and X chromosome amplicon origins, peak acetylation is adjacent to a preferred replication initiation site, suggesting that histone acetylation may facilitate pre-RC binding or activation. Moreover, we have found that acetylation on some lysines is dependent on the pre-RC itself, suggesting that the pre-RC may recruit multiple co-activators, analogous to transcription factor regulation of promoters. Immunofluorescent and ChIP data indicate that histone variants H3.3 and H2Av, known components of hyperdynamic nucleosomes, are also enriched at the amplicon origins. We are using molecular and genetic methods to determine whether H3.3 enrichment simply represents deposition of this variant behind replication forks or contributes to origin specification. Answers to these questions are medically important because alterations of the epigenome and origin function cause genome instability and cancer.

363C

Interaction of the SIN3 histone deacetylase complex with the histone demethylase LID. Lori A. Pile, Ambikai Gajan. Dept Biological Sci, Wayne State Univ, Detroit, MI.

SIN3, which is conserved from yeast to mammals, is the key scaffold component of a histone deacetylase complex. SIN3 acts as a corepressor affecting global transcription. Purification of *Drosophila* SIN3 isoform specific complexes in our laboratory identified the association of LID, a histone H3 lysine 4 demethylase, with the largest SIN3 isoform. Purification of a LID complex in *Drosophila* validates this interaction identifying many components of the SIN3 complex along with unique proteins that interact with LID. This suggests that the demethylase functions together with the deacetylase to co-regulate transcription, possibly at a subset of their target genes. Supporting this, analysis of histone modification patterns at SIN3 target genes under SIN3 knockdown conditions show changes in both acetylation and methylation. Analysis of global histone modification changes upon LID knockdown in S2 cells shows a specific increase in H3 lysine 4 trimethylation, with no significant effect on global levels of acetylation. It is thus of interest to look at possible gene specific effects on acetylation levels upon loss of LID at target genes. We are currently analyzing global changes in gene expression upon loss of LID in S2 cells to identify potential genes co-regulated by LID and SIN3. Furthermore, LID may also be involved in regulating expression of SIN3 itself. Overexpression of LID in S2 cells leads to a switch from the predominantly expressed SIN3 large isoform to the smaller isoform. Apart from the biochemical interactions of LID with SIN3, interestingly, genetic analysis shows similarities in LID and SIN3 knockdown phenotypes. While ubiquitous knockdown of SIN3 by RNAi in flies is lethal, LID RNAi leads to reduced viability. Further, both SIN3 and LID knockdown in Drosophila wing discs leads to a curly wing phenotype. In S2 cells, both SIN3 and LID knockdown lead to a decrease in cell proliferation. These data suggest that the two enzymes may

function together in regulating cell cycle and developmental pathways.

364A

Studies into functional aspects of two isoforms of Drosophila SIN3. Nirmalya Saha, Lori Pile. Wayne State University, Detroit, MI.

SIN3 along with the histone deacetylase (HDAC) RPD3 form a co-repressor complex in which SIN3 functions as a scaffold protein. Previous studies identified two isoforms of SIN3, SIN3 220 and SIN3 187, which differ only in the presence of extra C-terminal domain in SIN3 220. The two isoforms are found in distinct complexes with some unique proteins (though many proteins are common in both SIN3 isoforms). Additionally the complexes showed differences in deacetylation potential. Given the differences in structure and protein binding capabilities, we hypothesize that SIN3 220 and SIN3 187 will exhibit significant differences in function in the context of repression of transcription. Assuming the functional differences, we are studying the binding sites of SIN3 isoforms using the ChIP-qPCR technique. Previous studies also suggested that acetylation and deacetylation affect the nucleosomal density along the gene. We are performing MNase mapping after over expressing the isoforms and knocking down SIN3 in Drosophila S2 cells to study the changes in nucleosomal density along various regions of a gene. Results from these experiments are anticipated to elucidate functional differences between the isoforms and provide insight into the mechanism of action by which the isoforms repress transcription.

365B

Elucidating the contribution of distinct Su(Hw) zinc fingers in DNA association and female fertility. Ryan M. Baxley[1], Alexey A. Soshnev[1], Michael W. Klein[2], Ashley B. Gaeth[2], Joel A. Morales-Rosado[2], Bing He[3], Kai Tan[3], Pamela K. Geyer[1,2]. 1) Molecular and Cellular Biology Program, University of Iowa, Iowa City, IA; 2) Biochemistry Department, University of Iowa, Iowa City, IA; 3) Internal Medicine 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 broadly distributed sites genome wide. Su(Hw) has two known functions. First, Su(Hw) establishes an insulator within the *gypsy* retrotransposon. Second, loss of Su(Hw) results in female sterility, characterized by apoptosis during mid-oogenesis. To better understand the role of Su(Hw) in oogenesis, an EMS mutagenesis screen was performed. This screen differed from previous studies because *su(Hw)* mutations were identified based on female fertility and insulator functions. From ~8,000 mutagenized chromosomes screened, four new *su(Hw)* alleles were identified. These include two new alleles that genetically separate Su(Hw) functions. One allele falls into a novel class that retains insulator function but is female sterile. Molecular characterization demonstrated that these novel alleles carried mutations in ZnF4 and ZnF8, respectfully. Our current studies focus on defining how changes in individual ZnFs alter Su(Hw) function. To this end we are analyzing DNA binding of Su(Hw) mutants in vitro and in vivo. We find that loss of a single ZnF decreases in vitro binding and alters the distribution of retained sites in the genome. In general, retained sites are enriched for Su(Hw) interaction partners Mod67.2 and CP190. Together, our data suggest that genome wide occupancy of Su(Hw) binding sites have distinct ZnF requirements.

366C

***In vivo* function of Homie, the *eve* insulator: Is it a PRE blocker?** Miki Fujioka, James B Jaynes. Dept Biochem & Molec Biol, Thomas Jefferson Univ, Philadelphia, PA.

Recently, we described the properties of an *even skipped (eve)* insulator, named Homie (Homing insulator at eve), located at the 3' end of the *eve* locus, between an *eve* PRE and the adjacent gene. Homie has both enhancer blocking activity and P-element transgene homing activity. The third feature of Homie is that it is capable of causing long-range enhancer-promoter (E-P) communication between genes located several megabases apart. Our studies indicate that a transgenic copy of Homie interacts physically with the endogenous Homie, which causes a transgenic promoter to communicate with the endogenous *eve* enhancers. Two transgenic copies of Homie can also interact to facilitate long-range communication. How do these properties of Homie relate to its *in vivo* function? In order to test this, we created a "native context" transgene that contains the entire *eve* locus and the 5' end of the adjacent gene *TER94*, including its 1st 2 introns, which contain enhancers that drive the near-ubiquitous *TER94* embryonic expression. In this transgene, the coding regions are replaced with reporter genes, so that promoter activity can be easily monitored. Using this construct, we see that Homie has the ability to block the activity of the *eve* PRE. Monitoring *TER94* promoter activity, when Homie is removed, the initially strong, ubiquitous expression that mimics *TER94* expression changes to mimic the *eve* pattern. When the PRE is also deleted, the pattern reverts to that of *TER94*. This suggests that one function of Homie is to shield the *TER94* promoter from repressive effects of the *eve* PRE. Furthermore, in keeping with its ability to facilitate long-range communication, results with these same transgenes suggest that in addition to blocking PRE-dependent repression, Homie facilitates interaction between the *eve* promoter and the set of enhancers located 3' of the *eve* coding region. Our working model is that Homie interacts with the *eve* upstream promoter region, looping out the intervening DNA.

367A

A Role for dCAP-D3/Condensin II in Preventing Natural Transposon Mobilization. Michelle Longworth, Andrew Schuster. Department of Molecular Genetics, Cleveland Clinic Lerner Research Institute, Cleveland, OH.

Condensin complexes are conserved from bacteria to humans and are well known for their role in promoting chromatin condensation at the beginning of mitosis. Recently we discovered a non-mitotic role for the *Drosophila* Condensin II complex: the Condensin II component, dCAP-D3, regulates transcription of clusters of genes, partly in combination with the *Drosophila* retinoblastoma protein homolog, RBF1. Surprisingly, approximately 1/3 of all dCAP-D3 targets are positioned within 5 kb of a natural transposon. We analyzed a number of loci containing dCAP-D3 target gene clusters in close proximity to natural transposons and found that the transposon DNA in the area had mobilized in dCAP-D3 mutants. Transposon mobilization events increase with decreasing expression levels of dCAP-D3 protein. In addition, mutants heterozygous for two other Condensin II subunits also demonstrate similar transposon mobilization events. Finally, we present data that acute knockdown of dCAP-D3 by RNAi results in partial loss of Transposon DNA sequence and generation of possible intermediates of a Homologous Recombination event. Our lab and others have previously demonstrated that *Drosophila* Condensin II subunits can influence the spread of chromatin marks in Position Effect Variegation assays and we are currently analyzing whether decreased dCAP-D3 expression levels result in changes in histone modifications following transposon mobilization. We propose a working model whereby dCAP-D3/Condensin II prevents the homologous recombination of transposons by acting to maintain a repressive chromatin state.

368B

Variegated transvection by the enhancer *GMR*. Jack R. Bateman, Justine E. Johnson, Melissa N. Locke. Dept Biol, Bowdoin College, Brunswick, ME.

In Drosophila, maternal and paternal homologs are intimately paired in virtually all somatic cells. Pairing of homologs can permit *trans*-interactions between an enhancer on one homolog and a promoter on another, a phenomenon known as transvection. We recently established a transgenic system that uses fluorescent reporters to assess the capacity of diverse enhancers to act in *trans* on a paired homolog. Using this system, we have shown that the eye enhancer *GMR* is capable of activating expression of *GFP* via the *hsp70* promoter in *trans*. However, when we compare fluorescent intensities of individual cells in the eye disc, we find that there is great variability in the strength of *GFP* activation from cell to cell. Such variation is not observed when *GMR* acts in *cis* at this genomic position, implying that the variability results from mechanisms of transvection and not from local chromatin effects. Furthermore, using quantitative RT-PCR, we show that activation in trans is decreased when a competing promoter is placed in *cis* to *GMR*, implying that the capacity of an enhancer to act in *trans* is influenced by its local genetic environment.

369C

Histone Recognition and Nuclear Receptor Coactivator Functions of Drosophila Cara Mitad, a Homolog of the N-terminal Portion of Mammalian MLL2/3. Andrew K. Dingwall, Chhavi Chauhan, Megan Parilla, Manuel O. Diaz, Claudia B. Zraly. Stritch School of Medicine, Oncology Inst & Dept Pathology, Loyola Univ Med Ctr, Maywood, IL.

MLL2/3 histone lysine methyltransferases are conserved components of COMPASS-like nuclear hormone receptor coactivator complexes. In vertebrates, the paralogous ALR/MLL2 and HALR/MLL3 contain multiple domains required for proper epigenetic reading and writing of the histone code involved in hormone-stimulated gene programming, including receptor binding motifs, SET methyltransferase, HMG and PHD domains. The genes encoding MLL2 and MLL3 arose from a common ancestor. Phylogenetic analysis of MLL family proteins suggests that the ancestral gene underwent a fission event in some Brachycera dipterans including Drosophila spp., creating two independent genes corresponding to the N- and C-terminal portions. In Drosophila the C-terminal SET domain is encoded by trithorax-related (trr), required for hormone dependent gene activation. We identified the cara mitad (cmi) gene that encodes the previously undiscovered N-terminal region consisting of PHD and HMG domains and receptor binding motifs. The cmi gene is essential and its functions are dosage sensitive. CMI associates with TRR, as well as the EcR/USP receptor heterodimer and is required for hormone dependent transcription. Genetic tests reveal that cmi is required for proper global trimethylation of H3K4 and that hormone stimulated transcription requires chromatin binding by CMI, methylation of H3K4 by TRR and demethylation of H3K27 by the demethylase UTX. The evolutionary split of ALR/MLL2 into two distinct genes in Drosophila allows for important insight into the distinct epigenetic functions of the conserved readers and writers of the histone code.

370A

The piRNA is sufficient to guide Piwi to specific genomic sites to induce epigenetic changes. Xiao Huang, Haifan Lin. Yale Stem Cell Center, New Haven, CT.

Although the function of many epigenetic factors has been intensively studied, little is known about mechanisms that guide them to specific sites in the genome. Previously, we showed that a Piwi-piRNA complex in Drosophila specifically binds to a genomic site complementary to that particular piRNA1 and that Piwi recruits Heterochromatin Protein 1a (HP1a) to chromatin2. This led us to propose that the piRNA guides PIWI and its associated epigenetic factors to target genome1-3. To test this hypothesis, here we demonstrate that inserting piRNA-complementary DNA to an ectopic site either on the same chromosome or on a different chromosome leads to the recruitment of Piwi to these sites; whereas inserting a non-complementary sequence to the same ectopic site does not lead to Piwi recruitment. Piwi recruitment is abolished by RNase treatment, supporting the role of piRNAs in recruiting Piwi to chromatin3. The ectopic site without the piRNA-sequence insertions shows no Piwi binding; therefore the Piwi-piRNA complex is recruited to, but not originated from, the ectopic site. These observations together demonstrate that piRNAs are both necessary and sufficient to guide Piwi to specific genomic sequences by sequence complementarity. Furthermore, ectopic Piwi recruitment is accompanied by the enrichment of HP1a, Su(var)3-9, and repressive chromatin marks H3K9me2, H3K9me3 as well as reduction in Pol II transcription activity. These data implicate that Piwi-piRNA complexes guide epigenetic factors to specific genomic sties.

371B

PNUTS-PP1 associates with transcriptionally active sites on interphase chromosomes and is required for cell survival. Louise Rebecca Rawling[1], Anita Lucaci[1], Andrey Rudenko[2], Peter Glenday[1], Luke Alphey[2], Daimark Bennett[1]. 1) Inst Integrative Biology, Univ Liverpool, Liverpool; 2) Dept Zoology, Oxford University, Oxford.

Tight regulation of gene expression is critical for cells to respond normally to physiological and environmental cues and to allow cell specialization. Reversible phosphorylation of key structural and regulatory proteins, from histones to the transcriptional machinary, is acknowledged to be an important mechanism of regulating spatial and temporal patterns of gene expression. Protein Phosphatase 1 (PP1), a major class of serine/threonine protein phosphatase, is found at many sites on *Drosophila* polytene chromosomes where it has many important roles in controlling gene expression and chromatin structure. PP1 is targeted to different chromosomal loci through interaction with a variety of different regulatory subunits, which are thought to modify PP1's activity towards specific substrates. Here we describe the *in vivo* role of PNUTS (PP1 Nuclear Targeting Subunit), one of the most abundant PP1-binding proteins in the mammalian nucleus. *Drosophila* PNUTS is an essential gene. In proliferating tissues mutant cells become basally localised, express activated caspase and undergo cell death. Binding to PP1 is essential for PNUTS function. PNUTS protein is found in the nucleus and, during interphase, is chromosomally localised and associates with PP1 at active sites of transcription in an RNA-dependent manner, suggesting a role in transcriptional regulation. We will present here our results from genetic and cell biological experiments to identify potential substrates of chromosome-associated PNUTS-PP1, which help to explain the requirement for this holoenzyme in cell survival.

372C

Mapping the *Telomere elongation* mutation in Drosophila. Hemakumar M. Reddy, James M. Mason. Laboratory of Molecular Genetics, NIH/NIEHS, Research Triangle Park, NC.

Telomeres are necessary to prevent activation of the DNA damage response and to maintain chromosome length. Drosophila telomeres differ from those in mammals in the mechanism of telomere maintenance, as Drosophila lacks telomerase. Drosophila telomeres contain a terminal array of non-LTR

retrotransposons (HTT array). This array contains three elements, *HeT-A*, *TART*, and *TAHRE*, of which *HeT-A* is the most abundant. Transposition and gene conversion are the two major mechanisms of telomere elongation. A dominant mutation *Telomere elongation, Tel*, causes cytologically visible elongation of telomeres. *Tel* was mapped to 69 on the genetic map, corresponds roughly to 92 on the cytological map. It was further localized to a 318 kb region between 92A2 to 92A11, using *P* element mediated male recombination. To further map the *Tel* locus, we selected 3 *P* elements and 2 *Minos* elements that subdivide this region. Recombination was induced in males that carried each of these elements individually, and recombinants were put into stocks and DNA collected at intervals for 12 generations. Quantitative PCR was performed to estimate *HeT-A* copy number in these recombinants. The results show that *Tel* maps between the transposons *P{PZ}Dl*^{05151} and *P{XP}Ino80*^{d10097}, a 77 kb region that contains *Ino80*, a ncRNA, a pseudo gene, a miRNA, 8 unannotated genes, and a 40 kb well-conserved intergenic region. Next generation sequencing of *Tel* and a *y w* control was done to identify signature differences in the sequence by comparing them with FlyBase reference sequence and with DGRP sequences. DGRP is a resource panel of 192 wild-caught, inbred lines, whose sequences were available in a public database. *HeT-A* copy number analysis of DGRP lines identified 5 lines that showed elongated telomeres, on par with *Tel*, and 3 others with moderately increased copy number. We expect that mapping of the genetic factor(s) responsible for the increased *HeT-A* copy number in these DGRP lines, coupled with subdivision of the 77 kb region using *Minos* element insertions that have recently become available will identify the *Tel* mutation.

373A

An RNA-seq screen for allele-specific parent-of-origin effects in *Drosophila melanogaster*. Kevin H.C. Wei[1], Julien F. Ayroles[1,2], Daniel A. Barbash[1], Andrew G. Clark[1]. 1) Molecular Biology and Genetics, Cornell, Ithaca, NY; 2) Harvard Society of Fellows, Harvard, Cambridge, MA.

Genomic imprinting is a non-Mendelian mode of inheritance where the expression of each allele depends on the parent-of-origin. In classical cases of imprinting, characterized mostly in mammals, epigenetic modifications are deposited in the parental germline causing parent-specific silencing of one allele in the offspring. Evidence for imprinting in *Drosophila melanogaster* comes from complex genetic crosses, where mutations that induce position effect variegation produce different variegating phenotypes depending on transmission from the mother or father. However, to date, no naturally occurring variants have been found to display genomic imprinting. Moreover, no genes have been identified to show parent-specific silencing. We generated transcriptome sequences by RNA-seq for offspring of reciprocal crosses and used read counts to identify distortion of allele proportions characteristic of imprinted expression. Chi-square tests of these counts show that in the reciprocal crosses between lines Beijing11 (B11) and Netherland4 (N04), 507 and 665 genes have significant distortions in daughters and sons respectively. As the chi-square test is expected to yield many false positives due to the compound binomial sampling in library preparation, cluster generation and sequencing, we focused on the 147 genes significantly distorted in both sexes of offspring. In this set, 140 (95%) share the same direction of distortion, and they cluster broadly along chromosome arms. Many of these display parent-specific silencing of only B11 but not N09 alleles, some of which we confirmed with allele-specific restriction enzyme digestion and pyrosequencing. However, in a separate reciprocal cross between Beijing14 (B14) and Tasmania9 (T09), the same tests fail to detect significant distortions of allele proportions. Unlike classical cases in mammals with species-wide imprinted loci, these results suggest that in flies “imprinting” is a strain-, and perhaps cross-specific phenomenon.

374B

De novo establishment of Polycomb-mediated repression. Jumana AlHaj Abed, Siddhi Desai, Judith Benes, Richard Jones. Biology, Southern Methodist University, Dallas, TX.

Polycomb group proteins (PcG) are 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. Although maintenance of repression by PcG proteins is well characterized, the mechanism by which PcG proteins initially recognize repressed state of a gene remains ambiguous. 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. We will report on our progress towards characterizing the molecular and biochemical events that lead to the initiation of PcG-mediated repression of *giant* (*gt*) in a genetic background with a homogenous population of cells with respect to PcG-mediated repression. Embryos will be collected over a time course where initially *gt* is repressed by maternally expressed Hb, and in which the PcG is required to maintain its ubiquitous repression at a later stage. Chromatin immunoprecipitiation (ChIP) will allow analysis of distribution of PcG proteins in addition to *gt* activators and repressors during the developmental window at which repression transitions from Hb to repression by PcG proteins. Whole-mount embryo staining of transgenic reporter lines will allow the functional identification of Polycomb Response Elements (PREs) within the upstream regulatory region of *gt*, and will aid in the interpretation of the ChIP data.

375C

Stuxnet destabilizes Polycomb-associated PRC1 complex to facilitate Notch receptor gene transcription. Juan Du[1], Junzheng Zhang[1], Feng Tie[2], Ying Su[1], Min Liu[1], Peter Harte[2], Alan Zhu[1]. 1) Department of Cell Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA; 2) Department of Genetics, Case Western Reserve University, Cleveland, OH, USA.

The activities of developmental signaling are controlled by a large array of post-translational modification events. By contrast, very little is known about the mechanisms that regulate the expression of their core pathway components. Using an in vivo RNAi screen, we identified 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. We generated a lethal null allele of stuxnet through an FLP/FRT-based technique and demonstrated that stuxnet is required for transcription of the Notch receptor gene in the wing imaginal disc. We found that 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. Consistently, we found that Stuxnet protein physically interacts with and subsequently destabilizes Pc protein and its associated PRC1 complex in vivo. Thus, Stuxnet protein facilitates Notch signaling by destabilizing the PRC1 complex, thereby reducing the repressive chromatin modification marker imposed on the Notch receptor locus. We named this novel gene stuxnet after the powerful virus that destroys PC computers. Our work identified a novel mechanism for the control of the activity and stability of the PRC1 transcriptional silencing machinery in development.

376A

Epigenetically-confused, an unusual Trithorax Group SET domain-containing protein, functions like a Polycomb Group gene. Hector Rincon-Arano, Jessica Halow, Jeff Delrow, Jorja Henikoff, Steven Henikoff, Susan Parkhurst, Mark Groudine. Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Polycomb (PcG) and trithorax (TrxG) group of proteins are the basic machinery of the transcriptional memory. PcG genes maintain gene silencing by modulating chromatin structure via repressive marks like H3K27m3, whereas TrxG genes support gene expression by establishing activating histone marks including H3K4m3. Here we describe the functions of the protein we named Epigentically-confused (Epic), which possesses a SET domain related to the H3K4 methyltransferase Trx. DamID chromatin profiling of Epic shows preferential recruitment to transcriptionally active genes overlapping with RNA pol II and H3K4m3, a typical feature of TrxG proteins. Surprisingly, in genetic interactions Epic behaves as a PcG protein. Accordingly, lack of Epic does not affect H3K4 methylation regardless its similarity to the Trx SET domain. By co-immunoprecipitation, we find that Epic interacts with the histone deacetylase RPD3 and Sin3. Chromatin immunoprecipitation experiments indicate that Epic is required for controlling acetylation levels of active promoters and their gene units, as its knock down increases H3K9Ac and H4K16Ac levels over promoter and neighbors regions. Paused genes are also targeted by Epic including the heat shock genes. Although the activation of heat shock genes is properly set up in Epic knock down cells, their turn off is impaired. Additionally, *epic* mutant flies are female sterile and exhibit an up-regulation of the Notch pathway, which affects cell lineage specification. Thus, continuous presence of Epic at active genes could be a fast way of modulating chromatin architecture during transcriptional shut down. Altogether, our data suggests that Epic belongs to new class of PcG gene that use their Trx features to bring the HDAC machinery to transcriptionally active regions in order to modulate the chromatin opening.

377B

The histone demethylase UTX and the chromatin remodeler BRM bind to Drosophila CBP and modulate the acetylation of histone H3 lysine 27. Feng Tie, Rakhee Banerjee, Patty Conrad, Peter Scacheri, Peter Harte. Dept Genetics, Case Western Reserve Univ, Cleveland, OH.

Trithorax-group (TrxG) proteins antagonize Polycomb silencing and are required for maintenance of transcriptionally active states. We previously showed that the histone acetyltransferase (HAT) CREB-binding protein (CBP) acetylates histone H3 lysine 27 (H3K27), thereby directly blocking its trimethylation (H3K27me3) by the Polycomb Repressive Complex 2 (PRC2) in Polycomb target genes. Here we show that H3K27ac levels also depend on other TrxG proteins, including the histone H3K27-specific demethylase UTX and the chromatin-remodeling ATPase Brahma (BRM). We show that endogenous CBP is physically associated with UTX and BRM in vivo and that UTX, BRM and CBP exhibit genome-wide co-localization on Polycomb Response Elements (PREs) and on many Polycomb target genes marked by H3K27ac. UTX and BRM bind directly to the conserved zinc fingers of CBP, suggesting that their individual activities are functionally coupled in vivo. BRM and histone H3 bind cooperatively to the PHD finger (C4HC3-type) of CBP, an integral part of the CBP HAT domain. BRM enhances in vitro acetylation of H3K27 by recombinant CBP. Drosophila brm mutations and RNAi knockdown of UTX and BRM reduce H3K27ac levels and increase H3K27me3 levels. Direct binding of UTX and BRM to CBP in vitro, and their physical association in vivo suggest that demethylation of H3K27me3 by UTX, acetylation of H3K27 by CBP and remodeling activity by BRM complex are coordinated for a rapid and efficient reversal of Polycomb silencing. We propose that UTX and BRM play an important role in antagonizing Polycomb silencing by positively modulating acetylation of H3K27 by CBP.

378C

***Drosophila* Myb interacts with NURF to repress cell cycle genes and transposons in non-mitotic tissues.** Juan Santana[1], Stephen Butcher[1], Scott McDermott[1], Mrutyunjaya Parida[1], Kristen Jogerst[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.

c-*Myb* is encoded by 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. siRNA knockdown of Dm-*Myb* was shown to reduce expression of genes with prominent roles in coordinating cell division in an embryonic *Drosophila* cell line. Along the same lines, Dm-*Myb* mutant flies display cell cycle defects such as aneuploidy and polyploidy, both hallmarks of cancer. Additionally, the Dm-Myb protein was identified in a complex containing a large number of proteins including the nucleosome remodeling factor NURF. Through yeast two-hybrid and genetic screens, as well as co-immunoprecipitations, we have now established that Dm-Myb specifically interacts with the major subunit of NURF (Nurf301). In light of these results, we performed gene expression analyses in Dm-*Myb* and *Nurf301* mutant animals under the assumption that a significant number of genes are co-regulated by both proteins. As expected but nonetheless striking, there is an almost 50% overlap of the genes regulated by these two proteins and, in contrast to the dogma in the field, we have observed a prominent transcriptional repression function for Myb and Nurf301 in non-mitotic tissues. These data suggest that, in addition to activation of cell cycle genes in dividing cells, Myb and NURF work to repress such genes in non-dividing cells. Even more surprising, tiling microarray and RNA-seq data indicate that Dm-Myb and Nurf301 are working in concert to silence transposable elements.

379A

Brahma (SWI/SNF) complex regulation of transcript elongation and pre-mRNA splicing is mediated by the SNR1 regulatory subunit. Claudia B. Zraly. Oncology Institute and Department of Pathology, Stritch School of Medicine, Loyola University of Chicago, Maywood, IL.

Drosophila Brahma (SWI/SNF) complex dependent chromatin remodeling is essential for the precise regulation of in vivo target genes during Drosophila development. Transcriptome studies in flies and mammals identified cell cycle and hormone responsive genes as important targets, while loss of SWI/SNF function has been linked to developmental abnormalities and aggressive cancers. The Brahma complex assists in reprogramming and coordinating gene expression in response to hormone signaling at critical points during development. We used RNAi knockdown in cultured cells and transgenic flies, and conditional mutant alleles to identify unique and important functions of two conserved Brahma (Brm) complex core subunits, SNR1/SNF5 and BRM/SNF2-SWI2, on target gene regulation. Unexpectedly, we found that incorporation of a loss of function SNR1 subunit led to alterations in RNA polymerase elongation, pre-mRNA splicing regulation and chromatin accessibility of ecdysone hormone regulated genes, revealing that SNR1 functions to restrict BRM-dependent nucleosome remodeling activities downstream of the promoter region. Our results provide evidence for critically important roles of the

SNR1/SNF5 subunit and the Brm chromatin remodeling complex in transcription regulation during elongation by RNA Polymerase II and completion of pre-mRNA transcripts that are dependent on hormone signaling in late development.

380B

CREB transcription factors and drug tolerance. Benjamin R. Troutwine, Yan Wang, Nigel Atkinson. ICMB, University of Texas, Austin, TX.

As a means to understand the mechanisms that underlie addiction, we study the acquisition of drug tolerance in Drosophila. Tolerance is defined as a reduced response to a drug caused by previous drug exposure, and is considered a core component of addiction. Tolerance is an adaptive process that works to dampen effects of a drug, but can cause the user to require a larger dose to achieve the desired effect and lead the user toward addiction. Drosophila acquire tolerance to the sedative effects of ethanol and benzyl alcohol after a single sedation, and previous work in our lab has shown that the BK-type Ca^{2+}-activated K^+ channel encoded by *slowpoke (slo)* is central to this process. Our lab has shown that *slo* is induced following sedation, that artificial induction of *slo* mimics the tolerant state, and that *slo* mutants do not acquire tolerance. Examination of the *slo* transcription control region identified three putative CREB binding sites, indicating that CREB transcription factors play a role in the drug-induced upregulation of *slo*. It has been shown in a number of systems that CREB signaling is important for neural processes linked to addiction; including drug reward, withdrawal and tolerance. A former member of our lab found that after sedation CREB isoforms are differentially regulated, CREB binding at the *slo* transcription control region increased and a CRE-regulated reporter was induced. Additionally, *Creb2* mutants failed to acquire tolerance and induction of a transgenic CREB repressor isoform *(Creb2b)* blocked the acquisition of tolerance and *slo* induction. We are using CREB mutants, RNAi constructs, and transgenes to further examine the roles of the CREB genes in the acquisition of tolerance. Additionally, we have generated an epitope-tagged CREB gene to further explore CREB regulation of *slo* following sedation and assay interactions with other transcription factors.

381C

Investigating the role of MRL proteins in invasive border cell migration. Lauren Dodgson, Eleanor Taylor, Daimark Bennett. University of Liverpool, Institute of Intergrative Biology, Liverpool, United Kingdom.

The invasion of cancer cells into surrounding tissues plays a causal role in tumour progression and is the initial step in tumour metastasis, which clinically is the most important process in the progression of cancer. For invasion to occur, cells must detach from the epithelium, acquire and regulate both their motile properties and affinity for other cell types as they migrate to a new location. Several lines of evidence indicate that the Mig-10/RIAM/Lamellipodin (MRL) family of adapter proteins transduce signals derived from growth factor receptors, via interactions with Ras GTPases and/or phospholipids, resulting in changes in the actin cytoskeleton, increased lamellipodia protrusion, cell motility and altered cell adhesion properties. *Drosophila* encodes only one MRL protein, encoded by *pico*, which we previously showed to have a role in the regulation of actin dynamics. Here we report on the role of *pico* in invasive border cell migration in the *Drosophila* ovary. During oogenesis, a pair of specialised cells differentiates at the anterior end of the egg chamber and recruits four to eight additional cells to form a border cell cluster. After detaching from their epithelial neighbours, border cells make their way to the oocyte-nurse-cell border, guided by redundant signalling through the PDGF/VEGF receptor (Pvr) and Egfr. We will present data from live imaging approaches that reveal the requirement for *pico* in the formation of actin-based protrusions and invasion in this system. This work points to the involvement of MRL proteins in tumour cell invasion and metastasis and is consistent with their known role in promoting lamellipodia-like structures at the leading edge of invasive cells, which provide the main driving force for cellular locomotion and invasion.

382A

Analyzing cancer stem cells using the Drosophila ovary. Rebecca L. Frederick[1], Allan Spradling[1,2]. 1) Carnegie Institution for Science, Department of Embryology, Baltimore, MD; 2) Howard Hughes Medical Institute.

A small subpopulation of cells known as cancer stem cells has been documented to sustain a few types of cancer, and their existence has been postulated in many other cases. Understanding the initial steps in tumorigenesis and whether a particular cancer is maintained by stem cells is critically important for designing optimal therapeutic approaches. However, the lineage tracing methods that can decisively delineate stem cells in normal or cancerous tissue have not been broadly applied to mammalian tumors because of technical difficulties. We have used lineage analysis in Drosophila to analyze the cellular basis of genetically induced ovarian dysplasias. In particular, mutations in the gene lgl transform the somatic follicle cells that surround the germ-line from a monolayered, polarized epithelium into a disorganized cellular mass. We find that lgl mutant cells display altered growth patterns even before they invade the follicle interior, a region normally occupied by the germ cells. Instead of remaining in a compact unit like the daughters of a wild-type follicle cell, lgl sister cells break apart and disperse over a larger region of the follicular surface. While all mutant cells do not grow equally, our results do not support the existence of highly specialized tumor stem cells. In marker gene expression studies, we observe that lgl dysplasias comprise heterogeneously differentiated cell populations. Our system also allows us to investigate differentiation state and epigenetic stability of normal and dysplastic cells. Our methods are applicable to many other types of dysplasias, including cells containing mutations in human oncogene homologs, indicating that Drosophila provides a robust system for systematically addressing the existence and genetic programming of cancer stem cells.

383B

Tumor suppressor mutations in *pebble*/*Ect2* activate Rac1 and reveal a mechanism of autoregulation. Jin-Yu (Jim) Lu[1], Michelle Pirruccello[2], Ming Wu[1], Jose C. Pastor-Pareja[1], Tian Xu[1]. 1) Dept. Genetics; 2) Cell Biology, Yale Sch Medicine/HHMI, New Haven, CT.

Proto-oncogene *Ect2*, the single human orthologue of *pebble* (*pbl*), is a Rho guanine nucleotide exchange factor required for cytokinesis. However, overexpressing full-length Ect2 does not lead to transformation, and its oncogenic form has not been found in human cancer. While a mutation ($Ect2^{T802P}$) is identified in the C-terminus of Ect2 in human breast cancer, its potential role and regulation in cancer remain unclear. Here we report that *pbl*/*Ect2*, despite being a proto-oncogene, also functions as a tumor suppressor. From a large-scale genetic screen, we identified multiple EMS-induced pbl^{tum} mutations that promote tumor growth and invasion in cooperation with Ras^{V12}. Distinct from null alleles, which do not cause tumors, pbl^{tum} are partial loss-of-function alleles with mutations affecting a highly conserved C-terminal region that is also mutated in $Ect2^{T802P}$. Importantly, expressing the $Ect2^{T802P}$-corresponding mutant Pbl also causes tumor growth and invasion. Genetically, pbl^{tum} mutations decrease Rho1 signaling but abnormally activate Rac1 signaling, suggesting indirect inhibition of Rac1 by Pbl. Blocking Rac1 or JNK signaling suppresses the tumor phenotype, indicating that pbl^{tum} mutations drive tumor growth and invasion via the Rac1-Pak-JNK pathway. To determine the function of the C-terminus of Pbl/Ect2, where pbl^{tum}/*Ect2* mutations cluster, we identified a conserved helical region that contains mitotic phosphorylation sites. We further discovered an intra-molecular interaction between the N- and C-terminus of Pbl/Ect2, suggesting an autoinhibitory regulation. Significantly, phosphomimetic mutations and serum stimulation both decrease the intra-molecular

interaction, partially opening the molecule to allow further activation. Our study reveals a tumor suppressor function of *pbl*/*Ect2* in inhibiting Rac1 and identifies an early step in its autoregulation.

384C

Tumorigenesis in the absence of the spindle assembly checkpoint. Sara Morais da Silva, Ricardo J. Sousa, Claudio E. Sunkel. Laboratory of Molecular Genetics, Instituto de Biologia Molecular e Celular, Porto, Portugal.

The spindle assembly checkpoint (SAC) mechanism prevents aneuploidy by only allowing mitotic progression if all chromosomes are attached to microtubules via the kinetochores, and aligned correctly at the metaphase plate. This mechanism ensures that the cell cycle does not proceed unless the correct conditions for progress are met. We have created a fly model of tumorigenesis where by weakening the SAC system and simultaneously inhibiting apoptosis we induce hyperplastic discs. The expression of *Bub3* or *BubR1* RNAi in wing discs causes loss of bristles and multiple empty sockets in the adult thorax associated with cell death. When inhibitors of apoptosis are expressed together with each of the SAC RNAi genes, the wing discs are hyperplastic, indicating that the absence of SAC together with the inhibition of apoptosis causes overgrowth. These hyperplastic discs when transplanted into adult hosts appear to grow indefinitely invading most of the abdomen. The cells from the hyperplastic discs as well as from the transplants display aneuploidies. In addition, gene expression profiling by micro-array technology was obtained for the hyperplastic discs and it has revealed several differentially regulated genes with roles in apoptosis, aneuploidy and tumorigenesis. We will discuss the link between SAC genes, apoptosis, aneuploidy and tumour development.

385A

Loss of Rabex-5 displays leukemia-like hematopoietic defects that involve dysregulation of Ras, Notch and groucho. Theresa Reimels. Oncological Sciences, Mount Sinai School of Medicine, New York, NY.

Leukemia is the most common childhood cancer but presents, and is more difficult to treat, in adults as well. In most cases the mechanisms underlying its development remain unknown. To address this problem we are examining leukemia-like phenotypes in Drosophila. Homozygous loss of the Ras regulator Rabex-5 in Drosophila larvae causes dramatic hemocyte phenotypes including overproliferation and mispatterning. When hemocyte lineages in this model of overproliferation are examined, no change in the percent of crystal cells and a statistically significant decrease in the percent of plasmatocytes coincident with differentiation of lamellocytes are observed. These data raise the possibility that a less differentiated progenitor hemocyte population is expanding and imply that Rabex-5 is negatively regulating the proliferation and/or differentiation of hemocytes. The requirement for Rabex-5 in hemocytes at various stages of their development is being investigated. Preliminary data suggest that Rabex-5 is not required in hemocytes to control proliferation and differentiation after the expression of the mature hemocyte marker Hemese, although it may make a small contribution potentially through its negative regulation of Ras activity. Rabex-5 null mutations cause melanotic masses, larval/pupal lethality and genetically interact with Ras, Notch and groucho. Ras, Notch and Wg are among the pathways involved in hematopoiesis that are implicated in human hematologic cancers. Since Rabex-5 loss results in a leukemia-like overproliferation of hemocytes and Rabex-5 expression is downregulated in primary human lymphomas and leukemia cell lines, we are examining Rabex-5 as a potential tumor suppressor in the hematopoietic system.

386B

Gene expression profiling in Drosophila models of human cancers associated with modulation of DCC/frazzled signaling. Joseph Sarro[1], Charles Tessier[2], Molly Duman-Scheel[1,2]. 1) Biological Sciences and Harper Cancer Inst, Univ of Notre Dame, Notre Dame, IN; 2) Med and Molec Genetics, Indiana Univ Sch Medicine, South Bend, IN.

We recently characterized two Drosophila models of human cancers associated with loss and gain of Deleted in Colorectal Cancer (DCC)/Drosophila Frazzled (Fra) signaling. In this investigation, we performed microarray analyses to investigate global changes in gene expression resulting from both loss and activation of Fra signaling. It was hypothesized that combinatorial analysis of these two experiments would reveal common mechanisms in our two Drosophila models of cancer. Activation of Fra signaling in wing disc clones resulted in 764 statistically significant differentially expressed genes, while 1,897 genes were differentially expressed in fra mutant embryonic cells. Significant genes were further analyzed with DAVID and MetaCore, which grouped genes into statistically significant disease networks, signaling pathways, and gene ontology (GO) processes. These analyses were performed for each array experiment separately, as well as for genes identified in both sets of array experiments. In addition to many cancer disease networks, including colorectal and breast cancer, many GO processes were deemed significant, including cellular morphogenesis, apoptosis, cell motion, axonogenesis, cytoskeletal organization, spindle organization, cell growth and cell adhesion. A number of Drosophila orthologs of genes that have been linked to human cancers, including several previously uncharacterized fly genes, were identified in both sets of experiments. These genes, as well as members of the signaling cascades Wnt, TGFβ, and Notch, all of which were modified in response to loss or activiation of Fra signaling, will be prioritized in ongoing secondary validation experiments in which gene expression and genetic interaction studies will be used to verify the array data. Analysis of these genes may provide new insight into human cancers associated with modulation of DCC signaling.

387C

Delineating the function of PRL-1 in *Drosophila*. Leslie J. Saucedo, Jake Goodchild, Krystle Pagarigan, Travis Edlefsen. Biology, University of Puget Sound, Tacoma, WA.

In the past decade, Phosphatase of Regenerating Liver (PRL) family members have emerged as molecular markers that significantly correlate to the ability of cancers to metastasize. In addition, PRLs are promising therapeutic targets; hindering PRL function in transformed cells has shown dramatic reduction in tumor formation in mice. However, contradictory cellular responses to PRL expression have been reported; in some mammalian cell lines, PRL instead inhibits cell cycle progression. An obvious culprit for the discrepancy is the use of dozens of different cell lines, including many isolated from tumors or cultured cells selected for immortalization. These studies each examine PRL in a different genetic environment, which may mean modulators and effectors of PRL function are missing or mutated. We created transgenic *Drosophila* to study the effects of PRL overexpression in a genetically controlled, organismal model. Our data support the paradigm that the normal function of PRL is to suppress cell growth. However, genetically altered environments can modulate the function of PRL; while PRL maintains its growth suppressive effect and counters the activity of the Src oncogene, it instead contributes to the activity of oncogenic Ras. Ongoing work includes how PRL function may be modified by additional genetic manipulations. In addition, because we have demonstrated

that the ability of PRL to slow cell growth under normal conditions is dependent on tight association of PRL with the plasma membrane, we will investigate if this localization is disrupted under conditions when PRL fails to counter oncogenic activity. A more complete understanding of PRLs will allow applications using these proteins as markers for metastasis to be better informed and thus, more effective.

388A

Neurofibromin (*Nf1*) function in Drosophila: Genetic and Physical Interactions Screens. James A. Walker[1,2], Jean Y. Gouzi[1], Robert Maher[1], Andre Bernards[1,2]. 1) Massachusetts General Hospital Cancer Center, Harvard Medical School, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, MA.

Neurofibromatosis type 1 (NF1) affects 1 in 3,000 life births and is the most common genetic disease associated with an increased cancer risk. NF1 patients are predisposed to developing multiple symptoms, including benign but often numerous and disfiguring tumors associated with peripheral nerves, termed neurofibromas, as well as malignant peripheral nerve sheath tumors. Loss of NF1 expression is also common in glioblastoma, neuroblastoma, and other non-NF1-associated tumors. The protein encoded by *NF1*, Neurofibromin, is a GTPase Activating Protein for Ras. However, the exact molecular and cellular defects responsible for NF1 are unknown. We have created a Drosophila model of NF1 and identified several phenotypes of null mutants, including an overall growth deficiency and a learning/memory deficit, both resembling symptoms of human NF1 and related RASopathies. Neurofibromin functions in larval neurons to regulate growth non-cell-autonomously. To identify dominant *Nf1* size modifiers we have conducted a genetic screen using deficiencies on the X and 2nd chromosomes. We have identified the neuronal receptor tyrosine kinase Alk and its ligand Jellybelly (*Jeb*) as rate-limiting upstream activators of Ras pathways responsible for NF1-regulated growth control, as well as learning defects in adult flies (Gouzi et al., 2011, PLoS Genet 7(9): e1002281). To complement our genetic screens, we have also conducted proteomic analyses to look for proteins that physically associate with Neurofibromin in neurons. Studies using Neurofibromin bearing conserved point mutations from NF1 patients have revealed specific altered interactions that may be relevant for disease. We will present results from both screens that implicate Neurofibromin in novel functions in neurons and discuss the possible implications for human disease.

389B

Suppression of DiscsLarge ovarian tumor invasion and growth by a novel class of "wounded tumor" loci. Min Zhao, Scott Goode. Dept Pathology, Baylor Col Medicine, Houston, TX.

Tumor suppressor DiscsLarge (Dlg) localizes at the basolateral junction of follicle cells (FCs) to stabilize the epithelium. In Drosophila, loss of Dlg results in invasive tumor to develop. To identify the molecules utilized by dlg tumor cells to grow and invade we completed a genome-wide suppressor screen. Here, we report a novel class of suppressors, named "wounded tumor", which had a unique phenotype of epithelium wound. Four genomic loci were recovered from the screen and CG11583 (renamed holey) was identified at one of them. Reducing Holey inactivated wound repair pathway JNK in dlg tumor cells specifically and the tumor atrophy and epithelium wound were observed during endocycle, a modified cell cycle in the absence of cell division under the control of endoreplication regulator dMyc (dm). Our data suggested that tumor shrinkage resulted from nuclear condensation whereas wound was caused by FC growth retardation and subsequent epithelium rupture. Holey also suppressed tumors derived from Dlg signaling pathway members Warts (Wts) or Yorki (Yki) during endocycle. Holey protein localized to the nuclei and was essential to maintain constitutive heterochromatin structure and ensure FC survival during endocycle. Holey phenotypes were likely to associate with dMyc in that they resembled each other's phenotypes and Holey expression was controlled by dMyc. To model "wounded tumor" in human, we knocked down (KD) hDlg and hBrix1, the human homologues of Drosophila Dlg and Holey in the Immortal Ovarian Surface Epithelial (IOSE) cells. The double KD of Dlg and Brix1 cells were less favorable to survive when co-cultured with Dlg single KD cells. Taken together, we propose that Holey was required for follicle cell growth and survival under the control of dMyc. Removal half of Holey was sufficient to induce dlg tumor dystrophy whereas it had no damage on animal's health. Since Dlg is lost in several human ovarian cancer cell lines and Holey is conserved in human, targeting "wounded tumor" human homologs may be a way to eliminate cancer cells.

390C

Using *Drosophila* to study functional relevance of conserved heart genes. James H Catterson[1], Pierre O Bagnaninchi[2], Anthony J Harmar[1], Margarete MS Heck[1], Paul S Hartley[1]. 1) Centre for Cardiovascular Science, Queen's Medical Research Institute, University Of Edinburgh, 47 Little France Crescent, Edinburgh, United Kingdom, EH16 4TJ; 2) MRC Centre for Regenerative Medicine, Chancellor's Building, University Of Edinburgh, 49 Little France Crescent, Edinburgh, United Kingdom, EH16 4SB.

Drosophila melanogaster is increasingly utilised as a model of heart development and function due to its unparalleled genetic toolbox and fast experimental turnaround. We utilised the Fly Atlas database (the fruit fly gene expression atlas) to identify genes enriched in the adult fly heart that have high sequence homology with human genes. We are screening these genes experimentally to establish if they have a role in heart development and/or function. We identified 5927 genes represented 4/4 times in Fly Atlas microarrays. Of these, 164 genes showed heart-enriched expression (>5-fold vs. whole fly), 149 of these genes were protein-coding, and 59 of these genes had clear human homologues. Genes identified included well-known 'heart' genes, e.g. Tinman, Neuromancer, Pannier, Seven Up - thus validating our *in silico* strategy. One candidate heart gene identified, Fermitin 1 (*Fit1*) - an integrin-associated protein with 47% identity with its human homologue (Kindlin 2) - was shown to display the same localisation at z-disks in cardiomyocytes as KIND2. In agreement with circadian microarray data on mouse heart and liver tissue, *Fit1* displayed a diurnal rhythm in expression and this diurnality (of KIND2) was confirmed in HepG2 cells. Kindlin-2$^{-/-}$ null mouse embryos die before cardiogenesis, making it very difficult to investigate how Kindlin-2 is involved in heart development and function. Therefore, we anticipate that study of *fit1* animals will shed additional light on the function of this gene during development and tissue morphogenesis. With our tools, the fruit fly will be an excellent model to study the function of these genes in the adult heart and will lead to further enlightenment of their function in the mammalian heart.

391A

DHR96 regulates cellular cholesterol homeostasis via the Niemann-Pick disease Type C genes. Akila Gopalakrishnan, Kirst King-Jones. CW405 Biological Sciences Building, University of Alberta, Edmonton, Alberta, Canada.

Cholesterol is an essential component of animal cell membranes and the principal precursor for steroid hormone synthesis, and cellular cholesterol levels

have to be strictly controlled to ensure cellular viability. We study the roles of nuclear receptor DHR96 (*Drosophila* Hormone Receptor 96) in the regulation of cellular cholesterol homeostasis and metabolism. *DHR96* mutants are viable and display no obvious phenotypes when reared on a normal fly food medium, but arrest development when reared on a low-cholesterol diet. DHR96 binds cholesterol in vivo, suggesting that cholesterol or a related molecule acts as a ligand for this receptor. We are now employing genome-wide strategies to identify and validate direct targets of this transcription factor. One strategy utilizes a transgenic line expressing DHR96 fused to the VP16 activation domain, which allows us to screen for highly activated gene targets via microarray analysis. We found several Niemann-Pick disease type C2 (*NPC2*) genes, which encode midgut-specific cholesterol transporters, to be likely direct targets of DHR96. We then examined whether misregulation of *NPC2* genes is the cause for the lethality of *DHR96* mutants on low cholesterol media. We found that ubiquitous knockdown of one of the *NPC2* genes causes lethality specifically on low-cholesterol media, which is rescuable by cholesterol supplementation. Consistent with this observation, knocking down *DHR96* specifically in the midgut is sufficient to recapitulate the cholesterol hypersensitivity phenotype of *DHR96* mutants. We are now conducting genetic epistasis studies to examine whether *NPC2* gene function is required for DHR96-mediated responses to changing levels of cholesterol. Our studies demonstrate that *DHR96* resides at the top of a genetic hierarchy controlling cholesterol homeostasis, allowing us to dissect the complex regulatory pathways that maintain a healthy cholesterol balance in all cells.

392B

The Role of *slowpoke* Encoded BK Channel in Heart Function. Santiago Pineda[1], Karen Ocorr[1], Diane Fatkin[2], Rolf Bodmer[1]. 1) Sanford Burnham Medical Research Institute, La Jolla, CA; 2) Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010.

Abnormalities in the ion channels that modify cardiac conduction are a common cause of cardiac arrhythmias. One channel that may have an important role in heart function is the BK channel encoded by the *slowpoke* gene, a K^+ channel that is both Ca^{2+} and voltage gated. Previous studies have identified the BK channel as important in heart function of larva and adult fly. Yet, it remains unclear whether this effect is due to altered neuronal signaling or cardiac specific. Heart specific RNAi knockdown of the BK channel in a denervated, semi-intact heart prep, we observed a significantly increased diastolic interval and heart period (decreased rate); suggesting an effect on cardiac repolarization. In agreement with this pharmacological activation of the channel resulted in decreased heart period (increased heart rate). Yet, the mechanism of BK channel function in the heart is still unknown. We discovered an association between non-synonymous variants in this channel gene and heart arrhythmias in humans and have expressed both the mutated and wild type human BK channel gene in flies lacking *Drosophila* BK channel expression. Flies with the mutated channel had poorer heart function compared with controls. We are using recombineering genetic tools to tag the BK channel in vivo while expressing the channel under control of its native promoter in order to determine cellular localization. Our preliminary research shows that modification of BK channel activity affects heart function in the fly suggesting that the human BK mutation is responsible for the arrhythmia seen in the human cohort. Using the fly heart model we should be able to elucidate the specific component of BK channel function that contributes to these patients' heart disease.

393C

Modeling reductive stress induced heart disease in flies. Heng Xie, Kent Golic. Dept Biol, Univ Utah, Salt Lake City, UT.

Alpha-B crystallin (*CryAB*) is a mammalian small heat-shock protein. The R120G amino acid substitution mutation of *CryAB* (*CryAB*^{R120G}) is associated with multiple diseases, including cataracts and cardiomyopathy [1]. Heart specific expression of *CryAB*^{R120G} in the mouse resulted in cardiomyopathy, including cardiac hypertrophy, progressive heart failure and premature death. Hearts expressing *CryAB*^{R120G} had an excess of reducing equivalents in cells, and mutation of *G6PD*, one of the primary sources of NADP reduction to NADPH, suppressed the phenotype [2]. These results led to the conclusion that *CryAB*^{R120G} cardiomyopathy results from reductive stress, defined as an excess of reducing equivalents in the cell. To investigate the cellular mechanism of reductive stress pathology, we expressed the human *CryAB*^{R120G} gene in flies. Expression in the eye results in an obvious rough eye phenotype. We found that the eye phenotype was suppressed by mutation of G6PD, and enhanced by overexpression of G6PD, indicating that the basis of cellular dysfunction is similar in mouse heart and fly eye. We found that this phenotype is connected with NADPH levels rather than the G6PD protein itself, because reduction of other NADPH producing enzymes (phosphogluconate dehydrogenase (6PGD), malic enzyme (MEN) and isocitrate dehydrogenase (IDH)) also suppressed the phenotype. Our results extend the mouse experiments to show that all major sources of NADPH affect the *CryAB*^{R120G} phenotype, strongly supporting the reductive stress hypothesis. [1] Vicart, P., Caron, A., Guicheney, P., Li, Z., Prévost, M.C., Faure, A., Chateau, D., Chapon, F., Tomé, F., Dupret, J.M., Paulin, D., Fardeau, M., 1998 A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat. Genet. 20(1):92-5. [2] Rajasekaran NS, Connell P, Christians ES, Yan LJ, Taylor RP, Orosz A, Zhang XQ, Stevenson TJ, Peshock RM, Leopold JA, Barry WH, Loscalzo J, Odelberg SJ, Benjamin IJ., 2007 Human alpha B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. Cell 130(3): 427-39.

394A

The inwardly rectifying potassium channel, Irk2, contributes to development of the adult wing in *Drosophila*. Emily A. Bates[1], Giri Dahal[1], Joel Rawson[2], Brandon Gassaway[1], Ben Kwok[1], Emily Bates[1]. 1) Chemistry and Biochemistry, Brigham Young University, Provo, UT; 2) UT Health Science Center, San Antonio, TX.

There is currently no defined role for potassium channels in developmental signaling. However, mutations that disrupt function of a human inwardly rectifying potassium channel, Kir2.1, are associated the morphological defects of Andersen-Tawil Syndrome: cleft palate, incomplete dentition, skeletal fusion and abnormal curvature of digits. We use *Drosophila melanogaster* to determine how inwardly rectifying potassium channels affect development. In *Drosophila*, the Irk2 inwardly rectifying potassium channel is a homolog to Kir2.1. *irk2* deficient lines, *irk2* RNAi, and expression of a dominant negative Irk2 subunit demonstrate that Irk2 function is necessary for pattern formation in the development of the adult wing. Compromised Irk2 function causes wing-patterning defects similar to those found when Decapentaplegic (Dpp) signaling is disrupted. To determine if Irk2 plays a role in the Dpp pathway, we generated animals that were deficient in both Irk2 and in Dpp signaling. Irk2 dominant negative phenotypes are enhanced by reduced Dpp signal. In wildtype animals, the Dpp signal can be detected in a stripe along the anterior/posterior boundary of the larval wing imaginal disc. The Dpp signal is reduced in irk2 deficient animals. Expression of dominant negative Irk2 completely eliminates the Dpp signal in the larval wing disc. TUNEL staining reveals that expression of a dominant negative *irk2* causes cells in the wing disc to die via apoptosis. Wing phenotypes could be explained by disruption of the Dpp signal or aberrant apoptosis. Blocking apoptosis with overexpression of P35 in animals that also express the dominant negative *irk2* reveals that apoptosis

occurs first and prevents Dpp signaling leading to wing phenotypes. It is likely that this same mechanism explains the morphological defects of Anderson-Tawil Syndrome.

395B

Interactions between the HuD homolog *fne* and the *dNab2* polyadenosine RNA binding factor in a fly model of human intellectual disability. Rick Stephen Bienkowski[1], Callie Wigington[2], Anita Corbett[2], Ken Moberg[1]. 1) Cell Biology, Emory University, Atlanta, GA; 2) Biochemistry, Emory University, Atlanta, GA.

Intellectual disability (ID), previously referred to as mental retardation, is a broad term for a collection of diseases that are characterized by limited intellectual capacities and major constraints in adaptive behavior. We have recently found that inactivating mutations in the human gene encoding ZC3H14, a polyadenosine RNA binding protein, cause autosomal recessive non-syndromic form of ID (NS-ARID). We have created a *Drosophila melanogaster* model of this disease by removing the orthologous gene, *dNab2* (aka CG5720), and these *dNab2* mutant flies recapitulate key aspects of the human phenotype, including impaired neural function (see Pak et al, PNAS, 2011). Moreover in recent work we have also found that the form of ZC3H14 missing in human NS-ARID patients can functionally substitute for dNab2 in the fly nervous system, indicating that ZC3H14 is a true functional ortholog of dNab2. Our goal in future work is to understand the role of dNab2/ZC3H14 proteins in neurodevelopment and function. A key step in achieving this goal is identifying mRNAs targeted by dNab2 in fly neurons. In preliminary work, we have used siRNA knockdown in human cells to identify mRNAs regulated by ZC3H14 and by a second RNA binding protein, the human antigen-R (HuR) protein, which is a member of the Elav protein family and has strong affinity for adenylate and uridylate rich elements (AREs). Preliminary evidence from these microarray experiments suggests that ZC3H14 and HuR may regulate a common set of transcripts. In parallel, we have uncovered evidence of genetic interaction between dNab2 and the HuR homolog found in neurons (*fne*). In light of these data, we have undertaken efforts to generate alleles of *fne* and other genes we believe act with dNab2 to control neurodevelopment and function. We are also analyzing physical and functional interactions between these proteins in a cultured S2 cells and in cultured primary brain neurons.

396C

Modeling Degenerative Disc Disease in Drosophila melanogaster. Megan C. Donegan, Joseph A. Chiaro, Hemlata Mistry. Department of Biology, Widener University, Chester, PA.

Chronic back pain, associated with disorders of the muscles and vertebrae of the spine, is of great medical importance and results in annual societal losses of over $90 billion. Degenerative disc disease (DDD) is a leading cause of disability in middle age. DDD arises when the integrity of the intervertebral disc is compromised. The start of degeneration is not entirely environmental, since DDD can arise in juvenile adults. Studying the genetic basis of disc degeneration is an emerging field. We are investigating changes that occur in gene expression following trauma to the ventral nerve cord in late-stage Drosophila embryos. Although Drosophila is an invertebrate, our investigations are likely to be meaningful because a large proportion of human disease genes have a homologous Drosophila counterpart. Furthermore, gene regulatory pathways that control patterning and cell fate decisions during the formation of the nerve cord are conserved between humans and flies. We expect that similar changes in gene expression will occur in response to injury in both organisms. We are using microarray analysis to detect changes in gene expression between wounded and control embryos. We are investigating candidate genes of interest further via real time quantitative PCR. Understanding this genetic basis is crucial to identifying individuals at risk for degeneration, identifying potential genes for therapy, and understanding the contributions of various genes to DDD onset and progression.

397A

The mechanism of nuclei positioning during muscle development in Drosophila. Hadas Tamir, Yaxun V. Yu, Michael Welte, Talila Volk. Molecular Genetics, Weizmann Institute, Rehovot, Israel.

Dynamic distribution of cellular organelles during muscle development is of crucial importance for myotube migration, attachment, and function. KASH domain family members contribute to organelles dynamic localization in the cytoplasm. In this study we aimed to analyze the contribution of the two Drosophila KASH proteins, Klarsicht (Klar) and MSP-300 to nuclei rearrangement during muscle development. We find that in embryonic myotubes, Klar promotes nuclei migration to the plus-ends of microtubules, adjacent to the myotendinous junction. Subsequent muscle sarcomerization is then associated with a significant organelles rearrangement. At this stage, MSP-300 promotes nuclei and mitochondria anchoring to the Z-discs, whereas Klar is driving nuclei even spacing within the muscle cytoplasm. We present evidences suggesting that the two Drosophila KASH proteins are essential for proper muscle development at key developmental stages, by allowing nuclei movement in a Klar-dependent manner, and nuclei anchoring to the Z-discs by a MSP-300-dependent mechanism. In conclusion, our results provide a mechanistic explanation for the process of nuclei and mitochondria positioning during muscle development. Since all these proteins are well conserved throughout evolution, this mechanism is highly relevant to vertebrate muscle development.

398B

A Drosophila Model of Friedreich's Ataxia and Autophagic Heart Disease. Luan Wang. Inst Environmental Hlth Sci, Wayne State Univ, Detroit, MI.

Friedreich’s Ataxia (FRDA) is one of the most prevalent heritable neurodegenerative diseases in the United States. It is caused by the mutation of a mitochondria iron chaperone gene Frataxin . The mutation significantly reduces the frataxin protein level and renders the patients with muscle weakness, degeneration of neural cells, and heart disorder. But the details of the pathogenesis is still unclear and the disease is currently untreatable. Here we developed a Drosophila model for FRDA by reducing the frataxin level in vivo through UAS-Gal4 system, which could decrease the frataxin levels in specific organs such as the heart (tinman), neurons (elav), or the whole body (actin and daG32). The impact of neurotoxicity will be measured by the locomotive assay and the visualized heart assay. We discovered that under normal condition, the frataxin deficient flies, daG32-FhIR, shows no significant difference compared to the wild type flies. But after the treatment of 50 mM Paraquat, a reactive oxygen species (ROS) reagent, for 72 hours, the locomotive activity of daG32-FhIR are greatly decreased. At the same time, the longevity of the daG32-FhIR are also affected negatively. We also observed that the Tinman-FhIR flies, which is a relatively weaker promoter, shows no difference in development comparing to the wild type flies. In contrast, the stronger promoter line, daG32-FhIR is pupae lethal at 25 °C, and can normally develop at 18°C. This suggests that there is a threshold for frataxin level to maintain the normal function. Its reduction above the threshold is not essential at normal environment. But when the frataxin level decreases below the threshold, or the flies are challenged by the oxidative stress from ROS and locomotive activity will be affected. We also found that the phenotypes of the frataxin-deficient flies are very similar

to the flies with decreased activity of autophagy, which is a major role in defense against oxidative stress. So more work to explore the association of frataxin signal pathway and autophagy activity is needed.

399C

A Maternal High Sugar Diet Leads to Metabolic Defects in *Drosophila* Offspring. Jessica Buescher[1], Laura Musselman[2], Riddhi Mitra[1], Breckyn Robinson[1], Thomas Baranski[2], Jennifer Duncan[1]. 1) Department of Pediatrics, Washington University, St Louis, MO; 2) Department of Medicine, Washington University, St Louis, MO.

Obesity is a major health concern and leads to chronic health problems. Epidemiological and experimental data indicate the foundation for obesity and obesity-related diseases may be established early in life as a consequence of a suboptimal maternal-fetal nutritional environment. It is unclear how such a foundation is established but alterations in epigenetic programming mechanisms may be involved. Our hypothesis is that maternal caloric excess leads to epigenetic reprogramming in offspring, resulting in alterations in gene expression and increased susceptibility to metabolic disease. To test our hypothesis, we have established a novel *Drosophila* model in which virgin female w^{1118} *Drosophila* are fed a control, low sugar (LS) diet or a high sugar (HS) diet for 7 days before mating with male *Drosophila* w^{1118} on LS food. Importantly, all progeny develop on the control LS diet. Using established biochemical assays, we measured body composition of offspring. We observe that wL3 offspring from HS-fed maternal flies exhibit significant increases in glucose, trehalose, and triacylglycerol (TAG), reductions in cholesterol, hemolymph trehalose and hemolymph TAG, and a delay in pupariation. Interestingly, male offspring demonstrate more significant alterations in body composition than female offspring. Similar observations were noted in the *Canton S* Drosophila strain. Microarray analysis on mid-L3 male *Drosophila* w^{1118} offspring from HS-fed maternal flies and corroboration by tissue-specific qRT-PCR show reduced expression of midgut-expressed metabolic genes, including genes involved in glucose transport, lipid metabolism and cholesterol homeostasis. Furthermore, lipid staining of midgut and fat body show alterations in lipid content and lipid particle area, respectively. Taken together, our data indicate that maternal caloric excess results in metabolic alterations in male offspring exemplified by altered body composition and repression of metabolic regulators.

400A

Using Drosophila to Explore The Architecture of Natural Variations Influencing a Complex Disease Trait. Bin He[1], Michael Ludwig[1], Soo-Young Park[2], Pengyao Jiang[1], Cecelia Miles[1], Levi Barse[1], Desiree Dickerson[1], Sarah Carl[1], Honggang Ye[2], Graeme Bell[2], Martin Kreitman[1]. 1) Department of Ecology and Evolution, The University of Chicago, Chicago, IL; 2) Department of Medicine, The University of Chicago, Chicago, IL.

Most common human diseases have a multigenic basis with contributions to disease risk and severity by many individual mutations of small effect size. This makes it difficult to predict the disease risk or to understand the disease mechanism. Here we propose a novel approach to the genetic investigation of a human complex disease, by combining naturally occurring genetic variations in *Drosophila* and a fly model of human neonatal diabetes. We created a transgenic line that expresses a mutant (disease-causing) form of human proinsulin in the developing eye imaginal discs, which causes neuro-degeneration in the adult eyes that mimics the beta cell death in human patients. When we crossed this line to a panel of 180 wild caught inbred lines of *D. melanogaster* (DGRP), a nearly continuous spectrum of disease phenotypes was observed. Applying genome wide association analysis, we identified a 14bp length polymorphism and a linked SNP in the gene *sfl* to be strongly associated with the eye degeneration phenotype (raw p-value=1.9e-8, Bonferroni corrected $p<0.05$). RNAi knock-down of *sfl* confirmed its role of modulating the mutant-proinsulin-dependent eye phenotype. Because the two polymorphisms were located in the intron, we hypothesize that they exert their effects through changing the expression level of *sfl*. To test this, we are performing pyro-sequencing to compare the relative expression levels of the two *sfl* alleles in F1 (hybrid) flies. In addition to the major effect gene, a secondary analysis identified SNPs that interact with the *sfl* locus in the intergenic region between *rpr* and *grim*, two genes that are key regulators of apoptosis in *Drosophila* in response to cytotoxic stimulus. Our results demonstrated the power of a fly model in studying human complex diseases and provided a potential novel candidate for diabetes.

401B

Intermittent hypoxia alters the metabolism of Drosophila on a high-fat diet. Erilynn T. Heinrichsen[1], Gabriel G. Haddad[1,2]. 1) Pediatrics Dept, University of California, San Diego, La Jolla, CA; 2) Rady Children's Hospital, San Diego, CA.

Over 60% of the population in the United States is estimated to be obese or overweight, and with obesity come many disease complications, including sleep apnea, hypoxia, atherosclerosis, cardiovascular diseases and stroke. Several of these complications also involve hypoxia, yet the fundamental basic mechanisms underlying the interaction of obesity and hypoxia remain unknown. *Drosophila*, as a model organism, offers tremendous power in uncovering and studying these mechanisms, given the abundance of molecular tools available to delve into the roles of specific genes and the conservation of biochemical pathways. We have characterized the phenotype of *Drosophila* on a high saturated fat diet in normoxia and hypoxia using triglyceride levels, carbohydrate levels, response to stress and lifespan. We have found that, when female flies are put on a high-fat (HF) diet, they have significantly increased triglyceride and glucose levels and a shortened lifespan. The HF diet allows for increased survival during starvation, but significantly reduces tolerance to stress conditions such as anoxia and extreme cold. Exposure to intermittent hypoxia (IH), but not constant hypoxia, appears to rescue the response to cold stress in flies on both diets. This suggests that IH alters the expression of genes involved in cold tolerance in such a way to override the effect of the HF diet. Microarray studies of these flies have uncovered many candidate genes that may play a role in the phenotype of flies on a HF diet and the underlying interaction of hypoxia and the HF diet. It appears that immune and metabolic pathways are greatly affected both by a HF diet alone and hypoxia with a HF diet. We found several genes that were regulated in opposite directions depending on the experimental condition (diet alone or diet with IH), indicating that while similar pathways may play a role in the phenotype of both conditions, they appear to do so through different mechanisms. These results both confirm and expand upon our hypothesis that intermittent hypoxia alters the metabolism of *Drosophila* on a high fat diet.

402C

The fat body controls nutrient flux via transcriptional and biochemical mechanisms. Laura Palanker Musselman, Jill L. Fink, Thomas J. Baranski. Endocrinology, Metabolism, and Lipid Research, Washington University School of Medicine, St. Louis, MO.

Recent epidemiologic studies demonstrate that high glycemic diets increase the risk of developing type 2 diabetes (T2D), but how excess dietary carbohydrate leads to T2DM remains unclear. Drosophila metabolic pathways are highly conserved with those in vertebrates, but flies offer a simpler, more

rapid platform to test the effects of genes or drugs. We developed a Drosophila model of T2D (mT2D) by feeding developing larvae a high sugar diet (HSD, 1M sucrose, Musselman et al 2011). Tissue-specific loss-of-function studies reveal that the fat body plays an important role in controlling the whole animal response to dietary excess. Fat accumulation is a protective mechanism in the face of a HSD, because genetic manipulations that decrease the capacity of flies to store triglycerides result in enhanced diabetic phenotypes. Flux of dietary carbon into storage as fat is abrogated in larvae reared on the HSD despite an increase in expression of genes encoding glycolysis and lipogenesis enzymes. We present evidence for novel susceptibility genes and those that protect against diet-induced diabetes in Drosophila. These studies should provide insights into mechanisms of insulin resistance and should identify potential therapeutic targets.

403A

Lipid and carbohydrate analysis on a Drosophila melanogaster Type 2 Diabetes model. Alejandro Reyes De la Torre, Juan Riesgo-Escovar. Developmental neurobiology and neurophysiology, Instituto de Neurobiologia, UNAM, Queretaro, Mexico.

Type 2 diabetes is a chronic disease characterized by peripheral insulin resistance, high glucose, and elevated free fatty acids. Insulin is an anabolic hormone whose signaling pathway is highly conserved from nematodes to vertebrates. In Drosophila melanogaster insulin pathway mutants, larvae and 1-2 day old adults have elevated lipid and carbohydrate levels, yet chronic aspects of these type 2 diabetes models have not been investigated. In this work, we measured total lipid and carbohydrate levels from wild type and homozygous mutant chico1/chico1 adult female flies at 1, 7, 14 and 28 days. chico is the fly insulin receptor substrate homologue. Also, we studied lipid droplets in isolated abdominal adipocytes with Nile Red in homozygous chico1/chico1 flies, and heteroallelic mutant Dp110A/Dp1105W3, InRE19/InR3T5, PKB1/PKB3 and S6KL-1/S6KP1713one day old adult flies and controls. DP110 is the catalytic subunit of the fly PI3 kinase; InR is the fly insulin receptor, PKB is the fly protein kinase B, and S6K is the fly S6 kinase. Results show significant differences in total lipids between wild type and chico1/chico1 flies at 1, 14 and 28 days ($P<0.001$). Moreover, there are significant differences in total carbohydrates between wild type and chico1/chico1 flies at 14 days ($P<0.001$). We conclude that lipid and carbohydrate homeostasis is altered in chico mutant flies, as there are no significant changes in lipids or carbohydrates throughout time in adult wild type flies. Preliminary results also show that there are no significant differences in lipid volume in abdominal adipocytes between control and mutant flies. So, differences in total lipids may be given by total abdominal adipocyte number instead of lipid accumulation in mutant abdominal adipocytes, or by accumulation of lipids in other tissues in mutants.
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404B

Identifying Genes Involved in Central Nervous System Control of Obesity. Irene Trinh[1,2], Oxana Gluscencova[1], Gabrielle Boulianne[1,2]. 1) Hospital for Sick Children, Toronto, Ontario, Canada; 2) University of Toronto, Toronto, Ontario, Canada.

The increasing prevalence of obesity as well as its association with many chronic diseases have turned obesity into a major health concern worldwide. Obesity has many underlying environmental and genetic factors that both contribute to disturb the homeostatic mechanisms that maintain a balance between energy intake and energy expenditure. At the top of the hierarchy is the central nervous system which regulates energy homeostasis by integrating inputs from the periphery and producing the appropriate outputs to the metabolic organs. However, despite the amount of attention given to this neuronal circuitry, the signalling pathways and underlying mechanisms are still not clearly defined. The goal of my project is to help further our understanding of these CNS mechanisms by using the powerful tools available in Drosophila melanogaster to identify neuronal genes involved in energy homeostasis. Specifically, I screened to see if knockdown of individual genes in the CNS would produce obese or lean phenotypes defined as increases or decreases in fat stores respectively. To date, I have completed screening 1700 genes producing 107 hits that when knocked down in fru-Gal4-expressing neurons that results in greater than 30 percent change in the levels of stored lipids compared to a fru-Gal4/wt control.

405C

The effect of three types of diets on the phenotype of Drosophila melanogaster. Xiangpei Zeng, Sean Mendez, Laura Reed. Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

The increasing frequency of Metabolic Syndrome in modern society suggests that changes in the human environment can predispose individuals to express cryptic genetic variation for metabolic disease. This might be due either to alterations in diets or the interaction of diet with the underlying genetic variations in the population. To contrast the effect of different types of food on phenotype, we have focused on the effect of three types of food on several metabolic traits in several inbred lines of Drosophila melanogaster. Specifically, we are interested in contrasting the effect of added fat in a low sugar or a high sugar diet. We used three types of food in this experiment: normal food (N), normal food with high fat (NF), and low sugar food with high fat (OF). Three phenotypes were measured: pupae weight, total triglyceride content, and trehalose content. We found significant interactions between the genetic lines and the diets for the phenotypes. Differences between metabolic traits mean and variance in response to three types of diets indicates that alterations in diets could contribute to the changes in phenotype of four inbred lines of Drosophila melanogaster, with independent contributions of sugar and fat effects.

406A

Suppression of progressive motor neuron degeneration by Diferuloylmethane (Curcumin) in transgenic Drosophila expressing mutant human gene of neurodegenerative disease. Namita Agrawal, Anjalika Chongtham, Nidhi Paliwal. Dept of Zoology, University of Delhi, Delhi.

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by an expansion of a homopolymeric polyglutamine (polyQ) stretch within the huntingtin protein (Htt) The fruit fly, Drosophila melanogaster has been proved a very powerful invertebrate model for testing drugs. Drosophila transgenic model engineered to express mutant human genes of neurodegenerative disease like Huntington disease (HD) has been used previously by us to test effectiveness of the drug combinations for the treatment of the HD pathogenesis (Agrawal et al., PNAS 2005). This model has been proven to be excellent models of these largely dominant human diseases by replicating most of the disease symptoms, such as late onset, reduced longevity, neurodegeneration, and impaired motor function. To find a safe, non toxic drug for the pharmacologic treatment of devastating neurodegenerative diseases is a real challenge in modern medicine. To circumvent this problem, a safe and suitable approach was used by testing some popular phytochemicals which are known for several thousand years in the world and are being used successfully for the treatment of various health conditions instead of pharmaceutical drugs.

We established a dose regimens of Diferuloylmethane (Curcumin) to assess its effectiveness on Drosophila HD model. Our findings indicate that 3 and 10μM concentrations of curcumin significantly ameliorate HD pathogenesis including aggregation. Interestingly, 10μM concentration of curcumin prevented progressive impairment of motor function in "humanized" fly model. This data suggests that curcumin may serve as a potential therapeutic agent for patients in the treatment of neurodegenerative diseases like HD without any side effect.

407B

A Parkinson's Disease Model for the Characterization of Long Term Effects of Early Exposure to Environmental Toxins. James W. Anderson, Arati Inamdar, O'Neil Wright, Janis O'Donnell. Biological Sciences, University of Alabama, Tuscaloosa, AL.

The degeneration of dopaminergic neurons, the central pathological feature of Parkinson's disease (PD), leads to characteristic movement disorders only after approximately 75% of the neurons in the midbrain have been lost. It is likely that susceptibility to idiopathic PD is defined by genetic variation acting in concert with environmental triggers that may have occurred decades prior to the onset of symptoms. This extreme lag in manifestation of PD presents a barrier for the identification of environmental risk factors and biomarkers for early events that can predispose an individual to this incurable neurodegenerative disease. We employed a Drosophila PD model based on exposure to the herbicide paraquat (PQ), which triggers dopaminergic neurodegeneration and movement deficits, as a system for defining mechanisms by which early events lead to later disease. We investigated whether a single exposure to PQ during the larval stage had long-term consequences that led to the development of PD-like features or that sensitized individuals to secondary environmental challenges in adult life. We found that larvae, fed for 12 hours only on 1-10mM PQ, subsequently exhibited parkinsonian symptoms in adulthood, including persisting mobility defects and low dopamine levels in the adult brain. We find evidence of dopaminergic neurodegeneration, as suggested by the diminished dopamine pools. We show that treated larvae are more sensitive to secondary exposure to PQ as adults. Additionally, PQ treatment of larvae induces chronic neuroinflammation that persists through metamorphosis and into adulthood. This chronic inflammatory response exacerbates the PQ-induced degeneration of dopaminergic neurons. Our data support the hypothesis that early exposure to environmental toxins is a risk factor for developing PD. Furthermore, we provide evidence that altering the inflammatory response early in this process can ameliorate later parkinsonian symptoms. In addition, we provide evidence for biomarkers with long-term alterations, which may facilitate early diagnosis.

408C

Mutations that Destabilize Helix-3 Induce Aberrant Processing of the Prion Protein. Daniela Arbelaez[1,2], Jonatan Sanchez[2], Pedro Fernandez-Funez[2], Diego Rincon-Limas[2]. 1) College of Liberal Arts and Sciences; 2) Department of Neurology, University of Florida, Gainesville, FL.

Prion diseases are caused by the misfolding of the normal prion protein (PrPc), a glycolipid-anchored membrane protein, into a toxic conformation (PrPsc). The globular domain of PrP is stabilized by different intramolecular interactions, one being hydrophobic interactions between helix 1 and 3. *In vitro* studies have shown that this well conserved hydrophobic core of PrP is stabilized by two Methionines (M205 and M212) in the helix 3 and that introduction of a polar amino acid at those positions (M205S and M212S) significantly disrupts the native fold of PrP. To understand the role of these Methionines in the structural stability of PrP *in vivo*, we expressed PrP-WT, PrP-M205S and PrPM205, 212S in Drosophila. We hypothesized that these mutations would increase the structural instability of PrP, promoting misfolding and heightening neurotoxicity. First, we found that the Met to Ser mutants did not induce the progressive locomotor dysfunction and premature death typical of WT PrP. Subsequent to these observations, we performed biochemical assays and these showed the mutations affect PrP conformation; the flies showed aberrant development in the mushroom bodies, as well as shortened axons at the alpha lobe. Further results using immunostaining and the antibody 6H4 showed no detection of PrP in the mutants. We also looked at the glycosylation patterns of the mutant PrP and found that they showed aberrant glycosylation. Also, the mutant PrP contained the N-terminal signal peptide, which is normally detached in fully processed PrPc. These results demonstrate that the mutant flies carrying the Met to Ser substitutions do not follow the normal PrP biogenesis pathway. These mutations in the helix 3 seem to induce conformational changes that enhance the formation of ctmPrP, a neurotoxic form of PrP. These findings are relevant because the ctmPrP topology has been found in patients with prion diseases and may be critical for the conversion of native PrP.

409A

***Drosophila* β-secretase and the cleavage of the fly Amyloid Precursor Protein are required for glial survival.** Bonnie J. Bolkan[1], Tilman Triphan[2], Doris Kretzschmar[1]. 1) CROET, L606, Oregon Hlth & Sci Univ, Portland, OR; 2) Institut für Zoologie III, Universitaet Mainz, 55099 Mainz, Germany.

The presence of Beta-Amyloid (Aβ) containing plaques in the brain is one of the histological hallmarks of Alzheimer's Disease. Aβ is produced from the Amyloid Precursor Protein (APP) by β-secretase (or BACE) and γ-secretase cleavage and therefore BACE has become a major focus in studying Alzheimer's Disease. In vertebrates, BACE has been shown to affect myelination and neuronal activity, functions that have been associated with the cleavage of Neuregulin and the β-subunit of a voltage-gated sodium channel, respectively. Here we show that a knockdown of Drosophila BACE (dBACE) in photoreceptors results in progressive degeneration in the lamina cortex due to a loss of glial cells. Loss of APPL, the sole fly APP protein, suppresses the degeneration whereas overexpression of APPL enhances the phenotype. An enhancement of the degeneration is even more prominent with a secretion-deficient form of APPL, which also results in degeneration when expressed alone. We therefore, propose a model in which full-length APPL, which is expressed in neurons, induces glial death and that dBACE cleavage interrupts this process leading to glial survival.

410B

Assessing the ability of nicotine to increase lifespan and rescue olfactory and motor deficits in parkin loss-of-function Drosophila melanogaster. Lori M. Buhlman, Raegan P. Chambers, Gerald B. Call. Biomedical Sciences, Midwestern University, Glendale, AZ.

Parkinson's disease (PD) is the second most common neuromotor degenerative disease, existing in both sporadic and familial forms and affecting about 13 of 100,000 in the US (Van Den Eeden *et al*., 2002). *Drosophila melanogaster* is a particularly attractive model for familial PD, as unlike mouse parkin loss-of-function models, *park*^{25}/*park*^{25} *D. melanogaster* exhibit many of the pathologies found in familial PD patients, including mitochondrial pathology, motor deficits and decreased lifespan. Progressive motor deficits found in *park*^{25}/*park*^{25} *D. melanogaster* are thought to result from mitochondrial pathology that causes indirect flight muscle degeneration (Greene *et al*., 2003). Epidemiological studies suggest that tobacco smokers are dose-dependently less likely to develop PD (Hernan *et al*., 2001; Grandinetti *et al*., 1994; Rajput *et al*., 1987); subsequent in vitro and in vivo studies show that nicotine is protective in models of sporadic PD (reviewed in Quik *et al*., 2009). Since olfactory deficits are one of the initial symptoms of PD, we have, for the first time, assessed

whether $^{+}/park^{25}$ and $park^{25}/park^{25}$ (Greene *et al*., 2003) *D. melanogaster* also model this phenotype. Literature addressing the potential protection by nicotine in *Italic Text*D. melanogaster parkin*Italic Text* loss-of-function models spans limited concentrations of nicotine and selected time points and durations in the organism's lifespan. In order to more comprehensively address whether nicotine can protect against the various deficits in this model of familial PD, we have assessed viability, olfaction, climbing and flying in wild-type, $^{+}/park^{25}$ and $park^{25}/park^{25}$ *D. melanogaster* that have been exposed to a range of nicotine concentrations from eclosion until behavior assays are performed on days 5, 10 15 and 20 post-eclosion. Our results elucidate the suitability of $park^{+}/park^{25}$ and $park^{25}/park^{25}$ in the pursuit of the mechanism(s) by which nicotine is protective against PD.

411C

Human LRRK2 expression increased animal lifespan and enhanced the resistance to oxidative stress in the Drosophila. Hui-Yun Chang, Hung-Cheng Wang, Franziska Wolter. Institute of Systems Neuroscience, National Tsing Hua University, Hsinchu, Taiwan.

We study the function of Leucine-rich repeat kinase 2 (LRRK2) in the animal model of Drosophila. LRRK2 mutations have been shown to be the most common genetic causative effect to both familiar and sporadic forms of Parkinson's disease in human. However, little is known about its physiological function in the animal brains. In this study, we found that LRRK2 expression in the brains showed an increase of the animal life span compared to normal or driver alone in Drosophila. In addition, LRRK2 expression showed a protective effect to the environmental toxin of paraquat. Interestingly, LRRK2 expression could substantially suppress grim-induced apoptosis but not hid- and reaper- induced phenotypes in the fly eye. These data strongly support wild type LRRK2 may play a role in protection from paraquat toxicity and neuronal degeneration.

412A

The role of Superoxide Dismutase 2 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 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. SOD2 is expressed in the mitochondria, a likely location for increased reactive oxygen species. Mild, moderate, and strongly expressing UAS alleles of mutant and normal MJD as well as UAS-SOD2 were expressed in the fly eye using the gmrGal4 driver. Flies were aged for one or seven days and their heads were fixed and embedded in epon resin blocks. Ultramicrotome 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. Additionally we examined the influence of up regulated SOD2 on mutant MJD protein solubility. Other research has implicated superoxide in the autophagy pathway, and autophagy has been suggested to reduce the degeneration caused by MJD by removing aggregates. Therefore, we propose that the increase in SOD2 levels interfered with the autophagy pathway causing the increase in degeneration.

413B

The neurodegenerative AMPK mutant *loe* interferes with the RHO pathway and actin dynamics. Mandy Cook, Jill Wentzell, Doris Kretzschmar. CROET, Oregon Health and Science University, Portland, OR.

Isoprenylation is an important mechanism allowing intracellular proteins, like small G proteins (e.g. RHO) to associate with the membrane, which is then followed by activation of the protein. This step is critical for signal transduction of cellular hormones, growth factors, and cytokines from the cytoplasm to the nucleus and influences proliferation, differentiation and survival of the cell. The isoprenoid pathway is negatively regulated by AMPK (AMP- activated protein kinase), an inhibitor of HMG-CoA Reductase (hydroxymethylglutaryl-CoA Reductase). The *Drosophila* mutant *loechrig*, which lacks a neuronal isoform of the AMPK γ subunit, shows progressive neurodegeneration, neuronal cell death of the adult nervous system and a lower cholesterol ester level. In order to determine the correlation between the *loe* mutation, isoprenylation and the RHO1 pathway, we generated and analyzed flies with mutations in RHO and its downstream targets. We were able to show that the *loe* mutation interferes with the prenylation of RHO1 and the regulation of the downstream LIM-Kinase pathway, which plays an important role in actin turnover and axonal remodeling. In addition, we used western blotting to show the concentration of cofilin, which regulates actin turn over. Interestingly, all these defects including a behavior phenotype, can be detected before a severe neurodegeneration is histologically visible.

414C

Characterizing mitochondrial dysfunction in a Drosophila model for TBI. Vanessa T. Damm[1,2], Rachel T. Cox[1,2]. 1) Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD; 2) Center for Neuroscience and Regenerative Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD.

Traumatic Brain Injury (TBI) affects millions of people every year, yet there is still much about the downstream molecular effects that we do not understand. The primary injury from TBI is a devastating focal or diffuse injury to the brain caused by a blow to the head or shockwave, in the case of combat. The resulting damage can lead to secondary injuries that result in subcellular changes and tissue damage. Our focus is on understanding the cell biological changes post-injury that occur to mitochondria. An impact to the brain shears neuronal membranes which results in ionic misregulation, formation of reactive oxygen species and calcium overload. This homeostatic disruption has a striking negative effect on mitochondrial function resulting in loss of ATP production, dysregulated calcium storage, and apoptosis. In order to visualize the effects of TBI on mitochondria in real time, we are developing a TBI model using the Drosophila larval brain. We have created transgenic flies expressing fluorescently labeled mitochondria, including those that sense reactive oxygen species and calcium levels. We are using live confocal imaging to characterize mitochondrial dynamics in the larval brain pre- and post-injury. Once we have established this TBI model, we hope to use it to identify neuroprotective genes and pathways. This knowledge may lead to better therapies specifically designed to reverse mitochondrial damage post TBI.

415A

Screen for common genetic modifiers of polyglutamine diseases in Drosophila. Javier R Diaz, Ismael Al-Ramahi, Juan Botas. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Polyglutamine diseases are caused by expansion of a CAG repeat within the coding region of the disease-causing gene. Two of the nine known polyglutamine disorders are Spinocerebellar Ataxia type 1 (SCA1) and Huntington's disease (HD). The proteins affected in these two disorders are Ataxin-1 and Huntingtin respectively. Using Drosophila as a model system, we carried out genetic screens of all genes encoding kinases for potential modifier genes of SCA1 and HD. In the SCA1 Drosophila model we drive expression of mutant Ataxin-1 (SCA182Q) to the eye using gmr-Gal4 and we analyze the external structure of the eye. In the HD model, we drive the expression of mutant huntingtin (N-terminal Htt128Q) to the nervous system using elav-Gal4 and we assess motor performance using a climbing assay. As a result of these screens we obtained common modifier genes. Some of these modulate SCA1 and HD similarly, whereas other modifier genes modulate them antagonistically (e.g., suppressor vs. enhancer). We are currently analyzing the mechanisms by which these modifiers modulate disease phenotype; these studies include analyzing steady-state levels of the disease-causing protein, investigating mitochondrial/bioenergetic alterations, and assessing Ca2+ levels.

416B

The role of Swiss cheese, the *Drosophila* homologue of Neuropathy target esterase, in glia development. Sudeshna Dutta[1,2], Doris Kretzschmar[1]. 1) Center for Research on Occupational and Environmental Toxicology, Oregon Health and Science University, Portland, OR; 2) Department of Integrative Biosciences, Oregon Health and Science University, Portland, Oregon.

Neuropathy target esterase (NTE), a molecular target of organophosphates (OP) found in pesticides and nerve gases induce delayed neuropathy (OPIDN) 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 in flies but also glial degeneration. Previously we have shown a cell autonomous requirement of SWS in both neuronal and glial 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. We are also investigating the importance of various functional domains of the trans-membrane protein SWS in the glia specific phenotype. Our recent findings in mouse show presence of SWS/NTE in astrocytes in the sciatic nerve, suggesting a conserved role of SWS in glia in higher vertebrates. These studies, using both *Drosophila* and mouse model systems, will help us to understand the importance of the SWS protein in glia, its regulation in axonal-glial interaction and its pathogenic function in inherited spastic paraplegia and in OPIDN in humans.

417C

Oxidative Stress in a Drosophila Model of TPI deficiency. Isaac J Fisher[1], Daniel Long[1], Joshua Hutton[1], Zhaohui Liu[1], Sarah Johnson[1], Michael J Palladino[2], Stacy L Hrizo[1,2]. 1) Department of Biology, Slippery Rock University, Slippery Rock, PA; 2) University of Pittsburgh S.O.M. Department of Pharmacology and Chemical Biology, Program in Neurodegenerative Diseases, Pittsburgh, PA.

Triose phosphate isomerase (TPI) is responsible for the interconverstion of dihydroxyacetone phosphate to glyceraldehyde-3-phosphate in the glycolytic pathway. Point mutations in the gene encoding this enzyme are associated with a glycolytic enzymopathy called TPI deficiency. This study focuses on *TPI[sgk]*, a mutant allele with a missense mutation (M80T) that causes phenotypes in Drosophila melanogaster similar to that of TPI deficiency in humans. The pathology of TPI deficiency is still poorly understood. In this study, we examine the redox status of flies with the *TPI[sgk]* mutant allele in order to better understand the pathology of this disease. We hypothesized that *TPI[sgk]* animals would have sensitivity to oxidative stress due to the higher levels of the oxidized forms of various redox molecules. Supporting this, we examined the ratios of the reduced and oxidized forms of NAD+, NADP+, and glutathione. It was determined that *TPI[sgk]* animals exhibit higher levels of the oxidized forms of these molecules in an age dependent manner. In addition, we tested the effect of oxidizing and reducing stressors on the behavioral phenotypes of the *TPI[sgk*] animals. It was found that reductive stress improves the behavioral phenotypes of the mutant organism while oxidative stress worsens these phenotypes. In addition, we examined the stability of mutant protein when animals were treated with oxidizing and reducing stressors and determined that TPI[sgk] protein levels were unaffected by redox stressors. Overall, this data suggests that reduced activity of TPI causes an increase in oxidative stress in the organism and that alleviating this stress with reducing compounds improves the mutant phenotypes.

418A

Sleep defects in Drosophila models of Huntington's Disease reflect altered PKA signaling. Erin D Gonzales[1,2], Jerry C-P Yin[1,3]. 1) Dept. of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Program in Cellular & Molecular Biology, University of Wisconsin-Madison, Madison, WI; 3) Dept of Neurology, University of Wisconsin-Madison, Madison, WI.

Identification of early biomarkers of neurodegenerative diseases is essential to understanding early pathogenesis and developing therapeutic strategies. Studies in HD carriers show disturbances in sleep are among the earliest quantifiable symptoms and correlate with cognitive changes, indicating sleep changes may be used as a biomarker for early disease and testing of potential interventions. Drosophila HD models recapitulate many cell and molecular phenotypes of HD and mammalian HD models, showing that pathways mediating pathogenesis are conserved. These phenotypes include alterations in transcription, intracellular trafficking, metabolic regulation, & motor function. Using pan-neuronal drivers C155 or G28 elav-Gal4 with full-length (mutHttFL) and truncated mutHtt (mutHttNterm), our goals were: 1) to determine whether Drosophila HD models exhibit perturbations in sleep & activity similar to those in HD carriers & 2) to identify the underlying pathways. Pan-neuronal expression of mutHttNterm by C155 normally results in pupal lethality, whereas G28 expression results in adult viability. We show that mutHttFL & mutHttNterm flies exhibit abnormal sleep and activity patterns. These changes in sleep architecture and activity precede other systemic phenotypes and resemble deficits in human patients. Treatment with a dopaminergic signaling inhibitor reverses these alterations. This reversal, along with a dramatically reduced sensitivity to caffeine, are consistent with abnormally elevated PKA in HD flies. We show that RNAi against PKA catalytic subunits or overexpression of a PKA regulatory subunit rescues C155-driven lethality, and restores normal sleep patterns when driven by G28. We also show biochemical & imaging data that baseline levels of cAMP and phospho-CREB are elevated brain-wide in HD flies compared to controls. Our data indicate that cAMP/PKA signaling is dysregulated early in HD and that this pathway may present a target for therapeutics.

419B

Fatty acid activation and neurodegeneration. Hannah B Gordon, Anna Sivachenko, PhD, Anthea Letsou, PhD. Department of Human Genetics, University of Utah, Salt Lake City, UT.

Fatty acids are utilized for a variety of cellular needs from energy substrates to membrane components. When a tissue is particularly dependent on these requirements for fatty acids, it is rendered sensitive to fatty acid metabolism. One tissue that is highly dependent upon fatty acid metabolism is the nervous system; here fatty acids play a central role in neuronal insulation. We have recently characterized *double bubble* (*dbb*) a gene encoding an acyl-CoA synthetase (ACS) that displays a neurodegenerative phenotype in the adult fly. ACS proteins are responsible for activating fatty acids for subsequent utilization as membrane components, signaling molecules and/or energy substrates. *dbb* mutants exhibit a shared loss-of-function phenotype with a previously characterized mutant in a homologous ACS gene, *bubblegum* (*bgm*). Alone, each of these mutants displays fully penetrant neurodegenerative phenotypes that are variably expressed. Neurodegenerative phenotypes in *bgm dbb* double mutants, however, are more severely manifested, and thus the double mutant provides a clearer platform to characterize the roles of ACS proteins and lipid metabolism in neuropathology. In humans, mutations in conserved proteins in the ACS fatty acid activation pathway are associated with the human diseases adrenoleukodystrophy and adrenomyeloneuropathy; both of which are characterized by neurodegeneration and signs of altered lipid metabolism such as high circulating very long chain fatty acids (VLCFAs, >22 carbon chain length). The specific lipid alterations that result from disruption of ACS proteins, as well as the primary mechanism by which altered ACS activity leads to neurodegeneration are not currently understood in either the Drosophila model or the human disease. Here we provide evidence that the Drosophila *bgm dbb* double mutant model provides a powerful in vivo platform to elucidate the roles and toxicity of lipids in neuronal cells.

420C

Survival motor neuron protein controls stem cell division, proliferation and growth. Stuart J. Grice, Sian E. Davies, Jilong Liu. MRC Functional Genomics Unit, University of Oxford, Oxford, United Kingdom.

Survival motor neuron (SMN) protein facilitates the biogenesis of ribonucleoprotein (RNP) complexes such as the small nuclear RNPs (snRNPs) required for pre-mRNA splicing. Although SMN is required in all cells, the motor nervous system is particularly sensitive to SMN reduction, with SMN loss causing the neuromuscular disease spinal muscular atrophy (SMA).

SMN protein is highly upregulated in early embryogenesis and there is increasing evidence to suggest that high levels of SMN are essential for timely proliferation and growth in multiple tissues. With the current development of therapeutics for SMA, there is an urgent need to comprehensively understand the transient and local enrichments of SMN required for normal development.

We have previously reported that SMN is enriched in *Drosophila* stem cells, and its loss leads to fewer stem cell divisions, changes in RNP component levels and localization, and alterations in the timing of differentiation. Our current research is looking at the specific requirement of SMN to control the switch between proliferation and differentiation. Using clonal analysis in the larval CNS and imaginal discs, as well as *smn* mutants, we have analyzed how local changes in SMN effect specific stem cell and differentiation factors. We show that high endogenous SMN levels are required for efficient proliferation and that SMN reduction correlates with a switch to differentiation. From this we hypothesise how a systemic reduction of SMN in early development can contribute to targeted neuromuscular defects and how reducing SMN levels can modify ectopic proliferation in *Drosophila*.

421A

Genes *Sema-1a* and *Sema-2a* as modifiers of dystrophin gene function in *Drosophila melanogaster*. Olena Holub, Yaroslava Chernyk, Nataliya Holub. Genetics and Biotechnology, Ivan Franko National University of Lviv, Lviv, Ukraine.

Muscular dystrophy (MD) refers to a group of genetic diseases characterized by progressive damage and weakness of facial, limb, breathing, and heart muscles. It is due to the lack of a key protein dystrophin that is needed to maintain the integrity and proper function of the muscle. *Drosophila melanogaster* is an excellent genetically tractable model for searching new approach to treatment dystrophies such as using genes-modifiers of dystrophin gene function. The aim of our work was to check up influence of genes *Sema-1a* and *Sema-2a* (involved in neuronal migration) as an possible genes-modifiers on mutant phenotype of dystrophin gene. Mutant strain NH_2*-Dys* constructed after the method antisense-RNA were used. It is characterized by diminished on 30% expression of dystrophin gene, defective thorax muscle structure and decreased the index of physical activity (IPA). Offsprings F_1 which contained supplementary copy of gene-modifier and dystrophin gene inactivation construct were analysed after these indexes. In all crossing systems was observed restore of thorax muscle structure with the frequency 59% - 61% that is in 10 times higher comparing to the strain NH_2*-Dys*. In climbing-test was shown increasing of IPA in progeny NH_2*-Dys // Sema-1a* in the 2 - 4 times and for hybrids NH_2*-Dys// Sema-2a* - in 3 - 6 times comparing to strain NH_2*-Dys*. Previously was shown that genes *Sema-1a* and *Sema-2a* resumed of wing vein structure with frequency 16% and 44% in strain NH_2*-Dys*. It could be concluded that genes *Sema-1a* and *Sema-2a* manifested to be dystrophin - deficiency phenotype suppressors moreover gene *Sema-2a* is more active suppressor than *Sema-1a* gene.

422B

TPI[sgk] is degraded by the proteasome in a chaperone dependent manner. Stacy Hrizo[1,2], Daniel Long[1], Michael Palladino[2]. 1) Department of Biology, Slippery Rock University, Slippery Rock, PA; 2) Department of Pharmacology and Chemical Biology, University of Pittsburgh SOM, Pittsburgh, PA.

Triosephosphate isomerase (TPI) deficiency is a severe glycolytic enzymopathy that causes progressive locomotor impairment and neuromuscular degeneration, susceptibility to infection, and premature death. We previously identified a recessive missense mutation in the TPI allele in Drosophila called sugarkill that exhibits similar phenotypes as TPI deficient patients such as progressive locomotor impairment, neurodegeneration, and reduced lifespan. In previously published work, we showed that the TPI[sgk] protein is an active stable dimer however the mutant protein is rapidly turned over by the proteasome reducing cellular levels of this glycolytic enzyme. We have confirmed the instability of the TPI[sgk] protein with pulse chase analysis of the protein in primary culture cells derived from embryos. In addition, we hypothesized that TPI[sgk] is recognized by molecular chaperones and components of the ubiquitin proteasome pathway, resulting in the proteasomal degradation of the mutant protein. In support of this hypothesis, we have detected an interaction between Hsp70 and Hsp90 and the TPI[sgk] protein. In addition, data collected using complementary pharmacological and genetic experiments indicate that both Hsp70 and Hsp90 are important for targeting TPI[sgk] for degradation. We have conducted mechanical stress sensitivity assays and analyzed TPI[sgk] protein levels in order to ascertain whether inhibiting the proteasome with MG132 or the molecular chaperone Hsp90 with geldanamycin, results in a decreased or exacerbated phenotype. We observed that the mechanical stress sensitivity in TPI[sgk] animals with reduced proteasome, Hsp90 and

Hsp70 activity was diminished suggesting TPI[sgk] is a functional protein and that reducing TPI[sgk] degradation can abrogate disease pathogenesis. The autophagy pathway was also assessed for a role in TPI[sgk] degradation and no change in TPI[sgk] degradation was observed with pharmacological studies.

423C

Neurodegeneration in a Temporally-Controlled Fly Model of Huntington's Disease. Kurt Jensen[1], Diego Rincon-Limas[1], Pedro Fernandez-Funez[1,2]. 1) Neurology, University of Florida, Gainesville, FL; 2) Neurosciences, University of Florida, Gainesville, FL.

Huntington's disease (HD) is a devastating condition characterized by accumulation of Huntingtin-containing intranuclear inclusions in the brain neurons of affected individuals. Unfortunately, the exact mechanism by which the expanded Huntingtin protein (Htt) leads to neurotoxicity is not clear. HD is a complex disease, and early changes that lead to neuropathology are not obvious. These early changes are particularly difficult to detect in *Drosophila* since traditional UAS-controlled transgenes result in gene expression in developing neurons, potentially causing both neurodevelopmental and neurodegenerative phenotypes. We opted to overcome this drawback by inducing ubiquitous expression of mutant Htt under the temporal control of the $Gal80^{ts}$ repressor. In our system, $Gal80^{ts}$; da>Gal4 flies were mated to UAS-Htt flies, and the resulting progeny were kept at 18°C throughout development (Gal80 active, system off). Upon eclosion, the adult flies were shifted to 31.5°C (Gal80 inactive, system on) and aged for up to 20 days. First, we determined the "on" kinetics of mutant Htt at both the RNA and protein levels. Muatnt *Htt* RNA was detected soon after induction, and mutant Htt protein was detected shortly thereafter. Then, we characterized this HD fly model functionally, histologically, and biochemically over time. The mutant Htt protein formed high-molecular weight aggregates, and changes in other HD-related pathology markers were also observed. Minor ultrastructural changes in brain structure were observed in the photoreceptors; however, these flies demonstrated a pronounced lethality. These results demonstrate the utility of establishing a model for HD that more rigorously controls transgene expression, while still providing a relevant disease model. We hope that this system will be exploited to further dissect HD and other neurodegenerative disease pathways.

424A

A Drosophila model of Multisystem proteinopathy caused by VCP/p97 mutation. Nam Chul Kim, J. Paul Taylor. Developmental Neurobiology, St.Jude Children's Research Hospital, Memphis, TN.

Multisystem proteinopathy (MSP) caused by mutations in valosin-containing protein (VCP)/p97, formerly known as IBMPFD-ALS, is an adult onset progressive disease affecting muscles (inclusion body myopathy), bone (Paget's disease of bone), the brain (frontotemporal dementia) and motor neurons (amyotrophic lateral sclerosis). As a recent study indentified VCP/p97 mutations in sporadic and familial forms of ALS, we generated and characterized a Drosophila model of ALS caused by VCP mutation to elucidate the role of VCP in disease pathogenesis. In 3rd instar larvae, mutant model animals have developmental defects in their neuromuscular junction (NMJ) such as small and premature synapses and a reduction in active zones . In adult abdominal NMJs, we found unstable and denervated synapses called footprints. Motor neuron dendrite formation was also significantly reduced in 3rd instar larval stage. Electron microscopy study revealed that motoneuron axons in mutant animals were severely degenerated. In NMJs and axons, fewer mitochondrias were observed compared to wild type control and the velocity of their transport also decreased. These defects by overexpression of $dVCP^{R152H}$ mutant protein in motor neurons resulted in abnormal locomotor behavior in 3rd instar larval stage (crawling) and also in adult stage (walking and flying). Therefore, this Drosophila model successfully recapitulates several important disease phenotypes of ALS patients and will be beneficial for further characterization of the disease mechanism in motor neurons.

425B

Accumulation of insoluble forms of FUS protein correlates with toxicity in drosophila. Magalie Lecourtois, Laetitia Miguel, Tracey Avequin, Morgane Delarue, Sébastien Feuillette, Thierry Frébourg, Dominique Campion. UMR Inserm U614, IFRMP23, Rouen Institute for Medical Research and Innovation, Faculty of Medicine, University of Rouen, 22 Boulevard Gambetta, 76183 Rouen Cedex 01, France.

The Fused in Sarcoma/translated in liposarcoma (FUS) protein is highly conserved and ubiquitously expressed. Physiologically, FUS is primarily located in the nucleus, but continuously shuttles between nucleus and cytoplasm. The precise roles of the protein are not fully elucidated, although FUS is known to be involved in multiple levels of RNA processing including transcription, pre-mRNA splicing, transport to and rapid local RNA translation at the synapse, and decay. Last, FUS proteins may also be involved in microRNA biogenesis. Recently, FUS has been identified as a major constituent of nuclear and/or cytoplasmic ubiquitin-positive inclusions in patients with frontotemporal lobar degeneration (FTLD) or amyotrophic lateral sclerosis (ALS). In brain tissue of patients presenting FTLD with FUS pathology, pathological FUS proteins shift towards an insoluble fraction. To explore aspects of FUS pathogenesis in vivo, we have developed a new Drosophila transgenic model expressing a wild-type isoform of human FUS protein. We found that when expressed in retinal cells, FUS proteins are mainly recovered as soluble forms and their overexpression results in a mild eye phenotype, with malformed interommatidial bristles and the appearance of ectopic extensions. On the other hand, when FUS proteins are specifically targeted to adult differentiated neurons, they are mainly recovered as insoluble forms, and their overexpression drastically reduces fly lifespan. Importantly, FUS neurotoxicity occurs regardless of inclusion formation. Lastly, we showed that molecular chaperones reduce FUS toxicity by modulating protein solubility. Altogether, our data indicate that accumulation of insoluble non-aggregated FUS forms might represent the primary toxic species in human FUS proteinopathies.

426C

Use of *Drosophila* cultured cells to investigate homeostasis and toxicity of metals such as copper, manganese and zinc. Stephanie E. Mohr[1], Quentin Gilly[1], Benjamin McElvany[1], Claire Y. Hu[1], Ian T. Flockhart[1], Donghui Yang-Zhou[1], Norbert Perrimon[1,2]. 1) Dept Gen, Harvard Med Sch, Boston, MA; 2) HHMI, Harvard Med Sch, Boston, MA.

Metals such as copper, manganese and zinc are essential to cell survival but in excess, the same metals can result in cell death and disease. Thus, maintaining appropriate intracellular metal levels is critically important to cell survival. We have developed a platform for studying metal homeostasis and toxicity using *Drosophila* cultured cells. As expected, low levels of metal supplementation have beneficial or neutral effects on cell viability as measured by a total cellular ATP readout, whereas supplementation with high levels significantly reduces cell viability. To test the utility of RNAi screening as a method for identifying factors required for metal homeostasis and toxicity, we generated RNAi reagents for knockdown of 50 gene candidates identified based on similarity to genes conferring sensitivity or resistance to metal supplementation in other model systems. As expected, several membrane transporters and

members of the Tor signaling pathway were identified as modulating viability in the presence of metals. In addition, the results of our initial RNAi experiments suggest that a putative P-type ATPase cation transporter plays a general role in cation import. Specifically, we found that RNAi knockdown with either of two different dsRNAs directed against the putative transporter prevents cells from benefiting from the positive effects of low-level metal supplementation and conversely protects cells from the toxic effects of high-level metal supplementation. Intriguingly, the transporter is highly related to a mammalian protein associated with Parkinson's disease. Our results suggest that RNAi screening in cultured *Drosophila* cells is an appropriate platform for identification of new genes involved in metal homeostasis and/or toxicity and further, that the results of these studies may impact our understanding of neurodegenerative and other diseases. Efforts to develop *in vivo* assays of metal toxicity for validation are ongoing.

427A

The Etiology of Brain Degeneration in drd Mutant Flies. Sreejith Perinthottathil, Wijeong Jang, Jiyoung Kim, Changsoo Kim. Chonnam National University, Gwangju, South Korea.

The Drosophila drop-dead (drd) mutant undergoes massive brain degeneration. The etiology of brain degeneration in drd mutant flies is still unknown. We found that DRD protein is selectively expressed in cells secreting cuticular and eggshell layers that exhibit blue fluorescence upon UV excitation, which is reduced in drd flies. We found that the drd tracheal air sacs lacking blue fluorescence collapse and genes induced in hypoxia are up-regulated in drd flies. Feeding of anti-ROS agents partially rescued the drd from sudden death. We propose that drd flies can provide a non-invasive animal model for hypoxia-induced cell death.

428B

Identification of protective and pathogenic residues in the prion protein. Jonatan Sanchez-Garcia, Daniela Arbelaez, Kurt Jensen, Yan Zhang, Diego Rincon-limas, Pedro Fernandez-Funez. Department of Neurology, Univ of Florida, Gainesville, FL.

Prion diseases are transmissible neurodegenerative diseases caused by misfolding of the prion protein (PrP) into pathogenic conformations. Unfortunately, major gaps still exist in the knowledge of how PrP undergoes conformational changes. We have shown previously that transgenic flies faithfully reproduce the structural dynamics of pathogenic and stable PrP sequences. Here, we describe point mutations in loop 2 and helix 3 with dramatically opposing effects on PrP structural dynamics. Dog PrP possesses a globular domain with a highly stable tertiary structure. We identified a charged amino acid in loop 2 of dog PrP (D158) that is not conserved in other mammals (N158). To test if D158 could increase PrP stability, we introduced the N158D substitution in mouse PrP. PrP-WT accumulates in pathogenic isoforms but PrP-N158D does not, supporting its higher conformational stability. Also, PrP-N158D does not induce the aggressive locomotor dysfunction observed with PrP-WT, indicating that the N158D substitution is neuroprotective. We also investigated the consequence of destabilizing helix 3, which has been proposed to initiate PrP misfolding. For this, we introduced two Met, to Ser substitutions in helix 3 that destabilize key hydrophobic interactions in the globular domain. Surprisingly, the M205,212S substitutions affected not only PrP folding, but also its processing and topology. PrP is anchored to the plasma membrane by a GPI. However, PrP-M205,212S resulted in an abnormal transmembrane topology (CtmPrP) that plays a role in disease. Consistent with CtmPrP, PrP-M205,212S retained it signal peptide and produced a protease resistance fragment. PrP-M205,212S also induced aberrant development of mushroom lobes, showing that it is neurotoxic. Overall, this work identifies key residues in loop 2 and helix 3 with dramatic effect in the stability of the globular domain. These studies may provide critical information for the development of anti-prion therapies.

429C

Tau-induced neurotoxicity and apoptosis in a Drosophila model. Tzu-Kang Sang[1], Chien-Ping Hsieh[1], Ren-Huei Shiu[1], Hui-Yun Chang[2]. 1) Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan; 2) Institute of Systems Neuroscience,, National Tsing Hua University, Hsinchu, Taiwan.

Tauopathies are characterized by the intraneuronal deposit of fibrils that containing hyperphosphorylated tau in human brain. Tau is a microtubule-associated protein, which stabilizes the microtubules cytoskeleton and regulates the dynamic of tubulin assembly. Recent studies have demonstrated that mutations in certain sites of tau that derived from autosomal dominant tauopathy FTDP-17 (frontotemporal dementia with parkinsonism linked to chromosome 17) could lead to hyperphosphorylated tau aggregates in the neurons. While Tau protein phosphorylation is the major focus on tauopathies studies, several reports found that tau-induced cytotoxicity could be uncoupled with its phosphorylation state. Here, we have employed a well-characterized Drosophila model of tauopathy to investigate whether the toxicity of tau is due to the cleavage by Caspases. Overexpression of human tau recapitulates the features of tauopathies, and the phenotype could be suppressed by RNA interference (RNAi)-mediated knockdown of selected Drosophila Caspases. In addition, expressing tau that insensitive to the Caspase cleavage resulted only mild neurodegeneration as compared to that over expressing wild type Tau. These data support that processes of tau by caspase is crucial for the tau-mediated cytotoxicity. Furthermore, we also evaluated fly tau ortholog CG31057 in which has preserved microtubule-binding domains but lack of the putative Caspase cleavage sites. In our analysis in the visual system, fly Tau appeared to be dispensable and its overexpression did not cause evident abnormality. Together, we propose that Caspase activation may be an important trigger for human Tau mediated pathogenic mechanism.

430A

Glia-Mediated Neurodegeneration in the *Drosophila melanogaster* CNS. Ivan J. Santiago[1], Israel C. Nnah[1], Amandeep Kaur[1], Rosa Mino[3], Tadmiri R. Venkatesh[1,2]. 1) Biology, The City College of New York, New York, NY; 2) The Graduate Center of the City University of New York, New York, NY; 3) The University of North Carolina, Chapel Hill, NC.

Proper development, function and maintenance of the central nervous system (CNS) are reliant on the intricate interactions between glia and neurons. Glia cells perform a variety of key functions such as maintaining homeostasis, trophic support and the uptake and recycling of neuronal debris. Disruptions in glial function have been implicated in many neurological disorders. Despite their obvious importance in the CNS, glia remain much less characterized than their neuronal counterparts. Drosophila glia have been classified into distinct subtypes based on their position and morphology. We have examined the role of glia subsets in neuro-protection and neurodegeneration. Our studies show that glia specific expression of Drosophila-Cdh1(Rap/Fzr), a conserved regulatory subunit of the Anaphase Promoting Complex/Cyclosome (APC/C), an E3 ubiquitin ligase, results in the loss of glia in the CNS at the 3rd instar developmental stage. These larvae emerge into adult flies lacking subsets of glia and exhibit temperature-sensitive paralysis, age dependent

neurodegeneration and life span reduction. These flies also exhibit aggregate formation and age-dependent vacuolization of the brain. By employing glia subset-specific GAL4 drivers we have identified the Astrocyte glia as the critical glia subtype necessary for neuroprotection. We will present data from confocal microscopy, transmission electron microscopy and behavioral analyses. These data suggest a vital role for glia in neuroprotection, as well as a regulatory role for the APC/C in glial differentiation.

431B

Integrating Human and Fly Genetics to Understand Alzheimer's Disease Susceptibility. Joshua M. Shulman[1,2], Selina Imboywa[1,2], Allison E. Diamond[1,2], Portia I. Chipendo[1,2], Philip L. De Jager[1,2], Mel B. Feany[1,2]. 1) Brigham and Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA.

BACKGROUND: Advances in human genetics have made the discovery of susceptibility loci for Alzheimer's disease (AD) a reality. The critical next step will be to determine the responsible genes and mechanisms. We are therefore integrating the results of human genome-wide association studies (GWAS) with a simple but powerful functional screen in existing *Drosophila* models of AD. METHODS: From published GWAS, 130 candidate AD susceptibility genes achieved significant ($p<5x10^{-8}$) or suggestive ($p<10^{-4}$) associations, nominating 89 conserved fly gene orthologs for further study. Lines predicted to activate or disrupt gene function were screened for enhancement or suppression of the rough eye phenotype produced by expression of human Tau or Amyloid-Beta (Aβ), which form neurofibrillary tangles and amyloid pathology in AD. Selected genes were also evaluated for impact on age-dependent, Tau-induced neurodegeneration in the brain. RESULTS: Our ongoing screen has identified 11 novel genes demonstrating robust interactions with Tau or Aβ neurotoxicity *in vivo*. Disruption of the fly integrin receptor *scb* (orthologous to human *ITGAM* and *ITGA9*) or the integrin modulators, *Fit1/2* (orthologous to the kindlin, *FERMT2*), enhanced Tau toxicity. We also find that gain- and loss-of-function in *cindr*, a regulator of actin dynamics and the ortholog of *CD2AP*, reciprocally suppress and enhance Tau retinal toxicity, and *cindr* also modifies Tau-induced neurodegeneration. Our cross-species strategy highlights several additional molecular pathways, including kinases (*EPHA1*/*Eph*) the microtubule cytoskeleton (*MAST4*/*CG6498*), and RNA-binding proteins (*SNRPN*/*SmB*). CONCLUSIONS: Based on associations with human disease and functional interactions in *Drosophila*, we identify integrin-mediated adhesion and several other cellular pathways as potentially important in AD susceptibility. Integrating human and fly genetics is likely to be a powerful approach to enhance AD gene discovery in the future.

432C

Effects of HDAC inhibitor treatment on motor deficits and lethality in a *Drosophila* model of Parkinson's disease. Robyn St. Laurent, S. Tariq Ahmad. Department of Biology, Colby College, Waterville, ME.

Parkinson's disease (PD) is a neurodegenerative disorder affecting dopaminergic neurons in the substantia nigra and characterized by debilitating motor impairment. In *Drosophila*, chronic exposure to the pesticide rotenone, known to selectively deteriorate dopaminergic neurons, produces PD-like motor deficits. Genetic models of Huntington's disease in *Drosophila* have similar motor deficits. The deficits seen in Huntington's, however, are caused by an expansion of a glutamine repeat in the Htt protein resulting in degeneration of neurons in the striatum. These motor deficits are attenuated by treatment with histone deacetylase (HDAC) inhibitors such as sodium butyrate. This study aims to examine the effects of the novel treatment of HDAC inhibitors on motor deficits and lethality in a rotenone-induced PD model using *Drosophila*. 1-7 day old *Drosophila melanogaster* wildtype flies (*Canton-S*) were fed rotenone at 25 μM or 250 μM concentrations for 7 days. Motor functioning was tested using a negative geotactic assay to measure climbing and flight abilities. Exposure to 250 μM rotenone caused motor deficits and increased lethality compared to control and 25 μM treatment. Preliminary findings from simultaneous sodium butyrate and rotenone exposure suggest an improvement of motor deficits and lethality compared to rotenone exposure alone. We are currently characterizing the mechanism of sodium butyrate-mediated improvement in the rotenone-induced PD model.

433A

TDP-43 and FUS proteinopathies: biochemical studies and animal models. Mengxue Yang[1,2], Kazuo Fushimi[1], Xiaoping Chen[1], Tanya Monahiem[1], Jianghong Liu[2], Li Zhu[2], Jane Wu[1,2]. 1) Northwestern University, Chicago, IL; 2) Institute of Biophysics, CAS, China.

Recent studies have discovered mutations in genes encoding RNA binding proteins, TAR-DNA binding protein 43 (TDP-43) and fused in sarcoma/translocated in liposarcoma (FUS/TLS) in patients with TDP-43 or FUS proteinopathies, including Ub+ frontotemporal lobar degeneration (FTLD-u) and amyotrophic lateral sclerosis (ALS). To elucidate the pathogenic mechanisms, we have begun to examine molecular and biochemical features of TDP-43 and FUS in cultured cells. We have established transgenic fly models of these proteinopathies by expressing wild type (Wt) or ALS-mutant forms of human TDP-43 or FUS proteins respectively.

We have begun to systematically compare molecular pathology induced by expression of Wt and ALS-mutant TDP-43 in cultured cells or transgenic flies. Expression of the ALS-associated A315T mutant remarkably accelerates neurodegeneration in flies. The A315T mutant significantly enhances neurotoxicity and increases the formation of aberrant TDP-43 species. The C-terminal domain of TDP-43 shows sequence similarity to the prion protein. Synthetic TDP-43 peptides flanking amino acid residue 315 form amyloid fibrils in vitro and cause axonal damage and neuronal death in cultured neurons. Our work identifies an amyloidogenic and neurotoxic region in the C-terminal domain of TDP-43 flanking amino acid residue 315. These experiments reveal similarities between TDP-43 and prion proteins in their peptide sequences and biochemical properties. Our work suggests that decreasing formation of neurotoxic TDP-43 species and enhancing clearance of such derivatives may have therapeutic potentials. We have begun to characterize cells and flies expressing Wt or ALS-mutant forms of FUS. Flies expressing ALS-mutant FUS show accelerated and more severe phenotypes of age-dependent progressive neurodegeneration, recapitulating key features of human FUS proteinopathy. The relevance to human diseases and potential applications in developing new therapies will be discussed.

434B

Exploring the Pathogenic Role of Phosphorylated TDP in Drosophila Nervous System. Po-An Yeh, Pang-hsien Tu. Biomedical Sciences, Taipei.

TAR DNA binding protein (TDP) has been broadly shown as a main component of ubiquitinated deposits in the nervous system of patients with frontaltemporal lobar degeneration or amyotrophic lateral sclerosis. Although hyperphosphorylation of TDP is also present in those diseases, the function and consequence of TDP phosphorylation remain unclear. In this study, using Drosophila melanogaster as a model system, TDP was shown to be

phosphorylated by casein kinase I. Whist, substantially phosphorylated TDP is exclusively degraded compared to non-phosphorylated TDP. Furthermore, we also demonstrated that phosphorylated TDP is indeed mis-localized in the cytosol of fly neurons. Importantly, phosphorylation of disease-associated TDPs showed more deleterious effect compared to wild type TDP, suggesting phosphorylation plays an important role on TDP proteinopathy. Our studies open a window to explore the pathogenic role and the biological function of phosphorylated TDP in vitro. We hope that could give a clue for the possible therapeutic treatment and understand more detailed molecular mechanism of these devastating deposits.

435C

A combinatorial drug cocktail rescues Prion Protein neurotoxicity in flies. Yan Zhang[1], Pedro Fernandez-Funez[1,2], Diego Rincon-Limas[1]. 1) Neurology, University of Florida, Gainesville, FL; 2) Neuroscience,University of Florida, Gainesville, FL.

Prion diseases are infectious and neurodegenerative disorders in which the normal cellular prion protein (PrP^{C}) converts into a misfolded isoform (PrP^{Sc}) with unique biochemical and structural properties that correlate with disease. All prion diseases are fatal, with no effective treatments at this time. In humans, prion disorders such as Creutzfeldt-Jakob disease present typically with a sporadic origin, where unknown mechanisms lead to the spontaneous misfolding and deposition of wild type PrP. Expression of wild type PrP in flies induces progressive locomotor dysfunction, spongiform degeneration and changes in the biochemical and structural properties of PrP. We also found that overexpression of human Hsp70 prevents PrP misfolding and neurotoxicity, suggesting that Hsp70 could be a therapeutic target in prionopathies. To demonstrate this idea, we fed flies with Hsp90 inhibitors known to result in Hsp70 transcriptional activation. However, none of these treatments resulted in significant effects on PrP accumulation. Then, we tried combinatorial treatments with compounds that activate Hsp70 by different mechanisms. In these experiments the levels of PrP were reduced by 50 percent, suggesting that elevated Hsp70 contributed to PrP degradation. In fact, we confirmed that the levels of Hsp70 were significantly elevated by Western blot and quantitative PCR. We are currently analyzing the functional effects of the combinatorial treatment on PrP-induced neurotoxicity in flies. The results of these experiments will be discussed in the poster. We propose here that a combinatorial drug treatment can induce sustained high levels of Hsp70 and result in reduced levels of misfolded PrP. These findings can have important therapeutic consequences for these devastating disorders.

436A

Axonal Transport in Drosophila models of Parkinson's Disease. Eric Anderson, Delnessaw Hirpa, Shermali Gunawardena. Department of Biological Science, The State University of New York at Buffalo, BUFFALO, NY.14260.

Parkinson's disease (PD) is a common neurodegenerative disease that is characterized by loss of dopaminergic (DA) neurons in the substantia nigra par compacta and lewy body formation. Thus far more than eight genes with different functions have been implicated in PD. However, little is known about how these genes contribute to the same PD pathology. Using Drosophila models of PD, we tested the hypothesis that all genes involved in PD have a common role in axonal transport. We found that expression of human α-synuclein (α-synWT) induces axonal defects in larval segmental nerves. Moreover, Familial Parkinson's disease (FPD) mutations in α-syn (Ala30Pro and Ala53Thr) showed enhanced blockages compared to α-synWT. Strikingly, WT and FPD mutant α-syn genetically interacts with kinesin-1. Preliminary biochemical analysis suggests that α-syn is membrane associated. Further, expression of PINK (dpink) and DJ1a does not induce axonal defects, but expression of these proteins in the context of reduction of kinesin-1 caused significant axonal defects. In contrast to α-syn, expression of DJ1a in the context of reduction of dynein caused axonal defects. Together, our results show that although PD proteins have different functions, they also have a common role in axonal transport. Thus, our observations propose that disruption of the axonal transport pathway could contribute to early neuropathology observed in Parkinson's disease.

437B

SERF1 gene function in Drosophila melanogaster. Swagata Ghosh, Josh Titlow, Robin Cooper, Douglas Harrison, Brian Rymond. Biology, University of Kentucky, Lexington, KY.

Abstract: SERF1 is a genetic modifier of the autosomal recessive form of Spinal Muscular Atrophy (SMA), the leading genetic cause of human infant mortality. SERF is well-conserved across species ranging from baker's yeast to human but its natural biological function is not known in any organism. Recently it was shown in C. elegans that the loss of SERF/MOAG activity, while phenotypically benign, suppresses amyloid protein toxicity, consistent with SERF contribution to protein homeostasis (Van Ham et.al, 2010). Here we use a reverse genetic approach to investigate Drosophila SERF1 (dSERF1) activity. We have created a series of dSERF1 defective backgrounds by combining existing deficiency stocks, imprecise P-element excisions and RNAi co-expression. The preliminary results show that dSERF1 null mutants are viable and fertile but display locomotor defects that vary with genotype. Gene complementation and misexpression studies combined with anatomical and neurophysiological analyses are in progress to refine our understanding of dSERF1 function and investigate the impact of altered dSERF activity on a D. melanogaster model of SMA.

438C

Exposure to fungal volatile organic compound, 1-octen-3-ol leads to induction of NOS-mediated inflammatory response in larval respiratory system. Arati A. Inamdar, Joan Bennett. Department of Plant Biology and Pathology, Rutgers, The State University of New Jersey, New Brunswick, NJ.

Indoor air quality significantly influences human health. Today, average person spends about 90% of his time indoors. The growth of fungi and fungal contaminants pose serious health risks especially in children and older populations. The epidemiological studies support an association between molds and respiratory tract symptoms in sensitized asthmatic persons and hypersensitivity to pneumonitis in susceptible persons. Moreover, there is suggestive evidence of association between molds and respiratory illness in otherwise healthy children. We pioneered *Drosophila* model to study the toxic effects of fungal volatile organic compounds (VOCs), one of the fungal secondary metabolites. Fungal VOCs are emitted as a mixture of several different organic chemicals. Our inexpensive model seeks to gather data that could establish a causal relationship between fungal VOCs and the respiratory symptoms reported by occupants of damp and moldy buildings. This approach circumvents the shortcomings of questionnaire-based and correlation studies . We incorporate *Drosophila* larval tracheal system to determine the modulatory effects of fungal VOCs. Upon exposure to 1-octen-3-ol, the most common fungal VOCs, larval tracheal linings demonstrated morphological changes. We further determined the induction of inflammatory response mediated by nitric oxide synthase (NOS) by performing immunostaining and diaphorase staining for activated NOS in tracheal linings. In conclusion, we validate our model to further dissect the mechanistic studies to explore the causal relationship between te reported respiratory illness and exposure to fungal VOCs.

439A

Oxidative stress contributes to outcome severity in a *Drosophila melanogaster* model of classic galactosemia. Patricia P Jumbo-Lucioni[1], Marquise Hopson[1], Bill Liang[2], Dean Jones[2], Judith Fridovich-Keil[1]. 1) Dept. Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Pulmonary Division, Dept. Medicine, Emory University School of Medicine, Atlanta, GA.

Classic galactosemia is a potentially lethal disorder that results from profound loss of galactose-1P-uridylyltransferase (GALT), the second enzyme in the Leloir pathway of galactose metabolism. Despite decades of research, the pathogenic mechanism in galactosemia remains unclear. Previous publications suggest an association between galactose exposure and free radical generation in genetically normal animal models. Our goal was to explore the impact of dietary exposure to pro- or anti-oxidants on acute galactose-sensitivity using a GALT-deficient *Drosophila melanogaster* model of classic galactosemia. We tested supplements based on their established or predicted roles as oxidants (paraquat and DMSO), anti-oxidants (α-mangostin and vitamin C), or mitochondrial inhibitors (rotenone), and also tested additives that might bolster metabolism under conditions of mitochondrial dysfunction (uridine and glutamine). To test the impact of each supplement, GALT-deficient and control flies were raised in parallel on foods with and without each supplement that contained glucose as the sole sugar, or glucose plus galactose. Animals were scored for survival to pupation and adulthood. Our results showed that oxidants had a deleterious impact on survival rates of GALT-deficient animals exposed to galactose, while antioxidants improved the survival of these animals exposed to galactose. We saw little if any impact on GALT-deficient animals consuming glucose as the sole sugar, or wild-type animals regardless of sugar exposure. As a biochemical measure of oxidative stress in these animals we measured oxidized (GSSH) and reduced (GSH) glutathione levels and found a striking synergy between oxidant and galactose exposures. Combined, these results implicate oxidative stress in the mechanism of acute galactose toxicity in GALT-deficiency, and raise the intriguing possibility that appropriate dietary supplementation might benefit patients with classic galactosemia.

440B

In Vivo Exposure Impacts of Nano Silver on "Drosophila melanogaster". Denise K Reaves[1], John J Bang[2], Catherine S Silver Key[1]. 1) Biology, North Carolina Central University, Durham, NC; 2) Departments of Environmental, Earth, and Geospatial Science, North Carolina Central, University, Durham, NC 27707, USA.

Department of Biology, North Carolina Central University, Durham, NC 27707, USA; Departments of Environmental, Earth, and Geospatial Science, North Carolina Central, University, Durham, NC 27707, USA. Silver is an antimicrobial agent used in many consumer and medical products and causes a pigmentation defect in humans when ingested in large quantities. Increasingly, silver nanoparticles (AgNP) are permeating the environment as biomedical and commercial use in on the rise. Our lab has found that adult flies that emerged from silver nanoparticle (AgNP) exposed larvae have reduced locomotion in a climbing behavior assay and in comparison to control-fed animals, exhibited decreased pigmentation or completely lacked pigmentation. To investigate the possibility that either the melanin biosynthetic pathway, responsible for dopamine production, or a generalized stress response is involved we have conducted qPCR assays to assess the transcription levels of genes in these pathways. RNA from newly emerged exposed and non-exposed flies was used in qPCR to assess expression of the yellow-f, yellow-f2, hsp70, and ple genes. Gene expression patterns observed show variances between the AgNP- exposed cDNA to wild type with little to no expression in hsp70 and increased levels of expression in ple. Thus, we conclude that consumption of AgNP during larval development may affect transcription levels of genes involved in the melanin biosynthetic pathway.

441C

Muscle defects associated with human A-type lamin revealed by studies in Drosophila. Om K. Shrestha[1], George Dialyans[1], Monika Zwerger[2], Dylan Thiemann[1], Diane E. Cryderman[1], Jan Lammerding[2], Liping Yu[1], Lori L. Wallrath[1]. 1) Department of Biochemistry, University of Iowa, Iowa city, IA; 2) Cell and Molecular Biology and Department of Biomedical Engineering, Cornell University, Ithaca, NY.

Mutations in the human *LMNA* gene encoding A-type lamins cause a collection of diseases termed laminopathies, including several types of muscular dystrophy. Lamins are intermediate filaments, which line the inner membrane of nuclear envelope. They are responsible for maintaining the nuclear shape and regulating gene expression through interactions with chromatin. Heterozygous mutations *LMNA*, which result in single amino acid substitutions within the C-terminal Ig-fold domain, were identified in patients with muscular dystrophy. These substitutions were modeled in Drosophila and found to cause muscle defects. We have taken a multi-disciplinary approach to understanding the molecular basis of these muscle defects. Using biophysical techniques such as Nuclear Magentic Resonance (NMR) and Circular Dischroism (CD), we determined that the amino acid substitutions caused perturbations of the tertiary, but not secondary, structure of the Ig-fold. Surprisingly, these structural changes did not result in 'weakening' of the nuclear envelope within myonuclei. However, microarray analysis showed that expression of the mutant lamins in larval muscle caused changes in the expression of genes related to oxidative stress, neuromuscular junction function, and muscle development. Collectively, these data have revealed the mechanisms by which disease-causing lamin mutations alter muscle physiology.

442A

***dtorsin*, the *Drosophila* ortholog of the early-onset dystonia *TOR1A* (*DYT1*), plays a novel role in dopamine metabolism.** Noriko Wakabayashi-Ito[1,4], Olugbenga Doherty[2], Hideaki Moriyama[3], James Gusella[4], Xandra Breakefield[1], Janis O'Donnell[2], Naoto Ito[1,4]. 1) Dept Neurology, Massachusetts General Hosp, Charlestown, MA; 2) Dept Biological Science, University of Alabama, Tuscaloosa, AL; 3) School of Biological Science, University of Nebraska-Lincoln, Lincoln, NE; 4) CHGR, Massachusetts General Hosp, Boston, MA.

Dystonia represents the third most common movement disorder in humans. At least 20 genetic loci (*DYT1-20*) have been identified. *TOR1A* (*DYT1*), the gene responsible for the most common primary hereditary dystonia, encodes torsinA, an AAA ATPase family protein. However, the function of torsinA has yet to be fully understood. We have created a complete loss-of-function mutant for *dtorsin* (*torp4a*), the only *Drosophila* ortholog of *TOR1A*, by homologous recombination. dtorsin null mutant flies are semi-lethal at pre-pupal stage. The few surviving adults are sterile and slow moving with reduced cuticle pigmentation. Third instar larvae of the *dtorsin*-null strain exhibited locomotion defects that were rescued by feeding dopamine. Moreover, biochemical analysis revealed that the brains of third instar larvae and adults heterozygous for the loss-of-function *dtorsin* mutation had significantly reduced dopamine levels. The *dtorsin* mutant showed a very strong genetic interaction with *Pu* (*Punch*: GTP cyclohydrolase), the ortholog of the human gene underlying dopa-responsive (*DYT5*) dystonia. Biochemical analyses revealed a severe reduction of GTP cyclohydrolase protein and activity in *dtorsin*

mutants, suggesting that *dtorsin* plays a novel role in dopamine metabolism as a positive-regulator of GTP cyclohydrolase protein. The locomotion defect was rescued by cDNA for both *dtorsin* and human torsinA. Moreover, co-expression of wild type together with a human disease form of htorsinA (ΔE302) cDNA showed locomotion defect. Thus this *dtorsin* mutant line will be valuable for elucidating the function of torsin protein and the human disease mechanism.

443B

Rejuvenation of meiotic cohesion: a conserved mechanism to combat age related chromosome segregation errors? Katherine A. Weng, Charlotte A. Jeffreys, Sharon E. Bickel. Biological Sciences, Dartmouth College, Hanover, NH.

Chromosome segregation errors during female meiosis I are the leading cause of birth defects and miscarriages, and as women age, the risk of aneuploid pregnancy increases exponentially. 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 aneuploidy. 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 germ-line. Using the synaptonemal complex (SC) protein C(3)G as a cytological marker to monitor cohesion, we find that reduction of Deco after meiotic S phase causes premature disassembly of the SC. In addition, Deco knockdown during mid-prophase results in increased levels of meiotic nondisjunction (NDJ). A genetic assay that allows us to determine the recombinational history of missegregating chromosomes indicates that although chiasmata are formed, they are not maintained when Deco is knocked down after meiotic S phase. Moreover, SC defects and increased NDJ also occur when cohesin subunits are knocked down after meiotic cohesion is established during S phase. These data argue that turnover of chromatin-associated cohesin and rejuvenation of cohesive linkages are required during meiotic prophase to stabilize chiasmata and ensure proper segregation of meiotic chromosomes. We propose that these activities represent a critical mechanism that allows metazoan oocytes to counteract the deterioration of cohesion caused by aging.

444C

A new *Drosophila* model of Spinal Muscular Atrophy highlights the importance of non-snRNP related functions of Survival Motor Neuron in disease pathology. Kavita Praveen[1], Ying Wen[2], T.K. Rajendra[2], A.Gregory Matera[1,2]. 1) Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC; 2) Department of Biology, University of North Carolina, Chapel Hill, NC.

Spinal Muscular Atrophy (SMA) is a common neuromuscular disease that strikes one in 6,000-8,000 young children; most of whom die before the age of two years. Greater than 95% of patients with SMA carry deletions or point mutations in the *survival motor neuron 1 (SMN1)* gene. The SMN protein is essential for survival and has a well-characterized role in the biogenesis of small nuclear ribonucleoproteins (snRNPs), which are core components of the spliceosome. Numerous additional functions 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 developed a *Drosophila* model system to study the consequences of SMN loss of function mutations. In order to uncouple the housekeeping and tissue-specific functions of SMN, we have generated a series of transgenic flies that express human SMA patient-derived point mutations that are conserved in the fly. The transgenes are expressed using the endogenous *Smn* promoter and are integrated at the same genomic locus. Null mutants in *Smn* die as larvae, show significant motility defects, and have reduced levels of minor-class snRNAs, U4atac and U12. Surprisingly, we find that transgenic expression of relatively low amounts of wild-type SMN fail to restore snRNA levels but can rescue both the locomotor defects and lethality of the Smn null flies, producing fertile adults. Similarly, expression of an SMA point mutant construct, Smn^{T205I}, rescues the larval motility defects, but the majority of these animals die as pupae with an snRNA profile identical to that of the wild-type transgenics. These data demonstrate that the reduction in snRNA levels observed in *Smn* mutants is not a major contributor to lethality, and indicate that non-snRNP related functions of SMN play important roles in SMA pathology.

445A

Comparative studies of chromosomes of the tripunctata Species Group of Drosophila. Mitsue Taukeuti Brianti, Galina Ananina, Louis Bernad Klaczko. Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

In this work, we present detailed photomaps of the polytene chromosomes of five closely related species of the tripunctata group: Drosophila mediopunctata, D. roehrae, D. unipunctata, D. paraguayensis and D. mediosignata. The tripunctata group (Drosophila subgenus) comprises 79 species, being one of the largest Neotropical groups of species, second only to the repleta group. The tripunctata group with the groups: calloptera, cardini, guaramunu, guarani, macroptera, pallidipenis, rubifrons and sticta, form the tripunctata radiation. This work is pioneering in detailed study of chromosomes of this radiation species. We also analyzed the configurations of metaphase karyotypes of the five species. Moreover, using probes of genes from Drosophila melanogaster, we identified Muller's elements by fluorescence in situ hybridization (FISH), obtaining reliable evidence of homology for the chromosomes. Furthermore, we characterized the inversion polymorphisms found in the strains of this species. When we analyzed chromosomal settings, noticed that D. unipunctata karyotype presents a unique conformation showing an extra mitotic chromosome, which does not polytenize; and a pericentric inversion in the X chromosome, suggesting a very fast chromosomal evolution. We also found a pattern for the distribution of chromosomal inversion polymorphisms among Muller's elements in these species. Element E is the most polymorphic, with many inversions in each species. Element C is the second most polymorphic; B and D are the least polymorphic elements among the studied chromosomes. When we analyzed the distribution of chromosomal inversion polymorphisms noticed a tendency of linkage disequilibrium between arrangements of chromosomal inversions located in the regions distal and proximal of the E in all species analyzed. These results may be more general and are consistent with bibliographic data available for at least the subgenus.

446B

Deciphering B chromosome sequence of *Drosophila albomicans* by short-read sequencing. Li Zhao[1,2], Yue Zhang[2], Qi Zhou[2], Ruoping Zhao[2], Wen Wang[2]. 1) Department of Ecology and Evolution, University of California - Davis, Davis, CA; 2) CAS-Max Planck Junior Research Group, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China.

B chromosomes are extra chromosomes in autosomal karyotype with considerable instability in their existence, number and structure, it doesn't obey the Mendelian inheritance laws, thus is of great significance for genetics. However, little is known about the DNA sequences of B chromosomes and their transmission, origination and evolution. Here we use *Drosophila albomicans* as material to study this question. *D. albomicans* is the only species with B chromosomes in *D. immigrans* group. Using the next generation Illumina sequencing technology, we sequenced the genome of the male flies of strains with B chromosomes and the inbred female flies without B chromosomes but derived from the same strain. After filtering and mapping of the raw reads, we compared assembled male genome sequence with the female sequence and successfully identified a B-link SNP site in a candidate B chromosomal scaffold in which more than 20% B specific sequences were repetitive. Moreover, we collected about 1500 B chromosomes using microdissection technology, and amplified these chromosomes' DNA with linear amplification technique and sequenced the DNA by Illumina. A total 5,161,496 bp of scaffold was obtained and was aligned with the assembled whole genome sequence data of *D. albomicans* to screen B chromosome specific DNA. We compared the B chromosome assembling result with genome assembling sequences, and found that 0.55 MB chromosome sequences could blast to 1.6 M genome sequences. Finally, part of B chromosome sequence, mostly rDNA, were assembled again using B chromosome sequencing reads which are overlap with the genome sequencing reads, and supposed to be the B chromosome specific DNA. These B chromosome-specific sequences are all turned out to be rDNA localized in the X heterochromatin region or unmapped heterochromatin region, indicating that B chromosomes sequences are homologous to those of A chromosomes and B chromosome may partially originate from ancestor X chromosome.

447C

Characterization of the RNase T2 gene from *Drosophila melanogaster* and the evolution of this RNase family in protosomes. Linda Ambrosio, Ryan Bailey, Stephanie Moriss, Gustavo MacIntosh. Department of Biochemistry, Biophysics and Molecular Biology, Iowa State Univ, Ames, IA.

Ribonucleases of the T2 family are RNA degrading enzymes present in almost all eukaryotic organisms. The T2 RNases have been studied extensively in plants, but their role in animals is relatively unknown. Several of the biological functions fulfilled by RNase T2 proteins in plants seem to be carried out by members of a different RNase family, RNase A, in vertebrates. In zebrafish two RNase T2 paralogs have been identified with *rnaset2* implicated in lysosomal degradation of rRNA. In order to further understand the role of T2 RNases in animals, phylogenetic analyses and studies of gene expression were initiated in *D. melanogaster*. Only one gene of the T2 family, *RNaseX25* was identified in the Drosophila genome. We found that this gene was expressed in all life cycle stages examined, supporting the hypothesis that the T2 RNase enzyme may have a housekeeping function in flies. However, preliminary gene expression data suggest that *RNaseX25* may also be involved in different stress responses. Additionally, we used phylogenetic analysis to shed light on the evolution of the T2 family of ribonucleases in protostomes. Only one gene, absolutely conserved, is found for most of these animal genomes. Together with its ubiquitous expression, this conservation is consistent with a role for RNase T2 enzymes in basic cellular function. A special case of gene duplication and the possible evolution of new functions in the parasitic wasp *Nasonia vitripennis* will also be discussed.

448A

Comparative genetic architectures of similar and independently evolved morphological novelties. Laurent Arnoult, Caroline Minervino, Benjamin Prud'homme, Nicolas Gompel. IBDML, UMR CNRS 6216, Case 907, Parc Scientifique de Luminy, 13288 Marseille Cedex 9, France.

A striking and recurrent pattern in evolution is the repeated generation of near identical traits in independent lineages, such as flight in birds and insects or echolocation in bats and dolphins. Strong evolutionary forces may explain why, in face of similar environmental challenges, similar phenotypic solutions are being selected again and again. Here we ask: when evolution repeats itself, is it through similar genetic paths? Do developmental mechanisms constrain these genetic paths by defining a limited set of mutations that can make a new trait? We set to study five independent gains of a pigmentation spot located on the wings of different Drosophila species: we aim at drawing a fine comparison of the genetic mechanisms underlying these independent gains. Focusing on one of this species, D. biarmipes, we identified the homeobox transcription factor Distal-less as the major switch controlling the development of the wing pigmentation spot in this species, through transcriptional regulation of multiple pigmentation genes. We are now using RNA-seq to list new candidates involved in the making of the spot, and a small hairpin RNA interference technique to test their function in vivo. Confronting these results were are reconstructing the genetic architecture of the wing pigmentation spot of D. biarmipes; we are now testing whether the same genetic actors are playing the

same roles in the different Drosophila species; in particular we are testing whether Distal-less has been repeatedly co-opted to orchestrate the expression of the same effectors in a spot like pattern.

449B

Forces shaping a Hox morphogenetic gene-network during evolution. James Castelli-Gair Hombria, Sol Sotillos, Mario Aguilar, Filippo Foglia. CABD, CSIC/JA/UPO, Seville, Sevilla, Spain.

The Abdominal-B selector protein induces the organogenesis of the posterior spiracles by coordinating an organ specific gene-network. The complexity of this network begs the question of how it originated during posterior spiracle evolution and what were the selective pressures driving its formation. As the network probably formed piecemeal with elements being recruited sequentially during evolution, we studied the morphogenetic consecuences of expressing in naive epithelial cells individual effector targets of the posterior spiracle network. In most cases, single target expression has little morphogenetic effect. In contrast, expression of the Cv-c RhoGAP protein increases actin based motility but also leads to epithelial polarity and adhesion defects. We show that these defects do not occur in spiracle cells normally expressing Cv-c because they have developed compensatory mechanisms. These mechanisms include the organ specific upregulation of cell polarity and adhesion molecules, which help compensating the deleterious effects caused by the transient Cv-c induced Rho1 inactivation. We show that other epithelial cells like the salivary gland, the leading edge and the trachea which also have coopted Cv-c to their morphogenetic gene-networks are also resistant to Cv-c's deleterious effects on adhesion and epithelial polarity. We suggest that during evolution, Cv-c recruitment to any epithelium would have caused similar defects that resulted in a strong selective pressure that necessarily lead to recruit in those same cells downstream targets involved in the control of basic cell properties (adhesion and polarity regulators) to regain homeostasis. We propose based on our data, that when a selector gene cascade coopts a morphogenetic regulator, the instability caused requires the recruitment of various compensatory molecules that normalize it.

450C

***vestigial* ectodermal function is not limited to wing development in *Tribolium*.** Courtney M. Clark, Yoshinori Tomoyasu. Zoology Department, Miami University, Oxford, OH.

The *vestigial* gene (*vg*) is often referred to as a wing "master gene" because of its ability to induce wings in various locations in *Drosophila* when it is overexpressed. The ectodermal function of *vg* in *Drosophila* seems to be limited to wing formation. However, it is yet to be determined to what extent the function of *vg* is conserved among other insect species. We disrupted the *vg* function via RNA interference (RNAi) in the red flour beetle, *Tribolium casteneum*, and analyzed the phenotypes. Depletion of *vg* in the late larval stages led to a partial or entire deletion of the hindwings and elytra. Interestingly, we also found that *vg* RNAi induced novel body wall phenotypes in the first and third thoracic segments. We analyzed the *vg* RNAi phenotypes in *Drosophila*, and confirmed that the function of *vg* in flies is limited to wing development. Expression analysis has revealed that *vg* is expressed not only in dorsal appendage primordia, but also is expressed broadly throughout the thoracic and abdominal segments of the developing embryo of both *Drosophila* and *Tribolium*. Recently, it has been reported that *vg* is expressed in the body wall of bristletails, which do not possess wings (Niwa et al, 2010). These results suggest that unlike in *Drosophila*, *vg* function in the ectoderm is not limited to wing development in *Tribolium* and that *vg* has an important role in insect body wall development that has been lost in *Drosophila*.

Current evo-devo research using *Drosophila* studies as a paradigm has been quite successful for understanding the changes that have contributed to morphological evolution. However, this approach has a caveat of creating a fly-biased view of evolution. Our study shows the importance of analyzing gene function in organisms other than *Drosophila* to gain a more comprehensive view of morphological evolution.

451A

Modeling allometry using lessons from *Drosophila*. Austin P. Dreyer, Eli M. Swanson, Alexander W. Shingleton. Dept Zoology, Michigan State Univ, East Lansing, MI.

How natural selection alters scaling relationships among traits, called allometries, is a poorly understood question in evolution. Elucidating the evolution of allometries, however, is fundamental to understanding how morphological variation is generated and maintained. Though many scaling relationships are undoubtedly the result of selection, questions remain about which types of selection generate which allometric relationships. Specifically, it is unclear which selection regimes would tend to increase or decrease the extent to which one trait scales with another among members of a population. Such questions are exceedingly difficult to test with any degree of power in living systems. To circumvent this problem we used mathematical models to simulate the effect of different forms of selection on scaling relationships. The model is based on the developmental and genetic mechanisms that regulate body and organ size in *Drosophila*. Using this method we are able to test for the theoretical conditions that give rise to isometry, where organs maintain proportionality as they increase in size, hyperallometry, where one organ becomes proportionally larger in relation to another as they both increase in size, and hypoallometry, where one organ becomes proportionally smaller in relation to another as they both increase in size. We demonstrate that selection on the two traits concurrently results in more rapid responses in the slope of the scaling relationship than selection on any one of the traits alone. The results of the model allow us to make predictions about the nature of selection on scaling relationships and the nature of the mechanisms targeted by selection.

452B

Expression and Function of *fushi tarazu* in Diptera. Amanda Field, Leslie Pick. University of Maryland, 4112 Plant Sciences Building, College Park, MD.

Highly conserved regulatory genes direct body patterning of diverse animals. How these regulatory networks have evolved to direct the development of different body plans is a fundamental question in the evo-devo field. Two homeodomain genes, ftz and Antp, evolved from a common ancestral Hox gene. While Antp remained well conserved throughout arthropods, ftz rapidly evolved from a Hox gene in more basal arthropods to a pair-rule segmentation gene in Drosophila melanogaster. Changes in ftz function resulted from variations in its expression pattern and in its protein coding sequence, the latter leading to the acquisition of a new protein partner for Dm-Ftz, the orphan nuclear receptor Ftz-F1. Critical changes in ftz appear to have occurred at the base of insect radiation, in the holometabolous stem group and in Diptera (Heffer et al. 2010). Drosophila melanogaster and the dengue and yellow-fever vector mosquito, Aedes Aegypti, represent distant dipteran lineages, providing an opportunity to assess whether and to what extent the functions of ftz and Antp have

diverged. We are comparing the sequence, expression and activity of these genes, making use of tools being developed in Aedes for functional studies. To compare expression patterns, in situ hybridizations are being performed with Aa-ftz, Aa-Antp, and Aa-ftz-f1 in early mosquito embryos. To assess the function of these genes in mosquitoes, both loss-of function and gain-of-function approaches are being used. The former is being carried out by RNAi while the latter is being done by generating transgenic mosquitoes. These transgenic mosquitoes will be used to mis-express Aa-ftz and Aa-Anp to determine whether this results in pair-rule or homeotic transformation phenotypes, similar to those seen in Drosophila melanogaster. Together, these results will provide insight into the molecular mechanisms underlying evolution of regulatory genes in an insect lineage.

453C

Genome-wide comparison among *melanogaster* sibling species reveals novel genes involved in myogenesis diversification. Ryan M Haskins, Yunyi Yang, Youngmin Chu, Juan S Chahda, Joseph Schinaman, Mirela Belu, Lyndsie Haefke, Rui Sousa-Neves, Claudia M Mizutani. Biology, Case Western Reserve University, Cleveland, OH.

The *Drosophila* genus comprises a number of species with remarkable adaptations to unique environments. *D. melanogaster*, *D. simulans* and *D. sechellia* are three sibling species of particular interest since they exhibit significant developmental and behavioral differences despite their recent divergence. To begin probing these differences and gain a better understanding of the mechanisms that operate in species differentiation, we reconstructed the genomes of *D. sechellia* and *D. simulans* and made pair-wise BLAST analysis of coding sequences against the *D. melanogaster* genome (Sousa-Neves, R. and Rosas, A., 2010). By selecting the group of genes that are most similar between *D. simulans* and *D. sechellia*, but most divergent in *D. melanogaster* (i.e. ancestral alleles of *D. simulans* and *D. sechellia*), we expected to identify genes that distinguish these two newer species from *D. melanogaster*. In this work, we screened for candidate ancestral alleles to be involved in a novel phenotypic variation of myoblast fusion that distinguishes *D. melanogaster* from its other sibling species. We selected genes expressed in a narrow window of embryonic development when myogenesis takes place. Our screening led to the identification of two novel genes: *snail-minded* (*sami*) and *pick-up sticks*. *sami* is expressed in the presumptive mesoderm and mesodectoderm, in a combined pattern of both *single-minded* and *snail*, an essential gene for muscle development. *pick-up sticks* encodes a predicted EGF-like secreted protein that initiates expression during somatic myoblast fusion and remains exclusively expressed in the somatic body muscle fibers. Functional analyses of the two genes are currently under way. The screening method presented here could theoretically be applied to any biological processes relevant to the diversification of this group of species, and may provide a valuable tool in addition to classical mutagenesis screenings to identifying novel developmental genes.

454A

A Homeodomain-dependent Function in a Rapidly Evolving *Hox* Gene in Insects. Alison Heffer, Leslie Pick. Dept Entomology, Univ Maryland, College Park, MD.

Hox genes are considered to be evolutionarily constrained because of their importance in determining segment identity in metazoans, and because mutations or mis-expression result in homeotic transformations. *fushi tarazu (ftz)* is a rapidly evolving *Hox* gene in insects that has switched in both expression and function from *Hox*-like in basal arthropods to pair-rule segmentation in *Drosophila*. In addition to its early role in segmentation, *Dm-ftz* is also required later in embryonic development in the central nervous system (CNS), specifically in RP2 neuron formation. We previously isolated Ftz from several insects spanning 450 million years of evolution, and found great diversity in expression and protein sequence. Despite this, *ftz* expression in the developing CNS of arthropods has been retained. Here we examine which motif or domain in the Ftz coding region is important for CNS function. To do this, Ftz transgenes containing mutations in the segmentation LXXLL motif, degenerated homeotic YPWM motif, and DNA-binding homeodomain were made, and all were placed under the control of the *ftz* neurogenic cis-regulatory element. The LXXLL and FNWS in *Dm*-Ftz were mutated to abolish all function, and the Ftz homeodomain was deleted, changed to the sequence of the N-terminal arm of *Dm*-Antp, or to the entire *Dm*-Antp homeodomain. Using a mutant *Drosophila* line that lacks the *ftz* neurogenic expression, we tested the ability of each of Ftz transgenes to rescue the RP2- phenotype, marked by loss of Eve expression. Here we report that neither the LXXLL or FNWS motifs are required for Ftz CNS function. Rather it is the homeodomain, and not specifically the Ftz homeodomain, that is required for function in the nervous system. Together, these results suggest that *ftz* has been maintained in the genomes of all insects examined to date because of its role in CNS development, which is homeodomain-dependent. This constraint later in development did not prevent Ftz from being co-opted into earlier developmental pathways as long as variations in expression and protein sequence did not impact CNS function.

455B

How different are mosquitoes and Drosophila?—Evolution of mosquito early zygotic genes. Wanqi Hu, James Biedler, Zhijian Tu. Biochemistry, Virginia Tech, Blacksburg, VA.

The beginning of embryogenesis is controlled by maternal transcripts and proteins. The zygotic genome starts to take over during the maternal-to-zygotic transition. Early zygotic genes are considered to play essential roles in embryogenesis. We have identified more than 100 pure early zygotic genes (without maternal expression) in the yellow fever mosquito *Aedes aegypti* by RNAseq and subsequent RT-PCR. This gene list has little overlap with the known early zygotic genes in Drosophila, indicating rapid evolutionary turnover. Interestingly, these novel early zygotic genes tend to have domains or characteristics consistent with functions related to early embryonic development. Phylogenetic analysis confirmed that many of the pure early zygotic genes are *A. aegypti* or mosquito specific. Gene duplication and subsequent acquisition of early zygotic expression profile may explain the origin of a number of the novel early zygotic genes.

456C

Functional genomic analysis of eye development in the red flour beetle *Tribolium castaneum*. Zahabiya Husain[1], Anura Shrivastava[1], Arun K Sasikala - Appukuttan[1], Bryce Daines[2], Rui Chen[2], Markus Friedrich[1]. 1) Wayne State University, Detroit, MI; 2) (2) Human Genome Sequencing Center, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.

The lineages leading to the red flour beetle *Tribolium castaneum* and the fruit fly *Drosophila melanogaster* separated at least 250 million years ago. Previous work in our lab suggests that this evolutionary separation resulted in major differences regarding the organization of the retinal determination gene network (RDGN). In *Drosophila*, mutations in the Pax6 transcription factor genes *eyeless (ey)* and *twin of eyeless (toy)* cause loss or reduction of the

compound eye. In *Tribolium*, by contrast, adult eye development is only mildy sensitive to RNAi mediated knockdown of *toy* and *ey* apparently due to redundant regulation which involves the transcription factor *dachshund (dac)*. We employed next generation sequencing to further elucidate the architecture of the *Tribolium* RDGN, comparing the pupal head transcriptomes of untreated, *dac* and *dac+ey+toy* knockdown animals. In addition, we investigated the transcriptome of eye- depleted pupae from animals injected with dsRNA targeting the *eyes absent (eya)* transcription factor genes. These experiments identified 2954 candidate eye developmental regulators, 557 of which appear to specifically depend on the synergistic input of *dac*,*ey* and *toy*. In addition, we identified 362 genes whose expression is enriched in the developing head of eye-depleted animals. We hypothesize that the latter define gene regulatory specification networks that are antagonistic to eye determination in the developing *Tribolium* head.

457A

New models to Diptera development: Clogmia albipunctata and Megaselia abdita. Eva Jimenez, Karl R. Wotton, Yogi Jaeger. Systems Biology Unit, Centre de Regulacio Genomica, PRBB, Barcelona, Spain.

We aim to study the evolution of gene networks by comparing the well characterised gene networks of the fruit fly Drosophila melanogaster with 2 further fly species: Megaselia abdita and Clogmia albipunctata. Clogmia belongs to a lineage of flies believed to have diverged early in the evolution of the dipterans, while the lineage leading to Megaselia branched intermediately; at the base of the cyclorraphans. To develop these species as models we are documenting some classic biology concerning their life cycles and early development. This includes the description of cleavage cycles and other morphological events using nuclear staining, fluorescence, confocal and time-lapse imaging. Furthermore, we are characterising the transcriptomes of pre-gastrulating embryos of both species. This morphological and molecular information will be used to aid the investigation into evolving gene networks such as those involved in segmentation, heart development and bristle patterning using traditional (in situ staining, RNAi) and computational approaches (reverse-engeneering).

458B

Common and distinct roles of juvenile hormone signaling genes in metamorphosis of holometabolous and hemimetabolous insects. Marek Jindra[1], Barbora Konopova[1], Vlastimil Smykal[2]. 1) Biology Center ASCR, Ceske Budejovice, Czech Republic; 2) University of South Bohemia, Ceske Budejovice, Czech Republic.

Insect larvae metamorphose to winged and reproductive adults either directly (hemimetaboly) or through an intermediary pupal stage (holometaboly). In either case juvenile hormone (JH) prevents metamorphosis until a larva has attained an appropriate phase of development. In holometabolous insects, JH acts through its putative receptor Methoprene-tolerant (Met) to regulate *Krüppel-homolog 1* (*Kr-h1*) and *Broad-Complex* (*BR-C*) genes. While Met and Kr-h1 prevent precocious metamorphosis in pre-final larval instars, BR-C specifies the pupal stage. How JH signaling operates in hemimetabolous insects is poorly understood. Here, we compare the function of *Met*, *Kr-h1* and *BR-C* genes in the two types of insects. Using systemic RNAi in the hemimetabolous true bug, *Pyrrhocoris apterus*, we show that Met conveys the JH signal to prevent premature metamorphosis by maintaining high expression of *Kr-h1*. Knockdown of either *Met* or *Kr-h1* (but not of *BR-C*) in penultimate-instar *Pyrrhocoris* larvae causes precocious development of adult color pattern, wings and genitalia. A natural fall of *Kr-h1* expression in the last larval instar normally permits adult development, and treatment with an exogenous JH mimic methoprene at this time requires both *Met* and *Kr-h1* to block the adult program and induce an extra larval instar. Met and Kr-h1 therefore serve as JH-dependent repressors of deleterious precocious metamorphic changes in both hemimetabolous and holometabolous juveniles, whereas BR-C has been recruited for a new role in specifying the holometabolous pupa. These results show that despite considerable evolutionary distance, insects with diverse developmental strategies employ a common-core JH signaling pathway to commit to adult morphogenesis.

459C

High Hemocyte Load Is Associated With Increased Resistance Against Parasitoids in Drosophila suzukii, A Relative of D. melanogaster. Balint Z. Kacsoh, Todd A. Schlenke. Department of Biology, Emory University, Atlanta, GA.

Among the most common parasites of Drosophila in nature are parasitoid wasps, which lay their eggs in fly larvae and pupae. D. melanogaster larvae can mount a cellular immune response against wasp eggs, but female wasps inject venom along with their eggs to block this immune response. Genetic variation in flies for immune resistance against wasps and genetic variation in wasps for virulence against flies largely determines the outcome of any fly-wasp interaction. Interestingly, up to 90% of the variation in fly resistance against wasp parasitism has been linked to a very simple mechanism: flies with increased constitutive blood cell (hemocyte) production are more resistant. However, this relationship has not been tested for Drosophila hosts outside of the melanogaster subgroup, nor has it been tested across a diversity of parasitoid wasp species and strains. We compared hemocyte levels in two fly species from different subgroups, D. melanogaster and D. suzukii, and found that D. suzukii constitutively produces up to five times more hemocytes than D. melanogaster. Using a panel of 24 parasitoid wasp strains representing fifteen species, four families, and multiple virulence strategies, we found that D. suzukii was significantly more resistant to wasp parasitism than D. melanogaster. Thus, our data suggest that the relationship between hemocyte production and wasp resistance is general. However, at least one sympatric wasp species was a highly successful infector of D. suzukii, suggesting specialists can overcome the general resistance afforded to hosts by excessive hemocyte production. Given that D. suzukii is an emerging agricultural pest, identification of the few parasitoid wasps that successfully infect D. suzukii may have value for biocontrol.

460A

The effects of temperature on developmental timing in species with different optimal growth temperatures. 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 species live in a variety of different climates and must adapt to their local environments. The genus includes tropical species that prefer relatively warm climates and temperate species that prefer relatively low temperatures. Species such as *Drosophila melanogaster* and *D. simulans* are able to adapt to a broad range of temperatures and have become cosmopolitan. Others, such as *D. sechellia* and *D. mojavensis* (subspecies *wrigleyi*), are restricted to small islands with a stable, uniform climate and may have lost the ability to radiate in the same way. The impact of temperature, which is known to affect the rate of development, on the embryonic development of species adapted to a single climate versus species adaptable to multiple climates is not known,

meaning cross-species comparisons at any temperature could be misleading. Numerous temperature-dependent embryonic events must be precisely tuned for proper development and viability, as the embryo is the most temperature-sensitive, but also most easily monitored, stage. With time-lapse imaging, we monitored 26 events spanning embryonic development in multiple species across their viable temperature ranges. We found embryonic development has a non-linear temperature dependence that changes between species, including the broader viable temperature ranges of cosmopolitan species compared to island species. We characterized the correlation between the native environment and the viable growth ranges, which informs how underlying adaptations may affect adaptation to new environments.

461B

Evolution of Mesoderm Invagination in the Insect Order Diptera. Steffen Lemke[1], Silvia Urbansky[1], Thomas Sandmann[2]. 1) Centre for Organismal Studies (COS), Universität Heidelberg, Heidelberg, Germany; 2) Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany.

In *Drosophila*, the onset of mesoderm internalization is marked by pulsed apical constrictions in a band of cells on the ventral side of the embryo. Following apical constriction, the nuclei of these initially columnar ventral cells drop basally, the cells assume a wedge-shaped morphology, and the ventral epithelium bends to form a shallow furrow along the ventral midline. This furrow then rapidly contracts into a deep invagination as cells within the furrow shorten by about 50% along their apical-basal axes. This internalization of mesodermal cells is mainly driven by the contraction of an apical and junctional actin network that coalesces after apical localization of non-muscle myosin II. Apical localization and activation of myosin II is promoted by apical RhoGEF2, which is independently controlled by *T48* and *folded gastrulation* (fog).

Mesoderm invagination without formation of a prominent ventral furrow has been reported for dipterans in the nematoceran suborder, including gnats and mosquitoes. To functionally test for putative genetic changes underlying different modes of mesoderm invagination within the insect order Diptera, we study these gastrulation movements in the nematoceran midge *Chironomus riparius*. We will present results from our ongoing study and the sequenced blastoderm transcriptome of *Chironomus riparius* as gene identification tool.

462C

Exploring the molecular basis of insect wing evolution: a comparison of beetle and fly wing development. David M. Linz, Yoshinori Tomoyasu. Zoology Department, Miami University, Oxford, OH.

We are studying the gene regulatory network of wing development in *Tribolium* (the red flour beetle) and comparing it to that of the fruit fly, *Drosophila*, to understand the molecular basis of morphological evolution. The wings of these two insects have become vastly different over evolutionary time. The fly has typical flight wings on the second thoracic segment (T2), but has intensively modified wings (halteres) on T3. In contrast, the beetle has a pair of hardened protective structures (elytra) on T2, and uses the T3 hindwings for flight. We have been analyzing the function of potential “wing genes” selected from previous *Drosophila* studies in *Tribolium* wing development (candidate gene approach). However, as these studies have progressed, the choices of candidate genes have become increasingly limited and also created a fly-biased view of insect wing evolution. To gain a more complete view of insect wing evolution, we have started exploring genes that could be unique to insects other than *Drosophila*. We first examined a class of developmental genes (toolkit genes) that are known to be important for embryonic segmentation in *Drosophila*. These toolkit genes tend to show a high degree of pleiotropy, therefore increasing the likelihood of finding novel wing genes in the beetle. Despite this, we found no definitive examples of wing specific genes. To obtain further insight into the molecular basis of insect wing evolution, we are adopting a *bona fide* non-candidate gene approach: RNA sequencing. By comparing the transcriptome from dorsal appendages in both the fly and beetle, changes in developmental mechanisms that have contributed to their morphological evolution can be fully explored and characterized.

463A

Evolution of Shape by Multiple Regulatory Changes to a Growth Gene. David Loehlin, John Werren. Biology, University of Rochester, Rochester, NY.

What genes and genetic changes are responsible for morphological differences between species? Here we identify a major gene that induces male-specific wing size and shape differences between *Nasonia* wasp species. Fine-scale mapping and in situ hybridization reveals that changes in at least three regions (two strictly non-coding) around the gene unpaired-like (upd-like) cause changes in spatial and temporal expression of upd-like in the developing wing and corresponding changes in wing width. Upd-like is a *Nasonia* homolog of *unpaired*, a well-studied signaling protein that regulates cell proliferation and differentiation. Our results indicate that multiple changes in the regulation of upd-like are involved in microevolution of morphological and sex-specific differences between species.

464B

Region specific patterning function of Pax6 in the developing embryonic head of Tribolium Castaneum. Qing Luan[1], Arun Sasikala-Appukuttan[1], Markus Friedrich[1,2]. 1) Biological science, Wayne State University, Detroit, MI; 2) Department of Anatomy and Cell Biology, Wayne State University, School of Medicine,Detroit, MI.

Because of this high degree of evolutionary conservation, major research investments have been made in studying the role of Pax6 in a variety of species. One consistent picture emerging from these studies is that Pax6 functions as high-level regulator. However, exactly how Pax6 executes its patterning role during visual system development and, by extension, how Pax6 acquired its role in eye development during evolution is still fundamentally debated. Previous work in our lab produced preliminary evidence that the Pax6 transcription factor paralogs eyeless (ey) and twin of eyeless (toy) are not only required for the normal development of the larval eyes but a larger region of the developing embryonic head. We have extended this analysis generating a high resolution map of ey+toy sensitive regions in the Tribolium larval head cuticle. These data demonstrate that Tribolium Pax6 is responsible for the normal development of a large region in the embryonic procephalon. We are now studying the mechanistic basis of these effects by examining pattern formation and marker gene expression in Tribolium ey+toy knockdown embryos.

465C

X-linkage and the evolution of sex-biased gene expression. Richard P. Meisel[1], John H. Malone[2], Andrew G. Clark[1]. 1) Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY; 2) Dept Biol Sci, Florida State Univ, Tallahassee, FL.

Because two out of every three X chromosomes in a population with 1:1 sex ratio are found in females, sex-specific selection pressures are expected to differentially affect X-linked and autosomal genes. Numerous studies have shown that genes with male- (female-) biased expression are under- (over-) represented on the *Drosophila* X chromosome, but the specific factors responsible are not known. Notably, genes with male-biased expression tend to be narrowly expressed in a limited number of tissues, and narrowly expressed genes are also under-represented on the *Drosophila* X chromosome. It is therefore unclear whether the non-random X-linkage of genes with sex-biased expression is the result of selection on genes with sex-biased expression, narrowly expressed genes, or some combination of the two. To address this problem, we measured sex-biased expression in multiple *Drosophila* species in head and whole fly and at different developmental time-points, and we obtained expression measurements from 14 adult tissues. Our results suggest that the paucity of X-linked genes with male-biased expression is driven primarily by a dearth of genes with accessory-gland-biased expression on the X chromosomes, and this dearth is a product of both male-specific selection pressures being weaker on X-linked genes and limits imposed by dosage compensation. In addition, there is also an excess of X-linked genes with female-biased expression, even after controlling for the confounding factor of expression breadth, suggesting that the female-biased transmission of the X chromosome favors the accumulation of female-beneficial substitutions in X-linked genes. These results demonstrate that the unique gene content of the X chromosome is a product of sex-specific selection pressures, and not an outcome of selection on narrowly expressed genes. Lastly, the expression levels of X-linked genes diverge faster between species than autosomal genes, suggesting that genes on the X chromosome are particularly evolutionarily labile, which may promote the unique gene content of this chromosome.

466A

Expression pattern evolution of three parent genes and their retrogene copies in *Drosophila* species. Ryan S. O'Neill, Denise V. Clark. Biology Dept, University of New Brunswick, Fredericton, NB, Canada.

Following gene duplication, gene copies may become specialized for different parts of the ancestral single copy function (subfunctionalization), or they may attain new functions (neofunctionalization). Retrogenes are gene copies that arise when processed mRNAs are reverse transcribed and inserted in the genome. While new genes generated through DNA-based gene duplication can retain the regulatory elements and introns of their parent gene, retrogenes lack these elements. A new retrogene is therefore unlikely to share its parent gene's expression pattern. In *Drosophila melanogaster*, retrogenes generally have narrower expression patterns than their parent genes, but, without knowing the ancestral pattern, we cannot determine whether the retrogene's expression is the result of subfunctionalization or neofunctionalization. In this study, the expression patterns of three parent genes and their retrogene copies are examined in several *Drosophila* species using *in situ* hybridization. In *D. melanogaster*, these parent genes and their retrogenes are *CG17734* and *CG11825*, *CG8331* and *CG4960*, and *Sep2* and *Sep5*. The genome sequences of species in the *Drosophilidae* lineage show that the three retrogenes arose within this lineage, while the parent genes have persisted in all *Drosophila* species. The parent gene *CG17734* is expressed during fewer embryonic stages in species with the retrogene compared to species without the retrogene, whereas the retrogene *CG11825* is expressed in those stages where *CG17734* expression is lacking, indicating that this gene pair may have evolved via subfunctionalization. For the other gene pairs, the orthologous parent genes have conserved expression patterns across species, regardless of whether or not a retrogene copy is present, suggesting that these parent genes have maintained their ancestral functions and have not undergone subfunctionalization following the arrival of the retrogene. However, retrogenes *CG4960* and *Sep5* have variable expression patterns across species, indicating prolonged functional diversification of these retrogenes.

467B

The molecular basis of speciation in Drosophila. Nitin Phadnis, Harmit Malik. Division of Basic Science, Fred Hutchinson Cancer Research Center, Seattle, WA.

Speciation, the process by which one species splits into two, involves the evolution of reproductive isolation between previously interbreeding populations. A central goal in evolutionary biology is to identify the genes and the evolutionary forces that cause reproductive isolation. The idea that genetic conflict involving segregation distorters may drive of the rapid evolution of genes underlying reproductive isolation is intuitively appealing, but empirical evidence is limited. Previously, we showed that a single gene *Overdrive* causes both male sterility and segregation distortion in *Drosophila pseudoobscura* Bogota-USA hybrids. Here, we perform a genome-wide genetic dissection to provide a comprehensive look at the genetic architecture of all components of the hybrid incompatibility underlying F1 hybrid sterility. Postzygotic isolation between Bogota and USA involves a single incompatibility consisting of only a handful large effect factors. The genetic bases of hybrid sterility and segregation distortion are largely but not completely overlapping. Identification and characterization of these genes, including *Overdrive*, are providing important insights into the molecular nature of reproductive barriers that isolate species.

468C

A possible contribution of *abrupt* in the evolution of beetle elytra. Padmapriyadarshini Ravisankar, Nagraj Sambrani, Yoshinori Tomoyasu. Zoology Department, Miami University, Oxford, OH.

Morphological innovation is a fundamental process in evolution, yet the molecular mechanism underlying the evolution of morphologically novel structures is still elusive. Coleoptera (beetles) is the most successful animal group on the planet, accounting for over 20 percent of extant animals. Innovation of elytra, which are highly sclerotized and modified forewings, is an important trait driving the successful radiation of beetles. We are using the red flour beetle, *Tribolium castaneum*, as a model system to understand the molecular basis of elytral evolution in beetles. *Tribolium* is rapidly gaining momentum as a genetic model system due to its availability of several modern genetic tools. Systemic RNAi technique is one of the important advantages in *Tribolium*, which has paved the way to create gene 'knock down' phenotypes by simple injection of double stranded RNA (dsRNA). Our initial RNA interference (RNAi) screening for genes important for the evolution of elytra has identified *abrupt* (*ab*) as a gene involved in the formation of unique elytral features. *ab* encodes an evolutionary conserved transcription factor that contains a BTB zinc finger domain. A mutation in *ab* in the fruit fly *Drosophila* results in the loss of a particular wing vein. Depleting *ab* function via RNAi in *Tribolium* also caused defects in some wing veins, suggesting that the function of *ab* in the wing vein formation is conserved among insects. In addition, we noticed that *ab* is essential for elytron and hindwing cell proliferation in the early dorsal appendage development in *Tribolium*. RNAi analysis in *Drosophila* has revealed that this novel *ab* function is indeed conserved even in *Drosophila*. Interestingly, *ab* RNAi also affected the formation of several features only seen in elytra. For example, the unique overall shape of elytra was altered by *ab* RNAi. This suggests that, in addition to several conserved functions, *ab* has gained a new function in the beetle lineage, which might have contributed to the elytral evolution.

469A

Ecdysone Signaling in Starvation Resistant *Drosophila*. Lauren A. Reynolds, Allen G. Gibbs. University of Nevada Las Vegas, Las Vegas, NV.

We selected for adult starvation resistance in replicated outbred populations of *Drosophila melanogaster*. These populations accumulate greater lipid stores as larvae that they can then use to survive adult starvation. Lipids are accumulated during the 3rd instar larval feeding period, which is ~24 hr longer in starvation-selected populations than controls; the rate of lipid accumulation during larval feeding is the same between starvation-selected populations and controls. To understand how the developmental delay is achieved we studied gene expression during the 3rd instar. Genes associated with the ecdysone pulse that results in puparium formation had delayed expression. We partially rescued delayed larval development by feeding 20-hydroxyecdysone (20E) to 3rd instar larvae, further suggesting a change in the timing of the ecdysone titer. We conclude that selection for adult starvation resistance has resulted in physiological changes in larvae that are mediated by ecdysone signaling.

470B

Keeping males and females the right size: A closer look at the mechanisms behind sexual size dimorphism. Nicholas D Testa, Shampa Ghosh-Modak, Alexander W Shingleton. Zoology, Michigan State University, East Lansing, MI.

Sexual size dimorphism (SSD) is an extraordinarily widespread and conspicuous phenomena in the animal kingdom, yet very little is known of the underlying developmental mechanisms that generate it. Such a proximate understanding of SSD is essential if we are to completely understand the ultimate causes of its evolution. Here, we investigate the underlying mechanisms of SSD in the fruit fly Drosophila melanogaster. One important mechanism for the regulation of SSD in Manduca sexta has been sex-specific differences in critical size, the point in development when starvation no longer delays development. Here we explore whether critical size also regulates SSD in Drosophila. Previously critical size has been difficult to determine in different sexes because of the low-survivorship of starved larvae and our inability to determine the sex of pupae that do not eclose. We circumvented this issue by identifiying the Y-chromosome in pupae using PCR. We show that critical size in Drosophila, as in M. sexta is significantly different between sexes. This suggests that change in critical size is an important, conserved mechanism in the evolution of SSD in holometabolous insects.

471C

Diverged developmental mechanisms underlying the conserved morphological structures in insect wings. Yoshinori Tomoyasu, Tingjia Lao, Matthew Korth. Dept Zoology, Miami Univ, Oxford, OH.

Two structures can be homologous when they have a common origin. Duplication and modification of homologous structures has been a driving force of animal evolution. Morphological similarity is an important characteristics used to identify homology (**morphological** or **classical homology**). However, morphological similarity can be deceiving because evolutionary modification can obscure the similarity of homologous structures. An alternative approach is to identify similarities in the developmental systems, which can be independent of morphological similarity (**developmental** or **deep homology**). Utilizing these two concepts is a powerful approach to understand the evolution of homologous structures, however, these two concepts often bring different, even controversial results. Insect wings are fascinating structures to study in regard to homology. All insect wings are considered to be monophyletic (*i.e.* homologous), and it is even possible to homologize each vein among different species. In fact, the vein pattern has been an essential trait for insect classification. However, the development basis of insect veins has never been explored in the evolutionary context. Studies in *Drosophila* have identified a battery of genes important for vein formation, some of which are important only for a particular vein (such as *sal, omb, Iro-C, kni, kn, cv* and *ab*). We depleted the function of these genes via RNAi in the red flour beetle *Tribolium*, and compared the phenotypes to those in *Drosophila*. RNAi for most of the genes in *Tribolium* resulted in vein abnormality, suggesting that the vein function of these genes has been descended from a common ancestor. Interestingly, however, the veins affected by the disruption of these genes varied between the two species. This is quite puzzling as these results suggest that a morphologically homologous structure could be produced by different developmental mechanisms, thus adding another complication to the relationship between two homology concepts. We will discuss a possible explanation for this apparent disagreement of the two concepts in insect wing veins.

472A

Dramatic Expansion and Expression Diversification of the Methuselah Gene Family During Recent *Drosophila* Evolution. Mark F. VanBerkum, Meghna Patel, Dana Hallal, Jeffery Jones, Denise Bronner, Rami Zein, Jason Caravas, Zahabiya Husain, Markus Friedrich. Dept Biological Sciences, Wayne State University, Detroit, MI.

In *Drosophila melanogastor*, the fifteen Methuselah/Methuselah-like (Mth/Mthl) genes are an insect specific family of GPCRs whose function is largely unknown. Using *in situ* hybridization techniques, we systematically evaluated the expression of each family member in the embryo and third instar CNS and imaginal discs. These receptors are expressed in diverse patterns from gastrulation to pupation, and while mesoderm expression (gastrulation and gut) predominates, expression in neuronal tissue (larval CNS) and the ectoderm (discs) is observed. Six genes (Mthl 1, 5, 9, 11, 13 and 14) are expressed only in the embryo, four (Mthl3, 4, 6 and 8) in larval tissue, and two (Mthl10 and Mth) in both embryos and larvae. To better understand the evolution of this expression pattern, we undertook a phylogenetic analysis of this family using receptor sequences from five *Drosophila* species as well as *Tribolium* and *Anopheles*. Mthl1, 5, and 14 are present in each species and form separate clades; a new Mthl gene (CG31720) also forms its own clade. All of the remaining *Drosophila* Mth/Mthl genes, along with a single gene in *Tribolium*, form a large clade of closely related sequences that are further defined by a conserved Mth ectodomain. The single *Tribolium* Mthl ortholog (Tc010567) provided a unique opportunity to compare the ancestral expression of this Mthl ortholog with the expression of *Drosophila* paralogs. The *Tribolium* Mthl gene is expressed in the hindgut and a bilateral cluster of mesoderm cells at the border between the procephalic and gnathal head region. The selective embryonic expression of Tc010567 is strikingly different from the wide spread expression of the Mthl superclade orthologs in *D. melanogastor*. Thus, our data suggest the acquisition of novel functionalities by gene family expansion during the evolution of the *Drosophila* lineage. Ongoing work is assessing the functional relevance of the expression patterns in *Drosophila*.

473B

Evolution of morphology and behavior in *Drosophila melanogaster* in response to predation. Michael DeNieu, Ian Dworkin. Zoology & Ecology, Evolution and Behavior, Michigan State University, East Lansing, MI.

Predation is a powerful selective force that has been shown to shape the evolution of many natural populations, yet its influence in the model system *Drosophila* is virtually unknown. A common thread among studies of antipredator adaptations in the wild is the striking parallelism i.e. in the phenotypic responses of independent populations. However, it is unknown whether these parallel phenotypic responses represent homologous genetic changes or whether common patterns of genetic and phenotypic correlations drive them. To address these questions, we initiated two replicate sets of populations of *Drosophila melanogaster* that have been subjected to predatory experimental evolution for over 70 generations. Each replicate consists of a predation population that undergoes episodic viability selection by 1st instar nymphs of the Chinese mantis (*Tenodera aridifolia sinensis*) and a control population that undergoes mock selection lacking the predator. We are undertaking an integrative approach measuring both behavior and morphology in tests of hypotheses regarding the nature of evolutionary trajectories in response to predation. Escape related morphology is one trait that has been shown to undergo repeated parallel evolution in many species, particularly fish, in which the morphology is directly related to escape performance. Consequently, we measured selection on wing size and shape of surviving and captured flies in order to assess how selection is driving changes in morphology. Though we found similar patterns of selection in the control populations as expected, patterns of selection in the predation populations differ dramatically resulting in divergence of wing size and shape. However, the behavioral responses of the predation populations in aggression, foraging and survival have been consistent suggesting that similar higher order phenotypes may be reached by multiple evolutionary paths.

474C

Alcohol consumption as self-medication against parasitic wasps In Drosophila melanogaster. Todd A. Schlenke, Neil F. Milan, Balint Z. Kacsoh. Biology Dept, Emory Univ, Atlanta, GA.

Organisms frequently utilize food resources that contain compounds toxic to other organisms. The ability to consume such toxins not only allows access to potentially underutilized resources, but can also provide protection against non-resistant predators and parasites. Given that larvae of the fruitfly Drosophila melanogaster live within rotting fruit and have evolved resistance to high levels of ethanol and other products of fermentation, we decided to test whether ethanol protects fruitflies from otherwise lethal parasites. Here, we show that environmental ethanol causes reduced infection of fruitfly larvae by endoparasitoid wasps. Furthermore, if infected, ethanol consumption by fruitfly larvae results in developmental retardation and death of wasps growing in the fly hemocoel, without need of the stereotypical anti-wasp immune response. This double protection afforded to fly larvae by ethanol is significantly more effective against a generalist wasp than a wasp that has evolved to specialize on D. melanogaster. Finally, fly larvae actively seek out ethanol-containing food when infected, showing they use alcohol as an anti-wasp medicine and self-medicate accordingly.

475A

Transcriptional profile during pachytene in Drosophila melanogaster females. Andrew Adrian, Josep Comeron. Biology, University of Iowa, Iowa City, IA.

We have recently obtained whole-genome, high-density recombination maps in Drosophila melanogaster separately for crossing over (CO) and non-crossover (gene conversion; GC) events. These maps based on more than 100,000 recombination events at a physical resolution down to 2.5 kilobases (kb) reveal highly variable CO rates along chromosomes, including coldspots within regions traditionally labeled as high-recombination. GC is more uniformly distributed across the genome than CO and detectable in regions where CO is severely reduced or completely absent (eg., the small chromosome four). At a micro-scale however GC events have a tendency to occur within transcript units. These differences in CO/GC distribution suggest the influence of spatiotemporal chromosomal properties. To gain insight into the possible causes for the CO/GC variation across the genome we first attempt to link variation in recombination with transcription levels in Drosophila females. Present transcriptomes however poorly characterize the relevant expression during recombination, with germarium cells representing only a tiny fraction of the gonadal (ovary) tissue. In particular, we sought to investigate the expression profile of Drosophila stage 2a oocytes utilizing Laser Capture Microdissection and mRNA-Seq. Our analysis provides a glimpse of the transcriptional landscape at the most relevant pattern of gene expression during pachytene—when double strand breaks are being formed and repaired.

476B

Tracing causative polymorphisms for allele-specific expression in Drosophila melanogaster. Daniel Campo[1], Justin Fear[2], Lauren McIntyre[2], Sergey Nuzhdin[1]. 1) University of Southern California, Los Angeles, CA; 2) University of Florida, Gainesville, FL.

Variation in gene expression is thought to be an important source of phenotypic diversity, playing a crucial role in population and species divergence. Unraveling the genetic mechanisms by which gene expression is regulated is therefore an important step in order to understand certain evolutionary processes such like local adaptation and speciation. Much of the effort done to date in this regard has been focused on interspecific variation. 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 resequenced the entire transcriptome of a large panel of F1 heterozygous individuals, derived from a set of crosses between 70 isogenic lines and a common standard strain. Whole genome sequences for all the parental isogenic lines are also 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 20% of the genes assessed in more than 50% of the genotypes at a FDR of 0.05. This amount of cis- regulatory divergence is surprisingly high, and 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, since we used a common parental fly line 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.

477C

Deficiency of X-linked inverted duplicates with male-biased expression and the underlying evolutionary mechanisms in the Drosophila genome. Zhen-Xia Chen. Center for Bioinformatics, State Key Laboratory of Protein and Plant Gene Research, College of Life Sciences, Peking University, Beijing, PR China.

Inverted duplicates (IDs) are pervasive in genomes and have been reported to play functional roles in various biological processes. However, the general underlying evolutionary forces that maintain IDs in genomes remain largely elusive. Through a systematic screening of the Drosophila melanogaster

genome, 20,223 IDs were detected in nonrepetitive intergenic regions, far more than expectation under the neutrality model. 3,846 of these IDs were identified to have stable hairpin structure (i.e., the structural IDs). Based on whole-genome transcriptome profiling data, we found 628 unannotated expressed structural IDs, which had significantly different genomic distributions and structural properties from the unexpressed IDs. Among the expressed structural IDs, 130 exhibited higher expression in males than in females (i.e., male-biased expression). Compared with sex-unbiased ones, these male-biased IDs were significantly underrepresented on the X chromosome, similar to previously reported pattern of male-biased protein-coding genes. These analyses suggest that a selection-driven process, rather than a purely neutral mutation-driven mechanism, contributes to the maintenance of IDs in the Drosophila genome.

478A

Neo-Y chromosome divergence among populations of Drosophila albomicans. Chia-Hao Cheng, Hwei-yu Chang. Entomology department, NTU, Taipei, Taiwan.

Drosophila albomicans (2n = 6) has a pair of metacentric neo-sex chromosomes with a large portion (i.e., about 40% of the genome) of neo-Y chromosome arm originally from an autosome. Due to the lack of recombination in Drosophila males, the divergence time between neo-X and neo-Y chromosomes was estimated to be about 0.07 MY based on the synonymous substitutions rate. Our previous study of crossing D. albomicans and its sibling species D. nasuta has shown high non-disjunction rate of sex chromosomes in hybrid males with a neo-Y chromosome. Although X,neo-Y/Y males are viable, not a single X,neo-Y/neo-Y male has ever been found in our experiments which implies the existence of recessive deleterious alleles on the 3rd arm of the neo-Y chromosome. The hypothesis we proposed here is that the 3rd arms of neo-Y chromosomes in different populations accumulated recessive deleterious alleles independently. This can be investigated by complementation tests between two strains. Different fragments of recombinant 3rd arm of the neo-Y chromosome can be obtained through a fertile 3,X,X/neo-Y female. Special cross schemes were designed to reveal differential evolution of the 3rd arms of the neo-Y chromosomes. Molecular markers were mainly PCR fragments, genetically mapped and confirmed by in situ hybridization on salivary gland chromosomes. Recombinant strains were established and with proper genetic markers a more detailed examination can be done by comparing different neo-Y segments. Our contemporary result showed that the recessive deleterious effect of neo-Y can be complemented in other words we did obtain X,neo-Y/neo-Y' individuals if the two Y chromosomes were from different populations. As for where the deleterious alleles located, it's roughly shown that they're at both the distal and the basal fragments but not the middle portion of the 3rd arm of the neo-Y chromosome. Further confirmation is needed for detailed mapping is still ongoing.

479B

Comparative and functional analysis of CTCF binding site divergence in the Drosophila genome. Eldon Emberly[1], Joyce Stamm[2], Nickodemo Pavoni[3], Kyrillos Awad[3], Amy Lloyd[3], Brittany Pasierb[3], Carlos Ortiz[3], Sheryl Smith[3]. 1) Physics, Simon Fraser University, Burnaby, B.C., Canada; 2) Biology, University of Evansville, Evansville, IN; 3) Biology, Arcadia University, Glenside, PA.

Insulator sequences have defined functions in genome organization, regulation of enhancer-promoter communication and in barrier activity to prevent the spread of repressive chromatin. We have previously conducted a genome-wide analysis of sequences that bind to the insulator-associated protein CTCF in *Drosophila melanogaster.* Our analysis has revealed that the majority of CTCF-associated sequences were not found within intergenic regions as predicted, but were biased toward genes. CTCF-associated sites were predominantly found within 1000 base pairs of a transcription start site (TSS), suggesting a more direct role for these sequences in regulating transcription of the nearby linked gene. We have recently conducted an analysis of the conservation of promoter-associated CTCF binding sites across 11 species of *Drosophila* and show that genes associated with highly conserved CTCF elements function in regulating essential metabolic processes, while genes associated with poorly conserved CTCF elements function in metamorphic/ post-embryonic developmental processes. These findings suggest that promoter-associated CTCF sites and their linked genes are subject to similar selective evolutionary pressures. To further investigate the role of promoter-associated CTCF binding on transcriptional regulation of linked genes we analyzed several odor receptor family loci with closely linked CTCF-associated sequences across the 11 *Drosophila* species. We found sequence divergence from homologous CTCF-bound loci in *D. melanogaster* is associated with loss of CTCF binding and changes in linked gene expression. A comprehensive analysis of the chromatin structure at these loci is currently in progress and will provide valuable insight into the role of CTCF binding site divergence as a potential evolutionary tool for altering gene expression.

480C

Identification of transcriptional regulatory networks using structural equation modeling along with priori biological knowledge. Justin M Fear[1], Daniel Campos[2], Sergey V Nuzhdin[2], Lauren McIntyre[1,3]. 1) Genetics & Genomics, Univ Florida, Gainesville, FL; 2) Section of Molecular and Computational Biology, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089; 3) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL 32611.

Assays for transcriptome profiling, such as microarrays or RNA-seq, generate lists of significantly differentially expressed genes (DEGs). These lists are then the starting point for making biological inferences. One means to extract biological knowledge utilizes Go Ontology (GO) enrichment of statistically significant genes. Combined with the investigator's biological knowledge, enrichment analyses can lead to hypotheses about underlying mechanism. However, genes connected in a regulatory pathway may not share GO terms given that the underlying premise of the GO is to describe function and not to describe interactions. Transcriptional pathways can be reconstructed algorithmically, but these computational exercises often lack incorporation of biochemical knowledge and ignore genetic variation. By integrating gene expression data with published interactomes, sub-networks responsible for transcriptional regulation can be identified. Inclusion of protein-encoding genes regulated by means other than transcription into the sub-networks grounds the work in biochemical knowledge and allows for the identification of transcripts connected by biological processes. These specific sub-networks can then be tested for directionality in transcript regulation using structural equations. We demonstrate the utility of this approach using RNA-seq data from female heads.

481A

Genomic evidence that heightened gene duplicate accumulation gave a boost to the energy metabolism of the higher Diptera. Markus H. Friedrich,

Riyue Bao. Dept Biological Sci, Wayne State Univ, Detroit, MI.

Gene duplication is an important source of evolutionary innovation. To explore the genetic basis of macroevolutionary differences between major insect orders, we performed a genome-wide study of lineage-specific gene duplications in *Drosophila melanogaster* (fruit fly), *Anopheles gambiae* (mosquito), *Tribolium castaneum* (red flour beetle), and *Apis mellifera* (honey bee). In 5547 gene families conserved across these species, we discovered 467 families with lineage-specific duplications in *Drosophila* compared to only 168 in *Anopheles*, 218 in *Tribolium*, and 120 in *Apis*. Based on sister-paralog synonymous substitution divergencies, most of the *Drosophila*-specific duplications are old, dating back to the early diversification of the higher Diptera. This inference is corroborated by the results of ortholog searches in the genome of the Hessian fly *Mayetiola destructor*. Gene ontology analysis reveals that energy metabolism-related genes are significantly enriched in the *Drosophila*-specific duplications. We conclude that the early evolution of the higher Diptera was exceptionally impacted by gene duplication. One of the consequences was an expansion of the energy metabolism related transcriptome. The correlated emergence of exceptionally fast and hence energy expensive flight capacities in the higher Diptera is highly suggestive of a causal link.

482B

Premature Stop Codon Mutations and Evolution in the Drosophila pseudoobscura Complex. Kenneth B Hoehn, Mohamed AF Noor. Biology Dept, Duke University, Durham, NC.

A number of recent studies have shown that loss of function mutations caused by premature stop codons (PSCs) can have a significant impact on adaptive evolution to new environments. Despite this, most PSC studies have been limited to specific genes within a specific species pair, and little is known about the adaptive effects of premature stop codon mutations across the full genomes of multiple species. This represents a large gap in our understanding of adaptive evolution, and investigating levels of polymorphism within species as well as differences between closely related species for PSCs can give us insights into the evolutionary forces acting upon them. Because of the recently available genome sequences, the fruit fly Drosophila pseudoobscura represents a promising system for investigating these effects. This project has two distinct phases. First, custom Perl scripts were written to detect premature stop codons mutations in 11 D. pseudoobscura, 3 D. persimilis, and 3 D. miranda full genome sequences. Once found, genes of interest were selected on the basis of a) known orthologs in D. melanogaster used to predict gene function which may have been lost, b) how early in the coding sequence the PSC occurs, and c) whether or not the mutation was identified in multiple genome sequences. Expression was then confirmed by reverse-transcriptase PCR in the published genome strain and at least one PSC strain. In total, our initial computational approach found 2114 unconfirmed PSC's affecting 748 genes. Of these, 299 PSC's affecting 130 genes passed initial filtering based on presence of orthologs and severity of truncation. Specific results are discussed in the context of the likely evolutionary forces acting on these genomes.

483C

Birth, death, and replacement of importins in Drosophila. Emily Hsieh[1,2], Nitin Phadnis[2], Harmit Malik[2]. 1) University of Washington, Seattle, WA; 2) Fred Hutchinson Cancer Research Center, Seattle,WA.

The nuclear transport pathway performs the fundamental function of moving cargo between the cytoplasm and nucleus in eukaryotes. Nuclear transport is an essential function and is carried out through a highly conserved mechanism across all eukaryotes. Yet, in *Drosophila*, several components of the nuclear transport apparatus evolve rapidly under positive selection. Genetic conflict with selfish elements has been suggested as a possible cause for this pattern of rapid evolution. Here, we present a comprehensive phylogenomic analysis of importin gene evolution in *Drosophila*. Importins are adapter molecules that directly mediate the transport of cargo into the nucleus. Our analysis reveals a recurrent gain and loss pattern of the copies of importins in *Drosophila* across independent lineages. Interestingly, we discovered that almost all new copies of importins have acquired a testes-specific pattern expression since their birth through gene duplication. This pattern of repeated gains of testes-specific copies of importins and signatures of episodic lineage-specific positive selection suggests a function in suppressing segregation distortion in the male germline. Segregation distorters such as *SD* in *Drosophila melanogaster* act by impairing nuclear transport in the testes. We are currently performing functional tests of the hypothesis that an increased dosage of these non-canonical importins in the testes may serve a role in suppressing segregation distortion in males through restoring nuclear transport during spermatogenesis.

484A

A second generation assembly of the *Drosophila simulans* genome and its implications for genome evolution studies. Tina Hu[1], Michael Eisen[2], Kevin Thornton[3], Peter Andolfatto[1]. 1) Department of Ecology and Evolutionary Biology and the Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 2) Howard Hughes Medical Institute and the Lawrence Berkeley Laboratory, University of California Berkeley, Berkeley, CA; 3) Department of Ecology and Evolutionary Biology, University of California Irvine, Irvine, CA.

The amount of divergence between species is a function of mutation rate and the direction and intensity of natural selection. Previous analyses surveying rates of sequence divergence between *Drosophila melanogaster* and its sister species *D. simulans* have suggested that rates of divergence for nonsynonymous and synonymous sites are somewhat accelerated along the *D. melanogaster* lineage relative to *D. simulans* (Begun et al, 2007). This trend has been interpreted as resulting from a relaxation of selection associated with a reduced effective population size in *D. melanogaster*. Most noncoding DNA in Drosophila has also been shown to be subject to weak purifying selection. However, unlike coding regions, Begun et al. paradoxically document accelerated evolution of noncoding DNA along the *D. simulans* lineage. We revisit these observations by combining Illumina short read with available Sanger data for *D. simulans* strain *wt501*. Using this improved *D. simulans* reference, we revisit levels of divergence along the *D. melanogaster* and *D. simulans* lineages.

485B

Genomic satellite DNA repeats and small RNAs: An evolutionary analysis of the *Responder* satellite in the *Drosophila melanogaster* genome. Amanda M. Larracuente, Daven C. Presgraves. Biology Department, 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*. *SD/SD*$^{+}$ heterozygous males transmit the *SD* chromosome to >95% of their progeny when the *SD*$^{+}$ chromosome bears a sensitive *Rsp* allele. *Rsp* copy number in the pericentric heterochromatin of chromosome *2* is positively correlated with the sensitivity to segregation distortion, although *Rsp* repeats have been found outside of the 2nd chromosome

pericentric heterochromatin using cytological methods. The molecular relationship between *Rsp* repeat number and segregation distortion is not understood. Recently, RNAi has been implicated in Drosophila meiotic drive systems, including *SD*. Small RNAs corresponding to *Rsp* repeats are found in both female and male flies, consistent with the involvement of RNAi in *SD*. We present a bioinformatics study with two objectives: 1) To study the evolution of the *Rsp* satellite; and 2) to determine whether the small RNAs corresponding to *Rsp* are consistent with their involvement in *SD*. To study the evolution of the *Rsp* satellite, we surveyed the *D. melanogaster* genome assembly and BAC sequences, and other Drosophila species genome assemblies for *Rsp* repeats. We found several *Rsp*-like repeat families on all major chromosome arms in *D. melanogaster*. Although components of the *SD* system are assumed to be specific to *D. melanogaster*, we find *Rsp*-like repeats in other Drosophila species, however their relative ages are unclear. To determine whether small *Rsp* RNAs correspond to the *Rsp* repeats on chromosome *2* targeted by *SD*, we mapped small RNAs to their respective genomic locations.

486C

Experimental study of evolutionary conflict between the mitochondrial and nuclear genomes. Aimee J. Littleton[1], Maulik R. Patel[1], Ganeshkumar Miriyala[1], Ala Soofian[1], Harmit S. Malik[1,2]. 1) Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute, Seattle, WA.

The eukaryotic cell is a product of symbiosis between an ancient protobacteria and the mitochondria. Despite being one of the most successful relationships in evolution, it is also rife with conflict. The nuclear DNA is transmitted through both parents, whereas the mitochondria possess their own genome (mtDNA) that is maternally transmitted. Since males are an evolutionary dead-end, mtDNA is predicted to skew the sex ratio of a population in favor of having more females. While this appears to be true in plants, it is unknown whether animal mitochondrial genomes can similarly act selfishly. We are currently conducting experimental evolution in Drosophila melanogaster to test whether the mitochondrial genome can be artificially selected to increase fitness of females at the expense of male fitness. We are doing this by crossing daughters from every generation to naïve males over the span of fifty generations. Mating the females to naïve males every generation allows the mitochondrial genome to evolve independent of the nuclear genome. As a control we are coevolving females with males. Every ten generations we are conducting a series of fitness assays to detect any phenotypic changes. This includes counting female to male ratios of the progeny and eggs laid by the evolved females. We are also measuring male and female fecundity. Here I will present the current status of this experimental evolution including preliminary results suggesting skewed male-female fitness in at least one of the replicate lines.

487A

Sex-specific embryonic expression at different stages of sex chromosome evolution. Susan E. Lott[1], Jacqueline E. Villalta[2], Doris Bachtrog[3], Michael B. Eisen[1,2,3]. 1) Molecular and Cell Biology, Univ California, Berkeley, CA; 2) Howard Hughes Medical Institute, Univ California, Berkeley, CA; 3) Department of Integrative Biology, Univ California, Berkeley, CA.

Sex chromosome dosage differences between males and females are a major form of natural genetic variation in many species. In *Drosophila*, females have two X chromosomes, while males have one X and one Y. Several fusions of sex chromosomes with autosomes have occurred along the branches leading to *D. pseudoobscura* and *D. miranda*. The resulting neo-X chromosomes are gradually acquiring the properties of classical sex chromosomes, and becoming targets for the complex molecular mechanisms that have evolved to compensate for the differences in X chromosome dose between sexes. We have recently shown that *D. melanogaster* possess at least two mechanisms for dosage compensation: the well-characterized MSL-mediated dosage compensation active in most somatic tissues, and a second mechanism during early embryogenesis. To better understand the evolutionary constraints on sex chromosome expression and evolution, we have used single embryo mRNA-seq to characterize gene expression in female and male embryos of *D. pseudoobscura* and *D. miranda*, from ~0.5-8 hours of development. Examining expression from these X chromosomes throughout embryonic development, we observe a relationship between the age of the X chromosome and the number of genes that are compensated. We also characterize what kinds of genes and processes are more likely to be compensated or have their expression level more constrained, at various stages in embryonic development, which we can then use to test whether expression constraint is predictive of fitness consequences.

488B

Is the Drosophila X chromosome demasculinized? Colin D. Meiklejohn, Daven C. Presgraves. Dept Biol, Univ Rochester, Rochester, NY.

Male biased genes— those expressed at higher levels in males than in females— are underrepresented on the X chromosome of Drosophila melanogaster. Several evolutionary models have been posited to explain this so-called demasculinization of the X. Here we show that the apparent paucity of male-biased genes on the X chromosome occurs for a simple developmental reason and thus requires no special evolutionary explanation. As most sex-biased genes in Drosophila involve those expressed in the germline, we studied the chromosomal distribution of genes whose expression was measured using RNA-seq and microarrays from adult testes, ovaries, and somatic tissues. We find, first, that the underrepresentation of testes-biased genes on the X disappears once the lack of dosage compensation in the Drosophila male germline is accounted for. Second, we find that computationally demasculinizing the autosomes is not sufficient to produce an expression profile similar to that of the X chromosome in the testes, whereas correcting for the lack of dosage compensation in testes does. These findings show that the lack of sex chromosome dosage compensation in the testes can explain the apparent demasculinization of the X, whereas any evolutionary demasculinization of the X cannot explain its reduced expression in the testes.

489C

Adaptive Evolution and the Birth of CTCF binding events in the Drosophila genomes. Xiaochun Ni[1,2], Yong Zhang[1], Nicolas Negre[2,3], Sidi Chen[1], Manyuan Long[1], Kevin White[1,2,3]. 1) Department of Ecology & Evolution, University of Chicago, Chicago, IL; 2) Institute for Genomics and Systems Biology, University of Chicago , Chicago, IL; 3) Department of Human Genetics, University of Chicago, Chicago, IL.

Changes in the physical interaction between cis-regulatory DNA sequences and proteins drive the evolution of gene expression. However, it has proven difficult to accurately quantify evolutionary rates of such binding change, or to estimate the relative effects of selection and drift in shaping the binding evolution. Here we examine the genome-wide binding of CTCF in four species of Drosophila separated by between ~2.5 and 25 million years. CTCF is a highly conserved protein known to be associated with insulator sequences in the genomes of human and Drosophila. Although the binding preference for CTCF is highly conserved, we find that CTCF binding itself is highly dynamic and has adaptively evolved. Between species binding divergence increased

linearly with evolutionary distance, and CTCF binding profiles are diverging rapidly at the rate of 2.22% per million years (Myr). At least 89 new CTCF binding events have originated in the Drosophila melanogaster genome since the most recent common ancestor with Drosophila simulans. Comparing these data to genome sequence data from 37 different strains of Drosophila melanogaster, we detected signatures of selection in both newly gained and evolutionarily conserved binding sites. Newly evolved CTCF binding sites show a significantly stronger signature for positive selection than older sites. Comparative gene expression profiling revealed that expression divergence of genes adjacent to CTCF binding site is significantly associated with the gain and loss of CTCF binding. Further, the birth of new genes is associated with the birth of new CTCF binding sites. Our data indicate that binding of Drosophila CTCF protein has evolved under natural selection, and CTCF binding evolution has shaped both the evolution of gene expression and genome evolution during the birth of new genes.

490A

Strong evidence of biased gene conversion in *Drosophila melanogaster*. Matthew C. Robinson, Eric A. Stone, Nadia D. Singh. Genetics Dept, NCSU, Raleigh, NC.

Gene conversion is the non-reciprocal exchange of genetic information between homologous chromosomes during meiosis. Biased gene conversion (BGC) reflects the favoring of certain alleles over others during this process. In particular, gene conversion appears to be GC-biased across a wide variety of eukaryotic genomes. Preliminary evidence in Drosophila from a subset of coding and noncoding loci are consistent with BGC, but it remains unknown whether BGC is a general feature of Drosophila genome evolution. Here we systematically test for BGC at a genomic scale in *D. melanogaster* using newly available population genomic data. We take advantage of the Drosophila Genetic Reference panel, a set of 162 fully sequenced lines derived from a natural population from Raleigh, North Carolina. We test for BGC in four types of sequences: short introns, long introns, intergenic regions, and four-fold degenerate synonymous sites. In addition, we explore the effects of genomic context including local recombination rate, GC-content, and chromosome, on the degree of GC-bias in gene conversion. To test whether patterns of polymorphism are consistent with BGC, we examined the site frequency spectra of bi-allelic single nucleotide polymorphisms (SNPs) (polarized to *D. simulans*) from different sequence types and from varying genomic contexts. BGC should result in an excess of high-frequency AT->GC polymorphisms relative to neutral expectation; the degree of this skew indicates the magnitude of the BGC. We quantify the skew of the right-handed tail of the site frequency spectrum using a summary statistic "Q" and test for differences in the strength of BGC by comparing the Q statistic among our four sequence types and across genomic contexts. Our results are consistent with pervasive BGC in *D. melanogaster*. The degree of bias appears exaggerated on the X chromosome relative to the autosomes, as well as in regions of high recombination versus low recombination. BGC is thus likely to be a significant contributor to genome evolution in *D. melanogaster*.

491B

Mutation accumulation reveals a large duplication bias and substantial variation in substitution rates in *Drosophila melanogaster*. Daniel R. Schrider[1,2], Michael Lynch[1], David Houle[3], Matthew W. Hahn[1,2]. 1) Department of Biology, Indiana University, Bloomington, IN; 2) School of Informatics and Computing, Indiana University, Bloomington, IN; 3) Department of Biological Science, Florida State University, Tallahassee, FL.

Because all genetic variation on which natural selection operates originates via spontaneous mutation, the rates at which mutations appear in natural populations have important evolutionary consequences. Genome-wide mutation accumulation (MA) experiments are a powerful method for estimating mutation rates, and can therefore improve our understanding of the rate at which adaptive alleles arise and illuminate how natural selection shapes variation within and among species. Unfortunately, the small number of generations captured by most MA experiments limits their statistical power. In addition, most of these studies used sequencing methods that do not allow for comprehensive detection of large genomic duplications and deletions that result in genomic copy number variants (CNVs). We present results from an MA experiment in *Drosophila* that does not suffer from these shortcomings. More generations are captured in this experiment (~1160) than in all other eukaryotic MA studies combined. This wealth of data reveals >2-fold variation in substitution rates between MA lines derived from different isofemale ancestors, suggesting that mutation rates can vary greatly among individuals within natural populations, and that the mutation rate of a species cannot be represented by a single estimate. We also present the first accurate estimates of the rates of large duplications and deletions. We confirm the previously observed bias of small deletions over small insertions; however, at scales larger than ~150 bp, we find that the rate of duplication is much higher than the rate of deletion, reversing the current view that *Drosophila* exhibits a deletion bias. While this result implies that mutational forces alone would cause the *Drosophila* genome to grow rapidly, we show that selection against large duplications has prevented this growth from occurring.

492C

Classifying the evolutionary causes of nucleotide fixation. Alexander Shanku[1], Andrew Kern[2]. 1) BioMaPS Institute, Rutgers University, Piscataway, NJ; 2) Department of Genetics, Rutgers University, Piscataway, NJ.

A long term goal of population genetics has been to determine to what extent natural selection impacts patterns of genomic variation within and between populations. In an attempt to localize the effect of selection within genomes, much attention has been paid towards detecting the tell-tale signatures of selective sweeps, whereby a newly arising beneficial mutation rapidly increases in frequency to fixation. Such efforts have classically used population genetic summary statistics (Tajima 1983, Fu and Li 1993,Fay and Wu 2000) or more recently likelihood techniques (Kim and Stephan 2002, Kim and Nielsen 2004, Nielsen et al. 2005). Formally there are at least three possible routes (in the evolutionary sense) by which a novel mutation may fix: 1) it may be a neutral mutation, or nearly so, and drift to fixation, 2) it may be an unconditionally beneficial mutation and rapidly sweep to fixation (hard sweep) or 3) it may be initially neutral, or nearly so, but at some later point in time (e.g. after an environmental change) becomes beneficial and then fixes rapidly (soft sweep). Here we describe the use of supervised machine learning algorithms for the classification of nucleotide fixations into these three classes based on combinations of population genetic summary statistics. We examine the efficacy of three classes of algorithms, logistic regression, support vector and relevance vector machines, first by training and testing these algorithms on simulated data generated from the coalescent, then applying these techniques to first generation DPGP data. In training our classifiers by integrating over model parameters and altering demographic histories, we demonstrate that we have power to classify hard vs. neutral sweeps at 90% accuracy and hard vs. soft sweeps at >80% accuracy out to 0.4 units of time since the sweep fixed in the population (time=2N generations, N=population size). This work represents a novel application of supervised classifiers in population genetics and a potential new tool in searching for selection across the genome.

493A

Characterizing the homomorphic sex chromosomes of *Aedes aegypti*. Melissa A. Toups[1], Matthew W. Hahn[1,2]. 1) Biology, Indiana University, Bloomington, IN; 2) School of Informatics and Computing, Indiana University, Bloomington, IN.

Genetic sex-determination has evolved multiple times in both plants and animals. In many species, there is a nonrecombining region surrounding the sex-determining locus where sexually antagonistic genes accumulate. The size of this nonrecombining region may contain only a small proportion of the sex chromosome, as in *Aedes aegypti*, or may include the entire sex chromosome, as in *Drosophila melanogaster* and *Anopheles gambiae*. However, why the extent of this nonrecombining region varies among species is unknown. We investigated this phenomenon in *Aedes aegypti*, which has been demonstrated to have only a small nonrecombining region for the last 100-150 million years. The sequenced *Aedes aegypti* genome contains over 1500 scaffolds, but only ~250 scaffolds are mapped onto chromosomes. We developed DNA (RAD) tags and used Illumina sequencing to map ~95% of the largest *Ae. aegypt*i scaffolds to chromosome using an F6 recombinant population. In order to determine which scaffolds map to the nonrecombining region of the X chromosome, we compared RAD tag read depth for a male and a female mosquito. Those RAD tags that are in the autosomes or the recombining regions of the sex chromosomes have equal coverage in both males and females. However, RAD tags that have twice the coverage in a female or are found only in males are in the nonrecombining region of the sex chromosomes. We used the scaffolds containing these RAD tags to explore the genetic content of the non-recombining region, gene movement on and off these proto-sex chromosomes, and sex-biased expression in and around the non-recombining region.

494B

Methodological studies on development and duplicate datasets revealed new evidence for Meiotic Sex Chromosomal Inactivation. Maria Vibranovski[1], Jun Wang[1], Timothy Karr[2], Manyuan Long[1]. 1) Ecology & Evolution, Univ Chicago, Chicago, IL; 2) Biodesign Institute, Arizona State University, Tempe, AZ.

The role of the Meiotic Sex Chromosome Inactivation (MSCI) during spermatogenesis proposed initially by Lifschytz and Lindsley [1] has recently attracted a lot of interest to test if it is an evolutionary force on the chromosomal distribution of sex-biased genes in *Drosophila*. Besides the evidence from gene expression in spermatogenesis [2,3], here we report our methodological studies on two sets of new supporting data. First, we analyzed the recently generated data on transcriptional profiling of testis development [4], which took advantage of the spermatogenesis timeline and obtained RNA from the first wave of germline differentiation in larva testis. In this nicely designed system, the amount of meiotic cells increases with developmental stages. Our statistical study showed a significant lower expression of X-linked genes in meiosis in comparison to autosomal genes in later developmental phases, which provided new evidence in support of the MSCI model. Second, our Bayesian models on the expression profile of DNA-based gene duplication in spermatogenesis using the stage-specific database [3] detected significant new signals from MSCI. If MSCI acts as a general force affecting gene distribution, it should affect the distribution of both RNA- and DNA-based duplication. Here we confirmed this prediction. These new lines of evidence, in addition to recent genetic analysis of MSCI and dosage compensation [5], built up further support of the MSCI hypothesis. We reviewed and discussed related experimental and statistical methods and the application of Bayesian models in testing the MSCI hypothesis. 1.Lifschytz and Lindsley. PNAS USA 1972. 2.Hense et al. PLoS Biol 2007. 3.Vibranovski et al. PLoS Genetics 2009. 4.Mikhaylova and Nurminsky. BMC Biol. 2011. 5.Deng et al. Nature Genet. 2011.

495C

Conservation and expression pattern of overlapping genes in the *Drosophila* genome. Luyi Wo[1,2], Yihan Li[3], Stephen Schaeffer[1,2]. 1) Department of Biology, Penn State University, University Park, PA 16802; 2) Intercollege Program of Genetics, Penn State University, University Park, PA 16802; 3) Department of Statistics, Penn State University, University Park, PA 16802.

Drosophila has compact genome among which 30 % of protein coding genes overlap with each other in a variety of arrangements including straight-overlapping genes, embedded genes within a parental gene, polycistronic genes and interdigitated genes with straight-overlapping genes and parent-embedded genes dominating the categories. The comparison of gene structure of overlapping genes among the 12 *Drosophila* genomes was used to determine the selective constraint on these arrangements. In general, overlapping genes are not strictly conserved although the degree of conservation is consistent with evolutionary distance. Straight overlapping genes are the most conserved type and embedded genes evolve the fastest. Annotation artifacts do not seem to confound the inference of conservation level. Levels of gene expression of overlapping genes were examined to determine if gene organization affects levels of expression and dictates levels of conservation. The expression pattern of different types of overlapping cases revealed from microarray data of *D. melanogaster* seems to be concordant with conservation analysis. Straight overlapping genes overall are expressed universally across various tissues and developmental stages while embedded genes have a much lower average expression and tend to be expressed in a tissue or developmentally specific manner. Moreover, median expressions of two counterparts of straight overlapping pair, particularly parallel straight-overlapping pair, are significantly coupled, while those of parent-embedded pair tend to be inversely related. These data suggest that gene organization can influence levels of gene expression. Our analysis indicates that there may be transcriptional interference between genes when one gene is embedded within another gene.

496A

Insights into the Mechanisms of Intron Gain and Loss Using Drosophila Genomes. Paul Yenerall[1], Leming Zhou[2,3]. 1) Department of Biological Sciences; 2) Department of Health Information Management; 3) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA.

Recently it has been shown that intron density may vary among orthologous genes and that the rate of intron gain and loss can vary dramatically in different species. However, identifying the exact mechanism(s) that generate these structural modifications has proved challenging. Introns are known to affect gene expression and harbor various non-coding RNAs. Therefore, identifying the mechanism(s) of intron gain and loss will provide novel insight into the evolution of greater regulation and complexity in eukaryotes. Using 11 Drosophila species and an outlier, Anopheles gambiae, we identified 189 intron gain and 287 intron loss events. We then analyzed these events to determine what mechanism(s) may have been responsible for these changes. These analyses enabled us to identify the first documented case of intron gain via transposon insertion in an animal and the first documented case of intron loss via non-homologous end joining. Our data also suggest that a novel mechanism of intron gain that relies upon or is enabled by transcription may operate in Drosophila. Furthermore, we have collected all intron gain and loss events reported in the literature that appear to have occurred via a previously proposed

mechanism and have organized them into a new database. This database will prove useful to researchers in validating or refuting each proposed mechanism of intron gain and loss.

497B

Statistical models for RNA-seq data. Rhonda L. Bacher[1], Justin Dalton[2], Rita M. Graze[3], Kurt Jensen[4], Jonatan Sanchez-Garcia[4], Pedro Fernandez-Funez[4], Diego E. Rincon-Limas[4], Michelle N. Arbeitman[2], Ann L. Oberg[5], Sergey V. Nuzhdin[6], Lauren M. McIntyre[3]. 1) Departments of Statistics and Mathematics, University of Florida, Gainesville, Florida, USA; 2) Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, Florida, USA; 3) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, Florida, USA; 4) Department of Neurology, McKnight Brain Institute, University of Florida, Gainesville, Florida, USA; 5) Department of Health Sciences Research, Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, Minnesota, USA; 6) Molecular and Computational Biology, Department of Biological Sciences, University of Southern California, Los Angeles, California, USA.

RNA-Seq is a tool used for assessing gene expression based on read counts from high throughput sequencing. Many analysis methods to detect differential expression thus far have focused on using the Poisson distribution, or the negative binomial distribution for analysis of differential expression. Looking at both the underlying data and the measurement on which we perform the analysis, we propose that RNA-Seq data can be considered continuous. The underlying libraries are made from a solution of mRNA quantified by concentration. This solution is sampled and sequencing technology is used to estimate the number of molecules in the sample for a particular gene. Normalization techniques, which result in non-integer values, are often applied to RNA-Seq data in order to account for systematic effects on the total number of counts, for example the length of the exon/transcript and the total number of reads mapped to the reference per sample. Furthermore, the raw read counts themselves take on a large range of values (0, 1, to 6 and 7 digit numbers). We examine three different experiments in drosophila and using a mixed effects model with a normal distribution, we find the residuals do conform to underlying assumptions when data are complete. When some observations are missing residual assumptions are often violated.

498C

Laboratory selection on Drosophila melanogaster using Bacillus cereus spores: direct response to selection and correlated life history trait responses. Lawrence Harshman, Junjie Ma, Andrew Benson, Stephen Kachman, Zhen Hu. Univ Nebraska - Lincoln, Lincoln, NE.

D. melanogaster is a model for laboratory selection experiments. We selected for adult fly survival after tungsten needle-mediated infection with spores from the bacterium, Bacillus cereus, a gram-positive species closely related to Bacillus anthracis. There were nine lines (populations): three lines were selected for survival after spore infection, in addition there were three sham-infected control lines and three control lines that were not infected or poked. After 15 - 20 generations of selection, a strong response was observed for infection survival with > 10-fold increase in the LD50 value for live spores. All lines were assayed for correlated responses in life history traits after inoculation with autoclaved spores, inoculation with water, or no inoculation. Life span differences among the lines were observed only when autoclaved spores were introduced. In terms of fecundity, selected lines exhibited considerably higher levels of life-time egg and progeny production than the control lines. Development time (egg-to-adult) was also investigated. Males and females from the selected and control lines where separately subjected to three environmental conditions and thus there was a complex matrix of outcomes. One result was that when selected line males were exposed to autoclaved spores their progeny developed more rapidly. However, when selected line females were exposed to autoclaved spores their offspring developed more slowly. Two results are potentially noteworthy from an evolutionary standpoint. The first is that there was no trade-off between evolved survival after spore infection and progeny production. Instead elevated levels of egg and progeny output apparently compensate for the negative effects of spores on fecundity. The second is the observation of contrasting maternal and paternal effects on progeny development time in the selected lines.

499A

Computational modeling of *cis*-regulatory modules from 3D expression data in a *Drosophila* blastoderm atlas. Soile V E Keränen, Oliver Rübel, Mark D Biggin, David W Knowles. Lawrence Berkeley Natl Lab, Berkeley, CA.

Animal *cis*-regulatory modules (CRMs) function in the 3D context of the whole organism. To generate correct spatial outputs, CRMs must exploit the spatial information in expression patterns of *trans*-regulatory proteins. Based on the assumption that CRMs are optimized to interpret spatial information, we used computational methods to evolve 'CRMs' that generate *in silico* 3D spatial expression patterns that match selected 3D target patterns as closely as the simulation conditions allow. As input data, we used 3D quantitative expression patterns recorded in a VirtualEmbryo; a cellular resolution morphology and expression atlas of *Drosophila melanogaster* late blastoderm generated by the Berkeley *Drosophila* Transcription Network Project (BDNTP) (http://bdtnp.lbl.gov/Fly-Net/). Our algorithm used a simple mutation-selection approach to evolve black-box 'CRMs' that could take 3D expression data for 17 transcription factors and use it to generate a 3D output pattern resembling one of the *in vivo* target patterns. To test for convergence and parallelism, we repeated the evolution 50 times for each target pattern. Overall, normalized, most of the evolved interactions were weak or moderate whereas only few were strong, but even quite weak interaction could have a selectable effect on output pattern. This behavior is in agreement with the Continuous Network model proposed based on ChIP-seq data in which CRMs are bound and regulated by large numbers of transcription factors, many of which have only modest quantitative effects. Moreover, for a given target pattern and a set of transcription factors the solutions often tend to resemble each other, indicating the existence of a favored solution. This phenomenon may have implications on convergence and parallelism also *in vivo*.

500B

RNA-seq: the challenge. Lauren M. McIntyre[1], Rita Graze[1], Luis Novello[2], George Casella[2], Kenny Lopiano[2], Linda Young[2], Ann Oberg[3], Sergey V. Nuzhdin[4]. 1) Dept Molec Gen & Micro, Univ Florida, Gainesville, FL; 2) Dept Statistics, Univ Florida, Gainesville, FL; 3) Mayo Clinic Rochester, MN; 4) University of Southern California.

RNA-seq is revolutionizing the way we study transcriptomes. mRNA can be surveyed without prior knowledge of gene transcripts. Alternative splicing of transcript isoforms and allele specific expression are two applications of this new technology that are particularly exciting. Reports of differences in exon usage, and splicing between samples as well as differences among alleles and the best way to model and quantify these differences are a subject of great interest. This new technology has novel challenges. Some challenges are bioinformatics (e.g. map bias); some are technical (e.g. lane to lane variability), and

some are statistical (e.g. appropriate models for allele specific expression). Without proper consideration of all three sets of challenges bioinformatic, technical and statistical, inferences may be misleading. This presentation focuses on steps for dealing with these challenges that reduce or eliminate some of these concerns.

501C

Male-specific effects of mitochondrial-nuclear genetic interactions. Katelyn Mika, Sonya Joseph, Kristi Montooth. Biology, Indiana University, Bloomington, IN.

Over time, the majority of the original mitochondrial genome has migrated to the nuclear genome, but mtDNA still harbor genes specific to their metabolic function. Because of this, efficient oxidative metabolism in the mitochondria relies on interactions between protein products from both the nuclear and mitochondrial genomes. If mitochondrial and nuclear genomes co-evolve, then mitochondrial-nuclear incompatibilities are predicted to result when divergent genomes are brought together. Montooth et al. (2010) have shown that a particular incompatibility between a nuclear genetic variant in *Drosophila melanogaster* and a mitochondrial variant in *D. simulans* affects female fitness and line productivity. We have since shown that this mitochondrial-nuclear incompatibility lengthens development time by approximately three days and decreases female fecundity by 50%. To test for effects of genetic interactions (or intergenomic epistasis) between the mitochondrial and nuclear genomes on male fertility in Drosophila, we mated males of each of 12 mitochondrial-nuclear genotypes with virgin females of a common genotype and counted the offspring produced. We did not find strong epistatic effects on male fertility. Rather we found strong main effects of mitochondrial genotype on male fertility. While mitochondrial-nuclear genotypes have defects in developmental timing, female fecundity and male fertility, remarkably we find no adverse effects on adult metabolic rate. We hypothesize that there may be strong homeostasis driving a set maintenance metabolic rate. For flies with compromised mitochondrial function, running this maintenance metabolic rate may result in resource allocation tradeoffs that compromise reproductive fitness. In our set of mitochondrial-nuclear genotypes there is evidence for both shared and distinct outcomes of this tradeoff across the sexes.

Montooth KL, Meiklejohn CD, Abt DN, and Rand. 2010. Evolution 64: 3364-3379.

502A

The how of the Y: Direct versus indirect effects of heterospecific Y chromosomes on gene expression in Drosophila. Timothy Sackton, Daniel Hartl. Organismic & Evol Bio, Harvard Univ, Cambridge, MA.

The Drosophila Y chromosome is a degenerated, heterochromatic chromosome with few functional genes. Nevertheless, we have recently shown that disruption of conspecific Y/autosome and Y/X interactions via interspecifc Y chromosome introgressions in Drosophila leads to significant effects on gene expression and male reproductive fitness. In particular, *D. simulans* lines carrying a *D. sechellia* Y chromosome have reduced expression of testis-specific genes, reduced lifetime fecundity, and reduced sperm competitive ability. These results imply a significant role for Y/X and Y/autosome interactions in maintaining proper expression of male-specific genes, but do not distinguish between direct effects on gene expression or indirect effects mediated by heterospecific Y chromosomes on male reproductive tissue development or function. To identify direct Y interactions and test the role of indirect effects on the gene expression, we used RNA-seq to estimate allele-specific expression in *D. simulans* / *D. sechellia* hybrids carrying either a *D. simulans* or a *D. sechellia* Y chromosome. Allele-specific expression in F1s allows us to estimate the relative proportion of *trans* and *cis* effects of Y regulatory divergence and infer the relative proportions of direct interactions, allele-specific chromatin modifications, and downstream effects.

503B

Mitochondrial-nuclear incompatibilities are worse when temperature accelerates the rate of life. Mohammad Siddiq, Luke Hoekstra, Kristi Montooth. Biology, Indiana University, Bloomington, IN.

An efficient and coordinated metabolism is essential for an organism's ability to develop and respond to environmental conditions. We are using *Drosophila* strains that pair divergent mitochondrial and nuclear genomes to explore how unique mitochondrial-nuclear genotypes affect metabolism and life-history. Previously, we identified an incompatibility between *D. melanogaster* nuclear genomes and *D. simulans* mitochondrial genomes that significantly impacts fitness. Mapping the genetic basis in collaboration with Colin Meiklejohn (Univ of Rochester) and David Rand (Brown Univ) reveals that this incompatibility likely arises through compromised mitochondrial protein translation. Here we demonstrate that the phenotypic effects of this mitochondrial-nuclear incompatibility are conditional on environmental temperature. Using flow-through respirometry to measure larval metabolic rate, we find that mitochondrial-nuclear genotype significantly affects the ability of larval metabolic rate to acclimate to the thermal environment. Development time and pupation height, both of which are traits potentially associated with energetics, are also significantly affected by interactions between mitochondrial-nuclear genotype and developmental temperature. We find that the deleterious effect of mitochondrial-nuclear incompatibility increases with temperature, but there is also evidence that developmental plasticity provides homeostasis for metabolic rate. Together these results demonstrate thermodynamic constraint on performance *via* energy limitation, such that inefficiencies in metabolic processes are revealed when temperature accelerates the rate of life.

504C

Zinc finger proteins and the distribution of meiotic recombination events. Caiti Smukowski, Mohamed Noor. Duke University, Durham , NC.

The discovery of a 13-mer degenerate motif that recruits recombination events in the majority of human hotspots significantly advanced the study of meiotic recombination initiation. This motif binds to the Cys_2His_2 zinc finger (ZNF) protein PRDM9 in humans, and allelic variation in the protein affects hotspot activity in humans and mice. While transcription factors have long been recognized for their required role in yeast hotspots, the discovery of *Prdm9* is the first implication of ZNF proteins and sequence motifs as major determinants of hotspot location and usage in an animal. Recent findings on the ZNF protein *Trade Embargo* in *Drosophila melanogaster* recombination initiation further suggest a general role for ZNF proteins in the process of recombination. Using BLAST, several ZNF protein motif prediction programs, and other bioinformatic analyses, we identified all ZNF-protein-binding sequence motifs in *D. melanogaster* and *D. pseudoobscura* and tested for significant associations with regions of high and low recombination. We failed to find any ZNF motifs predictive of recombination rate. Hence, based on these results, we see no evidence that zinc finger proteins affect crossover rate variation within Drosophila genomes. We consider several limitations with our bioinformatic approach, but it is likely that other factors besides ZNF protein binding play a dominant role in the determination of regional recombination rates. These results could even suggest that Drosophila possess a unique recombination initiation system

differing from yeast and mammals.

505A

Men are messy: *Wolbachia* stem cell niche tropism in *Drosophila* is evolutionary conserved only in females. Michelle E. Toomey[1,2], Eva Fast[1], Horacio M. Frydman[1,2]. 1) Dept. of Biology, Boston University, Boston, MA; 2) National Emerging Infectious Diseases Laboratory, Boston University, Boston, MA.

The intracellular bacteria Wolbachia infect up to 70% of all insect species. Even though Wolbachia infections are the largest pandemic on this planet, the cellular and molecular mechanisms for bacterial spreading in nature are still unknown. Typically, Wolbachia are vertically transmitted through the female germline. We have previously reported that Wolbachia target the somatic stem cell niche in the Drosophila melanogaster ovary, facilitating germline infection and contributing to vertical transmission1. To assess if niche targeting is an evolutionarily conserved mechanism across the Drosophila genus, we investigated niche targeting in ecologically diverse Wolbachia strain-Drosophila species pairs. Our data revealed different types of niche tropism among naturally infected Drosophila species: 1) somatic stem cell niche tropism (Figure 1A); 2) germline stem cell niche tropism (Figure 1B); 3) hub tropism in the testes (Figure 8). Each host-bacteria pair displays qualitative and quantitative differences in niche tropisms. Phylogenetic analyses suggest that the different patterns of niche tropism are more closely related to the Wolbachia strain than the host species (Figure 2). Using hybrid introgression crosses and transinfection experiments we confirm that bacterial factors play a major role in determining the characteristics of stem cell niche tropism in both the female and male stem cell niches (Figures 3 and 7 respectively). This work highlights a widespread targeting of stem cell niches in the Drosophila genus, contributing to Wolbachia transmission in nature.

506B

Recessive lethal accumulation increases chromosomal inversion polymorphisms in *Drosophila melanogaster*. Shir-Fan Tung[1], Takahiro Miyo[2], Hsin-Yi Chi[3], Chau-Ti Ting[1,2,4,5], Shu Fang[3]. 1) Institute of Ecology and Evolutionary Biology, National Taiwan University, Taiwan, ROC; 2) Department of Life Science, National Taiwan University, Taiwan, ROC; 3) Biodiversity Research Center, Academia Sinica, Taiwan, ROC; 4) Institute of Zoology, National Taiwan University, Taiwan, ROC; 5) Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taiwan, ROC.

Gene coadaptation has been proposed as the major mechanism to maintain chromosomal inversion polymorphisms in natural populations. Genetic load, though less studied, is likely to be another as recessive deleterious mutations decrease the fitness of homozygotes, and in turn accumulate in the recombination-suppression regions of inversion heterozygotes, leading to the increase of inversion heterozygosity. If the latter mechanism is acting in natural populations, the recessive deleterious mutations would be expected to accumulate nearby the inversion breakpoints where recombination is greatly suppressed in inversion heterozygotes. The Afrotropical population of *Drosophila melanogaster* with high chromosomal inversion heterozygosity and high frequency of recessive lethals provides an ideal material to test this mechanism by examining whether recessive lethals located nearby the inversion breakpoints. By using recombination and deficiency mappings, we identified 14 recessive lethal alleles from eight lethal-bearing third chromosomes. Of which, 13 were mapped into the regions close to the breakpoints of inversions which were found to be polymorphic in the African population. This result provides strong evidence that the accumulation of recessive deleterious mutations can contribute to the maintenance of high chromosomal inversion polymorphisms.

507C

Evolution of the Hippo signaling pathway. Stuart J. Newfeld[1], Charlotte E. Konikoff[2], Billie J. Swalla[2]. 1) Sch Life Sci, Arizona State Univ, Tempe, AZ; 2) Biology Dept, Univ Washington, Seattle, WA.

Initially discovered in flies less than 20 years ago the Hippo kinase pathway has emerged as an important modulator of cell proliferation and apoptosis during development and as a regulator of homeostasis in adults. Here we report the first comprehensive phylogenetic analysis of the multigene families that constitute this pathway. Our data revealed that Hippo is younger than the TGF-beta and Wnt pathways with its origin in the Bilaterian lineage after its divergence from sponges and cnidarians. In vertebrate deuterostomes, well-known whole-genome duplications have expanded many pathway families while relationships between family members generally follow the species tree - as seen in the TGF-beta pathway. Alternatively, in protostomes gene loss appears to have occurred in several families - as seen in the Wnt pathway. In between these groups invertebrate deuterostome gene families show neither duplication nor loss, yet individual trees for many families are inconsistent with their respective species tree. Overall, the data suggest that the Hippo pathway arose late in animal evolution and likely played a role in the origin of the chordate bauplan.

508A

Genetic Population Structure of the Emergent Invasive Fruit Pest *Drosophila suzukii*. Jeffrey Adrion, Nick Haddad, Hannah Burrack, Nadia Singh. North Carolina State University, Raleigh, NC.

Biological invasions have been responsible for damaging modifications to ecosystem function in addition to diminishing native biodiversity. Understanding the colonization history of invasive species is an integral aspect of developing effective pest management strategies. Native to Japan, the soft-skinned fruit pest *Drosophila suzukii* has recently invaded the United States and Europe. The Eastern United States represents the most recent expansion of their range, which presents an exciting opportunity to test alternative models of their colonization history. Here we investigate the genetic population structure of an invasive fruit fly, with a focus on the eastern United States. We sequenced six 700 bp X-linked loci from 8-24 wild-caught males from each of 15 populations. Eight of these populations are from the East Coast of the US, and the remaining 7 include populations from Japan, Spain, the West Coast of the US and the Midwest US. We examine levels of nucleotide diversity in ancestral versus derived populations, and compare recently established populations such as those on the East Coast with less recent invasions such as that found in Hawaii. We estimate the degree of genetic differentiation among populations and explore alternative demographic models for the observed patterns of polymorphism. Moreover, we discuss implications for future range expansion and pest management.

509B

Temporal and spatial dynamics of adaptive evolution in temperate Drosophila. Alan O. Bergland[1], Katherine O'Brien[2], Emily Behrman[2], Paul Schmidt[2], Dmitri Petrov[1]. 1) Dept. of Biology, Stanford University, Stanford, CA; 2) Dept. of Biology, University of Pennsylvania, Philadelphia, PA.

The study of adaptation is the primary focus of evolutionary biology. However, despite a century of empirical and theoretical developments, we still lack a general and comprehensive view of the mode and tempo of adaptive evolution. The migration of the tropical fly Drosophila melanogaster to temperate climates is an excellent system in which to study adaptation because it has only recently colonized temperate climates, displays a well characterized set of traits and behaviors that promote survival over the harsh winter season and goes through a substantial number of generations (~10) through a single growing season allowing us to follow the action of natural selection in real time. To study the adaptive dynamics of Drosophila through space and time, we estimated allele frequencies with high accuracy genomewide from populations of Drosophila collected along a broad latitudinal transect and from seasonal collections across multiple years at a mid-latitude population. These data allow us to identify clinal variants to base pair resolution and, for the first time, to track the dynamic change in allele frequency over the course of the growing season. We identify known clinal variants and discover ~3000 novel clinal polymorphisms. Clinal polymorphisms are more likely to be functional (e.g., non-synonymous) than non-functional. Many genes have multiple clinal polymorphisms but, intriguingly, these polymorphisms are often unlinked or loosely linked in mid latitude populations suggesting selection is acting on multiple sites within a gene independently. Finally, we measure the strength of selection over the course of the growing season by examining changes in allele frequency between summer and fall. We find that, on average, sites have a more northerly allele frequency in the spring and a more southerly allele frequency in the fall; from these data we estimate very large changes in selection coefficients across the growing season.

510C

Genotype Evolution In Mimetic Ex Situ Conditions Gallia Butnaru 1*, Cristina Chelu1, Hildegard Herman1 1Department of Genetics, Banat University of Agricultural Sciences and Veterinary Medicine, Timisoara, 300634, Romania *Corresponding author: (E-mail: galliab@yahoo.com). Gallia A. Butnaru. Dept Genetics, Box 136, PO 1, Banat Univ Agricultural Sci, Timisoara, Banat.

The aim of this study was to determine the adaptation of Drosophila melanogaster raised on the medium with different concentrations of salt. Also we observed the phenotypic traits and molecular changes by RAPD method. In our study we used natural populations of Drosophila melanogaster collected from Socodor area (salty soils). The growth medium was supplemented with NaCl (Promega) as follows: 100 mM, 200 mM, 250 mM, 300 mM, 350 mM and 400 mM. DNA isolation was performed after Steller (1990) and five oligonucleotides were used in RAPD reactions for NaCl 100 mM variant. Total number of emerged individuals decrease in NaCl 100, 200 and 250 mM. The highest lethality affected larval stage (250 mM/NaCl). In NaCl 100 mM the sex-ratio was in male favor (1:1.61). Hunk thorax was seen in NaCl 200 mM and bubbled wings appeared in NaCl 300 mM. We also notice the presence of black spotted thorax in some individuals. The phenotypical expression of Ser gene was observed. The individuals with swollen abdomens appeared in variants with NaCl 200, 250 and 300 mM. The RAPD results revealed no significant differences in the molecular profile of individuals raised on medium with NaCl 100 mM. In oligomer four profile, with sequence 5'GGC-TTG-GCG3', we saw some unique bands (1800 bp in control and 2000 bp in case of 100 mM/NaCl variant). Conclusions. In high concentrations of NaCl (400 mM) Drosophila melanogaster adults did not survived. The small size of the body is an inherited particularity and different mutant forms were observed but a low amount of tumors were detected. An environmental condition related to phenotypic traits represents the results of the adaptive evolution. Keywords: Drosophila, NaCl, RAPD, evolution, genotype Acknowledgements: This work was supported by 52158/2008 PN II grant.

511A

Geographic subdivision among Drosophila melanogaster populations revealed by whole genome sequencing. Daniel Campo, Courtney Fjeldsted, Tade Souaiaia, Joyce Kao, Kjong Lehmann, Sergey Nuzhdin. University of Southern California, Los Angeles, CA.

Demography and selection can leave very similar genetic signatures in the populations making sometimes very difficult to distinguish their effects. Analysis of whole genome patterns of genetic variation within and between populations can help disentangle the relative role of such evolutionary forces since demographic processes are expected to affect the entire genome, whereas natural selection will only affect one or a few loci. For this work, we have generated a dataset of 35 individual whole genome sequences from highly inbred lines of Drosophila melanogaster from Winters, California (USA). This collection of genomes is the second dataset of this type made public so far, following the Raleigh set (http://www.dpgp.org/). We describe genome-wide levels of variation and divergence within and between these two North American populations, Winters and Raleigh. Both populations exhibited negative values of Tajima's D across the genome, which is a signature of demographic expansion. We have also detected a high level of genetic differentiation between the two populations. We found a region in the chromosome 3L, which contains a large number of highly differentiated positions, including fourteen non-synonymous changes. The same region also showed strong levels of linkage disequilibrium. This pattern strongly suggests an ongoing process of positive selection, that most likely started from standing variation. We have also found evidence for gene flow and introgression between Caribbean and Eastern North American fly populations, supporting the hypothesis of an admixture zone in the Southeast region of the US, already proposed by other studies. These results suggest that both evolutionary forces, demography and natural selection, play important roles in shaping genomic patterns of polymorphism within and between populations of D. melanogaster.

512B

The role of chromosome in the evolution of gene regulation, regulatory variation on the X. Rita M. Graze[1], Lauren M. McIntyre[1,2], Alison M. Morse[1], Sergey V. Nuzhdin[3], Marta L. Wayne[4]. 1) MGM, University of Florida, Gainesville, FL; 2) Department of Statistics, University of Florida, Gainesville, FL; 3) MCB, University of Southern California, Los Angeles, CA; 4) Biology, University of Florida, Gainesville, FL.

In Drosophila, as in other male heterogametic taxa, hemizygosity of the X chromosome in males results in differences between the X and the autosome in population size, average recombination rates, and dominance variation, all important parameters of evolutionary processes. X-linked genes may also be subject to a selective regime different from that of autosomal genes because they spend a disproportionate amount of time in females and are subject to X specific regulatory pathways in males. While the relative importance of each of these factors in the evolution of X-linked genes is not completely understood, it is clear that evolutionary dynamics differ for X-linked and autosomal (A-linked) genes. Patterns of regulatory variation may differ between chromosomes or between the sexes, but a focus on different chromosomes or on a single sex in expression experiments thus far has been a critical limitation preventing clear conclusions. Here we elucidate the role of chromosome in regulatory variation, separating X and autosomal regulatory variation in *D. simulans* males and females using a series of chromosome substitutions and analyses of overall, exon and allele-specific expression.

513C

Polymorphisms in chromatin accessibility state within *D.melanogaster*. Aaron Hardin[1], Xiao-Yong Li[2], Michael Eisen[1,2,3]. 1) Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) California Institute of Quantitative Biology, University of California, Berkeley, Berkeley, CA; 3) Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA.

The early embryo of *D. melanogaster* has several of the best studied gene regulatory networks in animals. In particular, the factors that regulate anterior-posterior patterning were identified by genetic methods several decades ago, and we now well understand many of their activities, expression patterns and targets. Previous work in the Eisen lab has shown that these factors bind to thousands of regions across the genome. Our lab has measured transcription factor binding in both *D. melanogaster* and the relatively closely related species *D. yakuba* but have been unable to predict the underlying sequence changes that influence these changes due to the large number of polymorphisms between species. However, correlated changes in binding across factors suggests that the underlying chromatin state may be playing a significant role in binding divergence. We have now examined chromatin state in several natural isolates of *D. melanogaster* by measuring genome wide DNase-hypersensitivity with high-throughput sequencing during the early embryo. The regions of hypersensitivity strongly correlate with nucleosome free regions and regions of bound transcription factors. In order to identify the sequence polymorphisms and correlate these with changes in chromatin state, we have pooled DNAse treated chromatin from several natural isolates and compared the frequency of recovered regions to the frequency of the polymorphisms present in our sample.

514A

Unpacking Estimates of Cis-regulatory Variation. Bradley J. Main[1], Andrew Smith[1], Rita Graze[2], Marta Wayne[2], Lauren McIntyre[2], Sergey Nuzhdin[1]. 1) MCB, Univ Southern California, Los Angeles, CA; 2) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL 32611.

The accumulation of regulatory variants in cis (at the gene) contributes to diversity between populations and divergence between species of Drosophila. In this study, we uncover potential non-additive cis-by-trans interactions within Drosophila simulans, which may be confounded in preliminary estimates of cis. Furthermore, we demonstrate that transcription start sites (TSSs) are not fixed genetic features between species, but rather, are likely an important component of functional cis-regulatory variation. We employ a custom, allele-specific microarray to survey allelic imbalance transcriptome-wide in a sample population of D. simulans. Cis-regulatory variation was exclusively estimated from allele-specific expression (ASE) assays in F1 hybrids, where the trans and environmental factors are shared equally between alleles. Then, we compared ASE between F1 hybrids and introgression F1 hybrids. The introgression F1's are identical to the full F1 hybrids, except they are homozygous for the tester line outside of the 12Mb introgression region on chromosome three. Thus, a change in ASE between these genotypes would only be explained by cis-by-trans interactions. To investigate changes in TSSs, we developed a new technique to target TSSs and applied this to four species within the D. melanogaster subgroup (D.melanogaster, D. simulans, D. sechellia, and D. mauritiana) and D. pseudoosbcura. This data in combination with publically available genome alignments between species was used to make inferences about the extent of TSS differences over evolutionary time. From these results, we show that TSS divergence increases with predicted phylogenetic distance, suggesting that changes in these cis-regulatory features are common and may contribute to species differences. Furthermore, among genes with divergent TSSs, we test for enrichment of low or highly expressed genes, association with gene duplication events, and enrichment of specific gene ontology categories.

515B

Population genomics of sub-Saharan Drosophila melanogaster: African diversity and non-African admixture. John E. Pool[1], Kristian A. Stevens[2], Marc Crepeau[2], Charis M. Cardeno[2], James J. Emerson[3], Russell Corbett-Detig[4], Pablo Duchen[5], David J. Begun[2], Charles H. Langley[2]. 1) Laboratory of Genetics, University of Wisconsin - Madison, Madison, WI; 2) Department of Evolution and Ecology, University of California - Davis, Davis, CA; 3) Department of Integrative Biology, University of California - Berkeley, Berkeley, CA; 4) Department of Organismal and Evolutionary Biology, Harvard University, Cambridge, MA; 5) Section of Evolutionary Biology, Ludwig Maximilians Universitat Munchen, Munich, Germany.

Populations from the African ancestral range of *Drosophila melanogaster* are the species' richest source of genetic variation, and may hold the keys to understanding the adaptation and demography in worldwide populations. We describe the preliminary analysis of >100 fully sequenced genomes from African populations of *D. melanogaster*.Sequencing utilized an Illumina Genome Analyzer IIx, in most cases using 76bp paired end reads with ~300bp inserts, with an average sequencing depth of 30X. Genomes were fully homozygous because genomic DNA was amplified from haploid embryos. These genomes originate from >20 sub-Saharan locations. Using a novel Hidden Markov Model admixture detection algorithm, we inferred high levels of cosmopolitan (non-sub Saharan) admixture in populations from across the African continent. Admixture proportions varied dramatically among samples, even within small geographic regions. Based on the megabase scale of admixture intervals, large-scale introgression appears to be a very recent phenomenon. Admixture proportions also differed starkly within samples, potentially indicating isolation mechanisms within populations. Populations from south of the Congo Basin were then found to have the highest levels of nucleotide diversity, and may represent the ancestral range of the species. Moderate levels of genetic structure were found across the African continent, and populations from central Africa were found to have the closest relationships with cosmopolitan populations. Evidence of adaptive differences between African populations was also apparent.

516C

Patterns of natural variation unravel strong ongoing genomic conflict in *Drosophila mauritiana*. Christian W. Schloetterer, Viola Nolte, Ram Vinay Pandey, Robert Kofler. Inst f Populationsgenetik, Vetmeduni Vienna, Wien.

Drosophila mauritiana, a close relative of *D. melanogaster*, is endemic to a few islands in the Indian ocean. Despite that *D. mauritiana* serves as an important model to understand the genetic basis of speciation processes, its genome sequence is not yet available, and natural variation has been characterized only for a few loci. We generated a draft genome of *D. mauritiana* and characterized the genome-wide polymorphisms by sequencing pooled individuals (Pool-Seq). We resolve the long-debated phylogenetic relationship within the *D. simulans* clade by showing that *D. mauritiana* and *D. simulans* are more closely related while *D. sechellia* is the most diverged species. Consistent with large amounts of shared polymorphism, we find no evidence for a faster X chromosome evolution within the *D. simulans* group, but a very pronounced effect in comparisons involving either *D. melanogaster* or *D. yakuba*. We demonstrate how the well-documented change in recombination landscape in *D. mauritiana* affects the portioning of variation along the chromosomes: regions close to the centromere which exhibit low variation and reduced selection efficacy in *D. melanogaster* show normal polymorphism levels and no enrichment of non-synonymous changes in *D. mauritiana*. Finally, we report the genomic signatures of ongoing genomic conflict in *D. mauritiana*. Two

windows larger than 500 kb around the genes *Dox* and *OdsH* show the signature of (almost) complete selective sweeps. Strikingly, the pattern of genomic conflict is not limited to *Dox* and *OdsH*, but we find an over-representation of nucleoporin genes, which are also involved in genomic conflict, among the most significant genes in genome-wide McDonald-Kreitman tests.

517A

Parallel Latitudinal Differentiation in Drosophila simulans. Alisa Sedghifar, David Begun. Evolution and Ecology, University of California, Davis, Davis, CA.

Patterns of latitudinal differentiation in species experiencing high gene flow provide evidence for spatially varying selection. We have compared genome-wide patterns of differentiation in D. simulans populations from Australia and North America. Genomic regions showing high levels of differentiation on both continents are very likely influenced by spatially varying selection. We describe patterns of continent-level convergent adaptive evolution at the nucleotide, gene and pathway levels and relate these patterns to the selection response to variable environments.

518B

Intraspecific structure of *D. littoralis* Meigen (Diptera: Drosophilidae). Svetlana Y. Sorokina[1], Boris V. Andrianov[2], Denis A. Romanov[2], Prohor A. Proshakov[1], Vladimir G. Mitrofanov[1]. 1) Dept Genetics, Koltsov Inst Dev Biology, Moscow, Russian Federation; 2) Dept Animal Genetics, Vavilov Inst Gen Genetics, Moscow, Russian Federation.

One of the main goals of evolutionary genetics is the study of mechanisms of speciation. In this context, the taxonomic systems that include intermediate stages between undifferentiated populations and reproductively isolated species deserve the special attention. *D. littoralis* Meigen is natural widely distributed palearctic species that forms fragmented, genetically heterogeneic populations in Eurasia from Iran to Northern taiga zone. In our previous study we have developed and used mtDNA markers to characterize the intraspecific polymorphism of *D. littoralis* at inter- and intrapopulation levels. Our data showed that Northern (European) and Southern (Caucasian) groups of populations are differentiated significantly and can be descendants of different refugial populations that diverged independently. In this study we include to the analysis the sample from Abkhazian population (Caucasian group) of *D. littoralis* and estimate its mtDNA polymorphism level. Also we use the nuclear polymorphic marker - LTRs of Tv1 retrotransposone that is specific for virilis group to study the genetic structure of *D. littoralis* species. In addition, we conducted a series of hybridization experiments to detect the signs of reproductive isolation between differentiated populations. Our data together with the data of allozyme (Goncharenko et al., 1989) and inversion (Mitrofanov, Poluektova, 1982) polymorphisms analyses as well as with the data of morphological analysis of male mating organ (Kulikov et al., 2004) allow us to divide *D. littoralis* species to subspecies: *D. littoralis littoralis* Meigen and *D. littoralis imeretensis* Sokolov. The study was supported by Russian Foundation for Basic Research (RFBR) grant 11-04-01630-a and Russian State grant "Gene pools and Genetic Diversity" Russian State grant "Gene pools and Genetic Diversity".

519C

Two types of *cis-trans* compensation in the evolution of transcriptional regulation. Toshiyuki Takano-Shimizu[1,2,3], K. Ryo Takahasi[1,4], Takashi Matsuo[5,6]. 1) Population Genetics, National Institute of Genetics, Mishima, Japan; 2) Department of Genetics, Graduate University for Advanced Studies (SOKENDAI), Mishima, Japan; 3) Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo, Japan; 4) Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Japan; 5) Department of Biological Sciences, Tokyo Metropolitan University, Tokyo, Japan; 6) Department of Agricultural and Environmental Biology, University of Tokyo, Tokyo, Japan.

Because distant species often share similar macromolecules, regulatory mutations are often considered responsible for much of their biological differences. Recently, a large portion of regulatory changes has been attributed to *cis*-regulatory mutations. Here, we examined an alternative possibility that the putative contribution of *cis*-regulatory changes was in fact caused by compensatory action of *cis*- and *trans*-regulatory elements. First, we show by stochastic simulations that compensatory *cis*-*trans* evolution maintains the binding affinity of a transcription factor at a constant level, thereby spuriously exaggerating the contribution of *cis*-regulatory mutations to gene expression divergence. This exaggeration was not observed when changes in the binding affinity were compensated by variable transcription factor concentration. Second, using reciprocal introgressions of Drosophila, we demonstrate that relative expression of heterozygous alleles from two distinct species often varied significantly between different species backgrounds, indicating the possible action of *cis*-*trans* compensation. Taken together, we propose that *cis*-*trans* hybrid incompatibilities are accumulating much faster than generally considered.

520A

Resequencing artificially selected populations to determine the genetic basis of quantitative traits. Thomas L. Turner, Andrew D. Stewart, Paige Miller. Ecology, Evolution, and Marine Biology Department, University of California Santa Barbara.

Genome-wide association studies hold the promise of comprehensive and systematic identification of the genetic basis of natural trait variation. However, good statistical power to identify variants with low population frequencies or modest effects requires sample sizes that are generally prohibitive. As a complimentary approach, we have combined population-based genome resequencing with large scale artificial selection on behavior (courtship song) and morphology (body size) in Drosophila melanogaster. Selection on body size (expected to be a highly polygenic trait) was performed on large populations for over 100 generations, while behavioral selection on courtship song was performed on more modest population sizes for 15 generations. This presentation will compare and contrast the whole-genome resequencing data for the two experiments in order to determine the power and prospects for future studies.

521B

Adaptation to mustard oils in the *Drosophila* radiation: ecological, genetic, biochemical, and metabolomics evidence across a specialization gradient. Andrew Gloss[1], Timothy Rast[1], Rick Lapoint[1], Michael Reichelt[3], Katharina Schramm[3], Daniel Vassao[3], Jonathan Gershenzon[3], Bill Montfort[2], Noah Whiteman[1]. 1) Dept of Ecology and Evolutionary Biology, Univ of Arizona, Tucson, AZ; 2) Dept of Chemistry and Biochemistry, Univ of Arizona, Tucson, AZ; 3) Dept of Biochemistry, Max Planck Institute for Chemical Ecology, Jena, Germany.

Mustard oils, the breakdown products of glucosinolates, are ecologically important defense compounds found in plants in the order Brassicales. Mustard oils (isothiocyanates) deter most generalist herbivores; however, molecular studies have revealed novel detoxification mechanisms in specialists preventing

mustard oil formation. Here, we investigate the mechanism of mustard oil detoxification in two herbivores, *Scaptomyza flava* and *S. nigrita*, nested within the *Drosophila*. Using *Arabidopsis thaliana* mutants deficient in glucosinolate biosynthesis, we show mustard oils induce glutathione-*S*-transferase activity in larvae coupled with formation of isothiocyanate-glutathione conjugates, implicating the general mechanism used to detoxify isothiocyanates and other toxins in many non-herbivorous organisms, including humans. Molecular cloning of Glutathione-*S*-transferase D1 (GSTD1), which catalyzes conjugation of electrophilic molecules to glutathione, reveals gene duplication followed by positive selection in the ancestor of these mustard-specialists. In addition, *S. flava* and *S. nigrita* GSTD1 enzymes conjugate glutathione to isothiocyanates in vitro more efficiently than GSTD1 from other *Drosophila* and *Scaptomyza*; this is, to our knowledge, the first demonstration that a single arthropod GST catalyzes isothiocyanate detoxification. A preliminary crystal structure shows some positively selected substitutions in a potential toxin binding pocket, at sites otherwise highly conserved across *Drosophila*. Together, these results suggest evolution of a canonical detoxification mechanism, rather than generation of a novel one, enabled specialization on a group of well-defended plants in these herbivorous drosophilids.

522C

Identifying 'soft sweeps' in egg size variation by re-sequencing experimentally evolved populations of Drosophila melanogaster. Aashish R. Jha[1,3], Cecelia Miles[2], Cristopher D. Brown[1,3], Kevin P. White[1,2,3], Martin Kreitman[2,3]. 1) Department of Human Genetics, The University of Chicago, Chicago, IL; 2) Department of Ecology and Evolution, The University of Chicago, Chicago, IL; 3) Institute of Genomics and Systems Biology, The University of Chicago, Chicago, IL.

Egg size is a classic quantitative trait that is directly related to fitness of both the parents and offspring. However, genetic factors influencing variation in egg size remain elusive. We undertook an experimental selection experiment in which nine Drosophila melanogaster populations derived from a single panmictic population (based on 120 isofemale lines) were selected for divergent egg volume in three treatment groups: large eggs, small eggs, and control (3 replicate populations per group). Ultra-deep population re-sequencing (~820X total genome coverage) with high power to detect variants segregating at low allele frequencies coupled with a novel method (MQVCCI) developed to detect variations and their frequencies from population re-sequencing data revealed a total of 1. 7M unique genetic variants of which a large proportion was shared between and among the treatment groups. Although the abundance of genetic variation suggests that there are plenty of genetic variations for positive selection to act upon, genomic scans in 100kb windows in all five chromosome arms in all evolved populations failed to identify classic sweeps in any of the populations suggesting that egg size is a complex and highly polygenic trait and adaptation may have occurred due to 'soft sweeps' which causes subtle shifts in frequencies of many alleles across the genome. We tested the hypothesis that soft sweeps are detectable by comparing frequency differences and their directions between the ancestral population and terminal populations. Genetic drift is expected to randomly change allele frequencies in control populations; however, in evolved populations alleles under positive selection will have shifted in the same direction more than expected by random drift. Candidate loci with shifts in frequencies will be further tested for potential functional roles.

523A

Emergence of essential mitotic function in the young gene Umbrea. Benjamin Ross[1], Leah Rosin[2], Danielle Vermaak[1], Mary Alice Hiatt[1], Barbara Mellone[2], Harmit Malik[1,3]. 1) MCB/Basic Sciences Dept, University of Washington/FHCRC, Seattle, WA; 2) MCB Dept, University of Connecticut, Storrs, CT; 3) Howard Hughes Medical Institute.

Genes that encode centromeric and kinetochore proteins are essential, yet can evolve rapidly as a consequence of selfish centromere competition during female meiosis in plants and animals. Nevertheless, their essential function in chromosome segregation restricts their adaptive landscape. We have found that the young gene *Umbrea/HP6* encodes a protein that has acquired centromeric localization and essential mitotic function in Drosophila within 10 million years of its birth. Born by gene duplication from *Heterochromatin Protein 1B (HP1B)*, *Umbrea* neofunctionalized by domain loss, the acquisition of two distinct gain-of-function interactions, and persistent selective pressure, resulting in a dramatic alteration in subcellular localization. While HP1B localized to heterochromatin in S2 cells, Umbrea colocalized with CenH3/Cid at all centromeres. Centromere localization may be important for Umbrea function, since loss of function of Umbrea in null mutants or constitutive knockdown by RNAi during development resulted in pupal lethality. Moreover, knockdown of Umbrea by RNAi in cultured cells induced an increase in chromosome congression defects during metaphase, and lagging chromosomes during anaphase. Despite these results, we found that positively selected residues fall in Umbrea's centromere targeting domains. Selective pressure may act on Umbrea to maintain centromere localization and function, since Umbrea orthologs lost centromeric localization with increased divergence when expressed in *D. melanogaster* cells. Our findings reveal the evolutionary steps underlying the acquisition of mitotic function in *Umbrea*, and suggest that centromere competition can drive not only diversifying selection in existing centromere genes but also essential neofunctionalization.

524B

Genetic basis for DDT resistance associated with CYP6g1 in Drosophila simulans. Julianna Bozler, Todd Schlenke. Emory University, Atlanta, GA.

Transposable elements can be a powerful adaptive force, and have driven the rapid evolution of many phenotypes. Previous studies have linked TE insertions to significant population-wide changes in the expression of metabolic and detoxifying enzymes. One classic example of such a mutation is the Accord transposon inserted in the 5' regulatory region of the DDT-resistance gene CYP6g1 of Drosophila melanogaster. This mutation is associated with constitutive Cyp6g1 over-expression and insecticide resistance. A similar mutation was found in Drosophila simulans, whereby a Doc transposable element inserted in the 5' regulatory region of Cyp6g1 also associated with increased Cyp6g1 expression and insecticide resistance. We have made several reporter constructs to identify the specific Doc gene sequences necessary for Cyp6g1 over-expression. Furthermore, to better understand the functional consequences of the D. simulans Cyp6g1 Doc insertion, we are characterizing the tissue-specific expression pattern of CYP6g1 in Doc+ and Doc- strains.

525C

The Influence of Feeding Rate on Dietary Restriction Treatments in Drosophila. Payal Daya, Mary Durham, Jeff Leips. Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD.

Dietary Restriction, a decrease in nutrient intake without malnutrition, has been shown to increase life span nearly universally among many species, including humans, and is highly linked to feeding behavior. Previous dietary restriction experiments have shown that spiders and other insects regulate and

compensate for the lack of protein in restricted diets by increasing food intake, and may store this excess energy as fat, often leading to obesity. Ongoing experiments in our lab investigate the dietary restriction response of life span and fecundity in Drosophila melanogaster using two different diets: one high in protein content (20% yeast) and one restricted in protein content (5% yeast). In order to substantiate if the observed dietary restriction response from those studies is influenced by differences in feeding behavior or if it is a result of the dietary treatment itself, we measured the feeding rates of several Drosophila genotypes on each dietary treatment. The flies were allowed to feed on radioactively labeled media for 24 hours and the total volume of food ingested by each individual was quantified by measuring isotope levels in a scintillation counter. We also measured the thorax length of each fly as an indication of body size, which was used as a covariate in our statistical analyses. This work provides evidence that the dietary restriction response of life span and fecundity observed in previous studies is largely an effect of the dietary treatment rather than changes in feeding rates of individuals. Flies reared on a high protein diet tend to eat more food than those reared on a restricted diet, so those reared on a restricted diet are clearly not compensating for the decrease in nutrients by eating a greater volume of food. Furthermore, since the genotypes we used in this study are part of a genome reference panel used for genome wide association, we can extend this study to gain a better understanding of how specific genes function and interact to regulate feeding rates and physiological responses to dietary restriction in Drosophila and many other organisms.

526A

Genome-wide association analysis of natural variation in tergite melanization in *Drosophila melanogaster*. Lauren Dembeck[1,2], Michael Magwire[1,2], Faye Lawrence[1], Richard Lyman[1], Trudy Mackay[1,2]. 1) Department of Genetics, North Carolina State University, Raleigh, NC; 2) W.M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC.

Pigmentation varies within and between species and is often an adaptive trait crucial for fitness. *D. melanogaster* females generally have light to medium stripes of melanization on the posterior end of each tergite. We measured natural variation in female abdominal pigmentation in 158 sequenced inbred lines of the *Drosophila* Genetic Reference Panel, derived from the Raleigh, NC population. We visually scored females for the proportion of melanization on tergites 4-6 on a scale from 0 (no melanization) to 5 (total melanization). We found significant genetic variation in melanization for each tergite, with broad sense heritabilities ranging from 0.239 - 0.876. We performed genome-wide association analyses for each tergite using 2.5 million single nucleotide polymorphisms (SNPs). We identified 30 SNPs associated with the proportion of melanization on tergite 6, the most significant of which was in *bric-a-brac 1*, a gene known to affect the proportion of melanization in female *D. melanogaster*. We also identified a SNP in the *cis*-regulatory element of *tan*, which was previously shown to affect interspecific differences in pigmentation. After accounting for linkage disequilibrium and imputing missing genotypes, we conducted a forward regression to estimate the fraction of variance explained. Five SNPs constituting 22 haplotypes account for 52% of the variance among the lines. We are currently conducting studies to further confirm the effects of these SNPs. This study will provide insight into the genetic architecture of melanization and also shed light on the question of whether genes causing variation within a species are the same as those involved in trait divergence between species. L.M.D. is supported by NIH Training Grant # GM045146.

527B

High levels of sex-specific additive genetic variation has strong implications for the heritability of lifespan in *Drosophila melanogaster*. Urban Friberg[1], Anne Lehtovaara[2], Holger Schielzeth[3], Ilona Flis[4]. 1) Ageing Research Group, Evolutionary Biology, Uppsala university, Uppsala, Sweden; 2) Anne.Lehtovaara@gmail.com; 3) Evolutionary Biology, Uppsala university, Uppsala, Sweden; 4) anolis.silf@gmail.com.

Understanding the genetic architecture of lifespan and rate of ageing is important both from the perspectives of evolutionary biology and medicine, as they both are central life history traits and sum over the expression of all genetic variants that contribute to mortal disease. In virtually all taxa, including humans, lifespan and rate of ageing are sexually dimorphic. Sexual dimorphism results from genes that are differentially expression in males and females. As a result the genetic architecture of a sexually dimorphic trait can be very different in the two sexes. Here we investigate the additive genetic architecture (AGA) of lifespan and rate of aging in males and females of *Drosophila melanogaster*. We show that the sexes have distinct AGAs for these traits and that these differ substantially across social environments. About half of the additive genetic variation was sex-specific. The high proportion of sex-specific additive genetic variation had a profound impact on the heritability of lifespan, which became highly dependent on the sex of the parent and the offspring considered. While father-to-son and mother-to-daughter heritabilities were moderate (ranging from 0.25 to 0.40), father-to-daughter and mother-to-son heritabilities were significantly and substantially lower (ranging from 0.10 to 0.12). In sum our results show that the AGA for lifespan and rate of ageing is very complex and depends critically on sex and social environment.

528C

Correlated changes in body melanization and mating success in *Drosophila melanogaster*. Babita Kajla, Ravi Parkash, Vineeta Sharma, Jyoti Chahal, Chanderkala Lambhod. Lab no. 19, Department of Genetics, M. D.UNIVERSITY, ROHTAK, Haryana, India.

Mating speed and copulation duration respond rapidly to laboratory selection in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), but there is a lack of data on the evolutionary response to natural selection in the wild. Further, it is not clear whether body melanization and mating behavior are correlated traits. Accordingly, we tested whether variation in body color impacts on mating latency, copulation duration, and fecundity in latitudinal populations of *D. melanogaster*. We observed geographical variation (cline) for mating propensity, i.e., mating speed as well as copulation duration increased along latitude. Phenotypic plastic responses for body melanization at 17 and 25 ° C also showed significant correlations with mating latency and copulation duration. Within population analysis based on assorted dark and light flies of five geographical populations showed significant positive correlations of copulation duration and fecundity with body melanization. To assess the role of males and/ or females on mating speed and copulation duration, we used atypical body color strains (i.e., dark and light males of *D. melanogaster*) for no-choice mating tests. Our data showed major influence of males for copulation duration and of females for mating speed. Furthermore, a difference in impact of body melanization on mating speed and copulation duration was demonstrated between species, i.e., low melanization in *Drosophila ananassae* Doleschall is correlated with lower mating speed and shorter copulation duration than in *D. melanogaster*. Geographical changes in mating propensity were significantly correlated with body melanization at three levels, i.e., within and between populations and between species. Thus, we have shown that a relationship exists between body melanization and mating success. Further, we found seasonal changes in temperature and humidity to confer selection pressures on mating-related traits.

529A

Pleiotropy effects of *Syndecan* on innate immune responses and life span of *Drosophila melanogaster*. Chia-Hua Lue[1], Maria De Luca[2], Jeff Leips[1]. 1) Biological Sciences, University of Maryland Baltimore County, Baltimore, MD; 2) Department of Nutrition Sciences, University of Alabama at Birmingham.

Life history theory is based on the premise that the competing energetic demands of growth, development, somatic maintenance, reproduction and storage give rise to trade-offs among traits. Genes controlling such trade-offs are expected to play an important role in life history evolution. In a previous mapping study we identified the gene *Syndecan* as candidate gene influencing natural variation in lipid storage in *Dorosphila melangoster*. Follow up studies showed that tissue specific alterations of the expression of *Syndecan* (in the fat body and brain) caused changes in a number of traits including metabolism, and sleep wake patterns. Given the influence of *Syndecan* on metabolism and lipid storage and known correlations between these traits with life span and immunity, here we explored the pleiotropic effects of *Syndecan* expression on these traits. We used the GAL4/UAS system in *Drosophila* to knockdown expression of *Syndecan* in the fat body and brain tissue then measured life span and age - specific immune responses in male and female virgin flies. For the immune response assay we measured the ability of flies to clear an *E. coli* infection at one, three, and five weeks of age. Our results indicated that the population with knockdown *Syndecan* gene in fat body tissue had poorer innate immune response and shorter life span than control population. The innate immune response displayed especially significant difference at early ages in both sexes. However, knockdown of *Syndecan* expression in central nervous system tissue caused a significant improvement of bacterial clearance capability and extended life span relative to that of control flies. Thus, variation in the expression of *Syndecan* has extensive pleiotropic effects with different consequences for life span and immune response depending on the tissue it is expressed in. We are currently doing studies to understand underlying mechanism of these pleiotropic effects.

530B

Naturally Occurring Mutational Variation in Sleep Traits in *Drosphila melanogaster*. Rachel A Lyman[1], Trudy F C Mackay[2,3], Mary Anna Carbone[2,3], Susan T Harbison[2,3], Matthew Jones-Rhoades[1], Richard F Lyman[2,3]. 1) Dept of Biology, Knox College, Galesburg, IL; 2) Dept of Genetics, NCSU, Raleigh, NC; 3) W M Keck Center for Behavioral Biology, NCSU, Raleigh, NC.

To provide a better understanding of spontaneous mutation rates and the types and locations of spontaneous mutations, we constructed a new set of 25 mutation accumulation (MA) lines from an inbred line of the *Drosophila* Genetic Reference Panel. The MA lines were maintained in small mass matings of 10 males and females per generation at a standard population density with discrete generations. We measured MA lines from generation 60, and subsequent generations, for behavioral (sleep, startle response, productivity), morphological (bristle number), physiological (body mass, gene expression) and a multigeneration competitive fitness assay. We found significant mutational variation for sleep traits. We performed analyses of genome wide variation in gene expression using Affymetrix GeneChip Drosophila Genome 2.0 Arrays to identify differentially expressed genes. We then determined for which loci variation in gene expression was associated with sleep traits to identify candidate *de novo* mutations associated sleep phenotypes.

531C

A Surprisingly Complex Genetic Architecture for Starvation Resistance Revealed by Multiple QTL Mapping Designs. Casey McNeil, Clint Bain, Stuart Macdonald. Molecular Biosciences, University of Kansas, Lawrence, KS.

In nature, animals must often survive periods of nutrient deprivation, and the ability to withstand starvation an important life-history trait. There is substantial genetic variation for starvation resistance in *Drosophila*, and several candidate genes related to nutrient acquisition, storage, and metabolism have been identified. To validate these genes, identify novel loci, and generate robust estimates of the effects and frequencies of causative alleles, we genetically dissected starvation resistance using the *Drosophila* Synthetic Population Resource (DSPR). The DSPR consists of recombinant inbred lines (RILs) derived from two highly recombinant eight-way synthetic populations, allowing for high-resolution mapping of QTL (quantitative trait loci). Assaying >1700 homozygous RILs we map 20, typically sex-specific QTL to small intervals (<0.5Mb), containing a median number of 68 genes. Individual QTL explain 3.5-13.3% of the phenotypic variation, and half are rare -- the minor allele is unique to a single founder line. To further characterize these starvation resistance QTL and potentially identify novel, context-dependent loci, we dissected the trait using ~2500 heterozygous genotypes over three additional mapping designs. First, pairs of RILs were intercrossed and heterozygous female progeny (RIX design) were assayed. Second, we separately crossed RILs to two isogenic reference strains, and assayed *trans*-heterozygous female progeny (backcross design). In the RIX design we confirm 3/5 QTL originally mapped in homozygous females, implying the two unconfirmed QTL may be products of inbreeding depression. In addition, we identify multiple novel, cross-specific QTL. Surprisingly, each backcross mapping design results in a genetic architecture distinct from that observed in any other design, suggesting a strong role for genetic background effects. Our work reveals an unexpectedly complex genetic architecture underlying an important life-history trait, and the fine-scale nature of mapped QTL advances our efforts to identify the causative sites.

532A

The impact of artificial selection for the wing shape on fluctuating asymmetry in four Drosophila species. Bianca F. Menezes, Blanche Bitner-Mathé. UFRJ, Rio de Janeiro, Brazil.

Fluctuating asymmetry (FA), the unassigned difference between the two sides of a bilaterally symmetrical trait, has been proposed as a useful tool for estimating changes in developmental instability and quantification of the degree of environmental and genetic stress that individuals experience during their development. Recent approaches have used left-right (L-R) variations in the size and shape of the Drosophila wings to describe levels of FA in natural populations under stress conditions. Whether artificial selection for the wing shape can increases FA remains an open question. In our laboratory, replicate lines were obtained by artificial selection for rounded or elongated wing shapes from natural populations of D. melanogaster, D. willistoni, D. hydei and D. mediopunctata. We used these lines to compare intra- and interspecific patterns of FA in wing morphology and whether there is difference between the long and rounded lines. All the wing traits were affected in different magnitudes within and among species. We detected significant intra- and interspecific differences in levels of FA in wing size and wing length. Only D. willistoni demonstrated fluctuating asymmetry on wing shape due to a possible antisymmetry, which might be correlated. Interspecific results presented significant levels of FA in all wing size traits among the rounded lines, including wing width and wing shape. Our results suggest that, under the same selection pressure, natural adaptation to artificial selection for the wing shape varies among species due to selection direction.

533B

Extending Association-Mapping: Genomics meets Phenomics. William R Pitchers[1], Eladio Marquez[2], Jessica Nye[2], David Houle[2], Ian Dworkin[1]. 1) Zoology, Michigan State University, East Lansing, MI; 2) Department of Biological Science Florida State University Tallahassee, FL 32306.

Genome-Wide Association (GWA) is currently a much-used methodology for mapping phenotype to genotype. Unfortunately, in most cases association mapping is carried out for a univariate metric of the trait of interest. Phenotypes are inherently multivariate, since traits are not expressed in isolation. We have shown that for our trait of interest - the shape of the *Drosophila melanogaster* wing - tests using univariate (or even low-dimensionality multivariate) approximations have a low resolution and can fail to detect interesting effects. We have therefore extended the toolkit of GWA to accommodate high-dimensionality morphometric data. It is also a feature of association analyses that their findings are specific to the population in which the mapping was performed, and associations are frequently found either not to generalise to other populations, or to explain a much smaller fraction of the trait variance when they do. In order to improve on this, we have independently replicated measures of all phenotypes, allowing us to model environmental variance in addition to genetic variance and map loci that are more likely to be of general importance. In combination, these new approaches make GWA a more powerful method for studying the genetics of complex traits.

534C

Body melanisation plasticity in generalist, cold and warm adapted *Drosophila* species. SEEMA RAMNIWAS, Ravi Parkash, Chanderkala Lambhod, Babita Kajla. Lab No. 19, Deptt of GENETICS, M. D.UNIVERSITY, ROHTAK, Rohtak, HARYANA, India.

Ectothermic drosophilids are profoundly affected by thermal selection (i.e., genetic effects) or through induced effects on phenotype (i.e., plasticity). Phenotypic plasticity is a powerful means of adaptation in diverse organisms but has received less attention for different drosophilids. We analyzed reaction norms of melanisation in *Drosophila* species which differ in developmental thermal range and geographical distribution. *D. ananassae* and *D. jambulina* are cold sensitive, and these species can be cultured between 18 to 32°C. By contrast, D. nepalensis is cold- tolerant and heat- sensitive species which can be raised between 12 and 25°C. The cosmopolitan species *D. melanogaster* has a broader thermal range (13 - -31°C). Significant differences were observed between reaction norms of melanisation in three anterior vs. three posterior abdominal segments in these species. In *D. nepalensis*, all the six abdominal segments (2nd -- 7th) are highly plastic. However, only the last three abdominal segments are plastic in D. melanogaster. In contrast, *D. ananassae* (a tropical species) lacks plasticity for all abdominal segments. Cosmopolitan species (*D. melanogaster*), even from much colder climates does not show darker phenotypes similar to that observed in *D. nepalensis*. The aim of this study is to understand the processes involved in generating the morphological diversity of color patterns and adaptation of *Drosophila* species to different geographical regions. Finally, comparing body melanisation patterns to phylogeny suggests recurrent adaptations for genetic polymorphism vs. phenotypic plasticity in different evolutionary lineages.

535A

Phenotypic McDonald-Kreitman tests of mitochondrial genotype effects on nuclear gene expression. David M. Rand, Patrick A. Flight, Nicholas Jourjine, Lei Zhu. Ecology & Evolutionary Biol, Brown Univ, Providence, RI.

Associating molecular genotypes with quantitative phenotypes is a general problem in quantitative and evolutionary genetics. Traditional association tests often lack a predictive model of the degree of divergence between DNA sequence variants and the level of phenotypic difference. We describe a phenotypic McDonald-Kreitman (MK) test of nuclear gene expression driven by alternative mitochondrial genotypes that scales trait variation based on level of sequence divergence. Four mitochondrial genotypes were introgressed in to a common OreR nuclear genetic background using balancer chromosomes: Dmel OreR, Dmel Zimbabwe, Dsim siI and Dsim siII. This 4-taxon sample of mtDNAs provides the minimum set of haplotypes needed for an MK test in each species. We extend the traditional 2x2 MK design of silent and replacement polymorphism and divergence to a 2x3 table that includes a row for intraspecific variation (Vp) and interspecific divergence (Dp) in nuclear gene expression values based on microarray data from each introgression strain carrying alternative mtDNAs. As reported in an earlier study of life history traits, the majority of nuclear genes show more variation in gene expression among mtDNA haplotypes within species than variation among the Dmel or Dsim mtDNAs. This pattern in consistent with ‘excess’ amino acid polymorphism for mtDNA in traditional MK tests, interpreted as evidence for selection against mildly deleterious variation in mtDNA. The expression profile data were also collected under varying levels of hypoxia to test the hypothesis that stressful conditions would shift the mtDNA effect from within- to between-species. In general this result was observed, as the number of phenotypic MK tests for nuclear gene expression showing ‘excess’ intraspecific variation was reduced under stressful hypoxic conditions. These analyses demonstrate an important retrograde signaling process between mitochondria and nuclear genes, and provide a simple means of making predictive tests of phenotype-genotype associations.

536B

Comparative analysis of sex-specific pigmentation identifies novel genes involved in phenotypic evolution. Sarah A. Signor, Arytom Kopp. University of California, Davis, Davis, CA.

The origin of diversity is one of the most central questions in evolutionary biology. In recent years the molecular changes responsible for the origin of some phenotypic differences has been identified. These studies typically proceed on a case-by-case basis making it difficult to infer the general rules and patterns of evolution. A systematic search for these patterns necessitates a comparative approach where multiple instances of phenotypic evolution can be examined in closely related species. Within the *Drosophila ananassae* subgroup there have been several independent changes in sexually dimorphic pigmentation. I am investigating the molecular basis of this color pattern evolution in three pairs of (sub)species using a combination of high throughput sequencing and genotyping assays. QTL analysis shows that the number of loci involved is moderate - ranging from two to four in different taxa. I find that sex-specific pigmentation is controlled by overlapping but clearly distinct sets of genes in different species. This genetic toolkit includes at least one previously unidentified member of the pigmentation pathway. The redundant structure of the pigmentation pathway appears to limit the role of constraint in its evolution and facilitate rapid change.

537C

Tipping the Scales: Artificial Selection on the Slope of a Wing Size-Body Size Scaling Relationship in *Drosophila*. R. Craig Stillwell[1], Alexander W.

Shingleton[1], Ian Dworkin[1], W. Anthony Frankino[2]. 1) Department of Zoology, Michigan State University, East Lansing, MI; 2) Department of Biology and Biochemistry, University of Houston, Houston, TX.

The scaling of body parts with body size is a fundamental aspect of biological form and function. The relationship between trait size and body size tends to be tightly correlated within species, and this scaling relationship can typically be described with a simple linear equation. Evolutionary theory predicts that such strong patterns of covariation should hinder the evolution of scaling relationships. However, the slopes and intercepts of these linear scaling relationships vary considerably among species, generating substantial morphological diversity. Consequently, it is unclear how selection acts on tightly correlated traits within a species to generate the changes in scaling relationships that we see among species. Here we use a novel artificial selection regime in an effort to alter the slope of the scaling relationship between wing size and body size in *Drosophila melanogaster* to determine whether the slope of a morphological scaling relationship can be modified by selection easily. After 15 generations of selection, we found that hyperallometric-selected (selected for increased slope) lineages had average slopes (across replicates and sexes) that were approximately 11% steeper than hypoallometric-selected (selected for decreased slope) lineages. Intriguingly, much of this response was due to a change of slopes in males; males from hyperallometric-selected lineages increased 15% in the slope compared to hypoallometric-selected lineages, while females from the same lineages increased only 7%. However, this difference was generated largely by the response of the hyperallometric-selected lineages; the hypoallometric-selected lineages were similar to the control lineages. Our data indicate that despite strong pattern of covariation between the size of the wing and body, changing the slope of the scaling relationship is possible.

538A

Polymorphisms Associated with Natural Variation in Olfactory Behavior in Drosophila melanogaster. Shilpa Swarup[1,3], Trudy F.C. Mackay[1,3], Robert R.H. Anholt[1,2,3]. 1) Department of Genetics,; 2) Department of Biology,; 3) W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC.

Natural variation in chemosensation provides a substrate for adaptive evolution. To identify polymorphisms associated with natural variation in olfactory behavior, we measured responses to a discriminating concentration of a standard odorant, benzaldehyde, in 168 inbred wild-derived lines with fully sequenced genomes of the Drosophila Genetic Reference Panel. We observed substantial variation in the behavioral responses of the DGRP lines. Genome-wide association (GWA) analysis identified 306 SNPs in 106 genes at a nominal P-value of 10-5. Further analysis revealed a preponderance of rare alleles with large effects. To verify causality of these alleles with phenotypic variation, we adopted an extreme QTL (xQTL) mapping strategy. We generated reciprocal advanced intercross line (AIL) populations derived from crosses between two DGRP lines with highest and lowest olfactory responses to benzaldehyde. We phenotyped 4000 flies from the AIL populations and performed massive parallel sequencing on pooled DNA samples collected from the top 10% and bottom 10% individuals. We used BWA and SAM tools to align genomic sequences and compare allele frequencies between the DNA pools. Combining information from GWA analysis and xQTL mapping can unambiguously identify polymorphisms causally associated with natural variation in olfactory behavior. Supported by NIH grant GM059469.

539B

The genetic architecture of diet-dependent immune defense in *Drosophila*. Robert L. Unckless[1], Susan M. Rottschaefer[1], Pavel Korniliev[2], Chloe Ota[1], Illana Porges[3], Jason G. Mezey[2], Brian P. Lazzaro[1]. 1) Department of Entomology, Cornell University, Ithaca, NY; 2) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 3) Jericho High School, Jericho, NY.

Dietary nutrition has profound impact on many traits, including the ability to resist and tolerate pathogenic infection. Understanding the role of nutrition in infection and disease is important in health contexts. Influences of diet and other environmental factors can additionally complicate the evolution of immune-related traits in natural systems. In the present work, we show that increasing dietary sugar results in dose-dependent increase in *D. melanogaster* susceptibility to chronic, but not acute, bacterial infection. We assayed defense against infection by a bacterial pathogen, *Providencia rettgeri*, in the *Drosophila* Genetic Reference Panel (DGRP), a collection of lines derived from a natural population whose genomes have been completely sequenced, after rearing on both high-sugar and low-sugar diets. We find considerable genetic polymorphism for defense on both diets, as well as a significant genotype-by-diet interaction that reveals a subset of lines whose defense becomes disproportionately poor on the high-sugar diet. We use genome-wide association mapping to preliminarily implicate genes underlying variation in defense on each diet, and test several organism-level metabolic indices for correlation with the defense phenotype.

540C

Introgression of Nuclear-Encoded Mitochondrial Proteins in *Drosophila yakuba* and *D. santomea*. Emily Beck[1], Aaron C Thompson[2], Joel Sharbrough[2,3], Ana Llopart[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) The Biosciences Graduate Program, University of Iowa, Iowa City, IA.

Introgression is the exchange of genetic information between different species through natural hybridization. Once viewed as a fortuitous *accident* due to incomplete reproductive isolation between species, it has become increasingly clear that introgression can potentially lead to ecological divergence, crop improvement or even invasiveness. Recent molecular genetic studies in plants indicate that introgression may allow populations to regain traits that have been lost by replacing the damaged alleles with functional copies from a closely related species. Novelty can also arise from introgression. Unique genetic combinations that result from the stable acquisition of genetic material from another species can produce new phenotypes and serve as source for novel adaptations. Hybrid zones, areas where two distinct species meet and hybridize, provide biologists with “natural experiments” and constitute ideal settings to study introgression. Unfortunately there is a dearth of hybrid zones. In 2000, however, a new unique hybrid zone formed by two species in the *melanogaster* subgroup, *D. yakuba* and *D. santomea*, was discovered in a small African island of the Gulf of Guinea. Previous studies in our lab showed that the mitochondrial genome of the former species had introgressed into the latter and replaced completely the native form. Since mitochondrial DNA products work intimately with nuclear DNA products in the oxidative phosphorylation pathway that takes place in mitochondria, we hypothesize that some nuclear genes in OXPHOS co-introgressed along with the mitochondrial genome. To test this hypothesis we have sequenced 12 genes of the OXPHOS pathway in a total of 33 *Drosophila* lines. Our preliminary results suggest that co-introgression has indeed occurred and support the idea of co-evolution between both mitochondrial and nuclear genomes.

Poster Full Abstracts - Evolution and Quantitative Genetics

Poster board number is above title. The first author is the presenter

541A

Screens to identify novel hybrid incompatibility genes in *D. melanogaster/D. simulans* interspecific hybrids. Tawny Cuykendall, Daniel Barbash. Molecular Biology & Genetics, Cornell University, Ithaca, NY.

Interspecific hybrids between *D. melanogaster* females and *D. simulans* males are characterized by F1 hybrid male lethality. 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)*. Both *Hmr* and *Lhr* were discovered in screens of natural populations for suppressors of lethality. There has been no systematic search for hybrid incompatibility (HI) genes similar to *Hmr* and *Lhr* in either the *D. melanogaster* or the *D. simulans* genomes. Two lines of evidence suggest that additional factors contribute to this lethal interaction: 1) expression of *D. simulans Lhr* in a *D. melanogaster* background is not lethal and 2) crosses between triploid *D. melanogaster* females and irradiated *D. simulans* males implicate the *D. melanogaster* X containing *Hmr*, the *D. simulans* 2nd chromosome containing *Lhr*, and also the *D. simulans* 3rd chromosome in lethality. Our goal is to uncover novel HI genes hidden in the genomes of these two species.

We are using the Bloomington Deficiency kit to screen for hybrid male rescue to identify regions in *D. melanogaster* which contribute to lethality. To date we have screened ~80% of the autosomal genome and found only one region that weakly suppresses lethality. This result suggests that there is not a large number of major effect HI genes in the *D. melanogaster* genome. In order to identify HI genes in *D. simulans*, we are performing an EMS mutagenesis screen. All live hybrid males are pre-screened for *Lhr* to verify rescue is due to a mutation in a novel HI gene. The males will then be subjected to whole genome sequencing to identify the candidate rescuing mutation. The putative HI gene will be verified by exploiting the RNAi tools available in *D. melanogaster* to specifically knock down the *D. simulans* ortholog in hybrids.

542B

Dissecting behavioral isolation in nature: Evolution of mate choice in the closely related species *Drosophila subquinaria* and *D. recens*. Kelly A. Dyer[1], Erin Giglio[1], Jacqueline Sztepanacz[2], Brooke E White[1], Emily R Peeden[1], Howard D Rundle[2]. 1) Dept Genetics, Univ Georgia, Athens, GA; 2) Dept Biology, Univ Ottawa, Ottawa, Canada.

The divergence among populations in male signal traits and female preferences may be an important source of behavioral isolation during the speciation process. Here we investigate the traits involved during mate choice within, and behavioral isolation between, the closely related species *D. subquinaria* and *D. recens*. In sympatric populations, *D. subquinaria* females discriminate strongly against both *D. recens* males as well as allopatric conspecific *D. subquinaria* males, consistent with a pattern of reinforcing selection to avoid mating with *D. recens*. Our long-term goal is to understand the mechanism(s) by which the male signal traits and female preferences diverge in nature to cause these changes in mate discrimination and ultimately reproductive isolation. To this end, we will present evidence that chemical forms of communication, namely male contact pheromones that consist of cuticular hydrocarbons (CHCs), are an important form of communication in these species, and likely form the basis upon which females choose among conspecific mates and discriminate against males of the opposite species.

543C

Testing the potential role of small RNAs in satellite DNA-based hybrid incompatibility between Drosophila melanogaster and D. simulans. Karina E. Gomez, Patrick M. Ferree. W.M. Keck Science Department, Claremont McKenna College, 925 N. Mills Ave, Claremont, CA 91711.

A central goal in speciation biology is identifying specific loci that cause post-zygotic reproductive isolation between species and understanding how these loci operate at the cellular and molecular levels in hybrids. Previous work has demonstrated that a large block of D. melanogaster X-linked satellite DNA kills hybrid female progeny produced from D. melanogaster fathers and D. simulans mothers. Unique to the D. melanogaster species, the 359-bp satellite block exhibits stretching defects in female hybrids that lead to X chromosome bridges and early embryonic death. We have proposed that these defects are due to mis-packaging of the 359-bp satellite DNA into heterochromatin by factors in the D. simulans maternal cytoplasm. Previous work in S. pombe and other model organisms suggest that small RNAs complementary to repetitive sequences are involved in their heterochromatic packaging. Based on this idea, 359-bp DNA mis-packaging in hybrid female embryos may result from a lack of 359-bp-derived small RNAs in the D. simulans cytotype. In order to test this specific model of hybrid incompatibility, we have made a set of transgenic Valium-22-derived constructs that can express small RNAs complementary to the 359-bp satellite. Our long-term goal is to place these transgenes into D. simulans in order to determine if 359-bp small RNAs expressed through the siRNA pathway can feed into the piRNA pathway and affect heterochromatin formation of the 359-bp satellite block in hybrids. As a step toward this goal, we have introduced several of these constructs into the D. melanogaster pure species. We are currently testing if overexpression of these small RNAs affects normal 359-bp packaging in D melanogaster.

544A

The effect of the X chromosome on regulation of gene expression in hybrids between *Drosophila yakuba* and *D. santomea*. Ana Llopart, Evgeny Brud, Emily Beck. Dept Biol, Univ Iowa, Iowa City, IA.

In interspecific crosses the *X* chromosome shows a disproportionately large effect on hybrid fitness, an effect known as 'Coyne's rule'. The faster-*X* hypothesis, proposed by Charlesworth and colleagues, posits that this large *X*-effect can be explained, at least partially, by *X*-linked genes showing a higher fixation rate of recessive favorable mutations than autosomal genes because they are hemizygous in the heterogametic sex. To determine the effects of the *X* chromosome on transcriptional regulation in hybrids we studied whole-genome patterns of gene expression in *Drosophila yakuba*, *D. santomea* and their hybrids using a combination of microarrays and mRNA-seq. We analyzed patterns of expression in female-, male- and nonsex-biased genes and took advantage of attached-*X* stocks to uncover the effects of *X*-linked recessive mutations in hybrid females. Our results provide new insight into the molecular basis of the Coyne's rule applied to gene expression.

545B

Design and construction of a new Drosophila species, D.synthetica, by synthetic regulatory evolution. Eduardo Moreno. Cell Biology, University of Bern, Bern.

Synthetic biology is an area of biological research that combines science and engineering. Here, I merge the principles of synthetic biology and regulatory evolution to create a new species with a minimal set of known elements. Using preexisting transgenes and recessive mutations of Drosophila melanogaster, a

transgenic population arises with small eyes and a different venation pattern that fulfills the criteria of a new species according to Mayr's “Biological Species Concept”. The genetic circuit entails the loss of a non-essential transcription factor and the introduction of cryptic enhancers. Subsequent activation of those enhancers causes hybrid lethality. The transition from “transgenic organisms” towards “synthetic species”, such as Drosophila synthetica, constitutes a safety mechanism to avoid hybridization and competition with wild type populations and preserve natural biodiversity. Drosophila synthetica is the first transgenic organism that cannot hybridize with the original wild type population but remains fertile when crossed with other identical transgenic animals.

546C

The Misshapen kinase negatively regulates integrins to promote follicle cell migration during egg chamber development. Lindsay K. Lewellyn, Sally Horne-Badovinac. Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Collective cell migration is critical to the proper formation of tissues and organs during development. These complex cell movements are orchestrated by the actin cytoskeleton and its dynamic interaction with the extracellular matrix (ECM) through transmembrane receptors such as the integrins. The egg chamber is a simple organ-like structure within the Drosophila ovary that is composed of a single oocyte and 15 nurse cells, surrounded by an epithelial layer of follicle cells. This epithelium undergoes a collective migration perpendicular to the egg chamber's A-P axis, an event that helps to transform this initially spherical structure into an elongated egg. Through a forward genetic screen, we have identified the Ste20-like kinase Misshapen (Msn) as a key regulator of egg chamber elongation. Live imaging revealed that loss of Msn leads to a cell autonomous defect in follicle cell migration. *msn* mutant cells show higher levels of integrins at the basal surface, and a disruption of ECM structure. Instead of being polarized around the circumference of the egg chamber, an arrangement that is believed to limit growth to the A-P axis, Collagen IV filaments are stuck around the edges of *msn* mutant cells. These observations suggest that the mutant cells are more tightly adhered to the underlying ECM, and that Msn negatively regulates integrin-based adhesion. Consistent with this model, reducing integrin levels by introducing one *myospheroid* mutant allele, partially rescues follicle cell migration and egg chamber shape in the *msn-RNAi* condition. Finally, observation of a Msn-YFP protein trap revealed that Msn is planar polarized at the basal surface, where it is enriched at the back of the migrating follicle cells. From these data, we propose that Msn negatively regulates integrins at the back of each follicle cell to promote collective cell migration.

547A

MIPP regulates tracheal morphogenesis through cell intercalation. Yim Ling Cheng, Deborah Andrew. Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD.

The *Drosophila* trachea is among the best model systems for studying tubular morphogenesis, an important process in the development of many organs. The major transcription factor regulating trachea formation is Trachealess (Trh), a basic HLH protein expressed in trachea from early development. We identified *mipp1* as one of the downstream targets of Trh in a global *in situ* hybridization screen. *mipp1* encodes a dual substrate specificity multiple inositol polyphosphate phosphatase that can dephosphorylate higher inositol polyphosphates to the Ca++ second messenger IP3 and dephosphorylate 2,3-bisphosphoglycerate to 2-phosphoglycerate. Although the MIPPs are very highly conserved, their biological function remains poorly understood. *Drosophila* encodes two *mipp* genes: *mipp1* and *mipp2*. To learn the role of *mipp1* and *mipp2* in tracheal development, we generated a knockout of *mipp1* by homologous recombination and obtained available *mipp2* mutant lines. Double *mipp1 mipp2* mutants have defects in dorsal trunk elongation, dorsal branch fusion and ganglionic branch migration that are more severe than observed with either single *mipp* mutant. *mipp2* is ubiquitously expressed, but *mipp1* is expressed specifically in the trachea. Initially, mipp1 is expressed in all tracheal cells but is downregulated by stage 14 in tracheal cells that do not undergo the process of cell intercalation to elongate (dorsal trunk and transverse connective). Based on the *mipp1* expression pattern, we speculated that the MIPPs are involved in promoting tracheal cell intercalation, which would explain the wide range of tracheal defects observed in the mipp mutants. Indeed, we observed both delays and failures in tracheal intercalation in $mipp1^{KO}$. To further characterize the roles of MIPPs and their substrates in cell intercalation, we are asking if the tracheal phenotypes can be rescued by enzyme-dead MIPP1 as well as the wild-type MIPP1, if *mipp1* is regulated by Spalt, the major negative regulator of cell intercalation in the trachea, and if the MIPPs affect the proteins known to be involved in tracheal cell intercalation.

548B

Tracheal Development in *Drosophila* Visual System. Wei-Chen Chu[1,2], Yuan-Ming Lee[1,3], Yi Henry Sun[1,2,3]. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Graduate Institute of Life Sciences, National Defense Medical Center, Taipei,Taiwan; 3) Department of Life Sciences and Institute of Genome Sciences, National Yang Ming University, Taipei, Taiwan.

Drosophila eye is a highly specialized neuronal system, and its neuronal activity should require lots of oxygen. The oxygen can be transported by trachea in insects. However, the distribution and development process of trachea in the *Drosophila* visual system have not been studied. To address this issue, we study the adult tracheal pattern, tracheal development process and its biological role in eye. These may provide a link between visual system and respiratory system. We have determined the retinal tracheal pattern and established the 3D model. And we also found the critical developmental stage for the trachea in *Drosophila* visual system. It has been shown that branching morphogenesis of the *Drosophila* tracheal system in embryo depends on the FGFR/FGF signaling pathway. Tracheal cells specifically express the FGFR (Breathless, Btl) that receives the FGF ligand Branchless (Bnl) signal. Bnl acts as a guidance molecule controlling tracheal cell migration. We have also found that *btl/bnl* signaling affected tracheal growth in eye. The flies with less retinal trachea can be created by manipulation of *btl* in trachea or *bnl* in eye. These flies show weaker ERG amplitude and age-dependent degeneration. It suggests that the trachea in the *Drosophila* eye is important for normal eye function. We speculate that as the eye grows in size, hypoxia will develop and induce tracheal ingrowth. This system may be used to study the role of hypoxia response in eye development, perhaps similar to mammalian angiogenesis.

549C

The role of Cad99C in 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 nine USH disease genes have been identified so far, most of which are highly conserved from flies to humans. Cadherin99C (Cad99C), the Drosophila orthologue of human Usher Cadherin PCDH15, regulates the length of microvilli in ovarian follicle cells. Cad99C is also strongly expressed in embryonic tubular organs including the salivary gland (SG) and trachea. The apical membranes of these tissues undergo dynamic changes during tube morphogenesis, suggesting a potential role for Cad99C in apical membrane dynamics. Although zygotic loss of *Cad99C* does not result in overt SG defects (perhaps because of maternal supplies), high-level expression of Cad99C results in profound changes in the polarity and shape of SG epithelial cells. Cad99C overexpression in the SG results in mislocalization of apical-basal markers such as SAS and α-Spec, and the epithelial cells become round, rather than maintaining their typical columnar shape. Apical actin and tubulin are also disorganized and, within each cell, is a small region of Cad99C staining of what appear to be elongated microvilli. Loss of *moe*, the single Drosophila member of Ezrin/Radixin/Moesin family of actin-binding proteins suppresses the Cad99C overexpression phenotype, suggesting a link between the actin cytoskeleton and Cad99C activity. We are currently exploring phenotypes associated with both maternal and zygotic loss of *Cad99C*

and examining the phenotypes of both loss and overexpression at the ultra-structural level. By learning how the USH genes function during the formation of the relatively simple Drosophila SG and trachea, we expect to gain important insight into how the USH genes function in human development and disease.

550A

A gradient of the transcription factor Cut programs patterning and growth of *Drosophila* airways. Chrysoula Pitsouli[1], Norbert Perrimon[1,2]. 1) Dept Gen, Harvard Med Sch, Boston, MA; 2) HHMI.

A fundamental question in developmental biology is how tissue growth and tissue patterning are coordinately regulated to generate complex organs with characteristic shapes and sizes. Evolutionarily conserved molecules control either growth or patterning, but few have been shown to coordinate both processes during organogenesis and their modes of action remain largely unclear. Here we show that in the developing primordium of the *Drosophila* adult trachea, the homeobox transcription factor Cut regulates both growth and patterning depending on its absolute expression level. We show that Cut is expressed in a gradient, the low and high expression domain of which defines the zones of highest and lowest proliferation, respectively, while absence of Cut expression enables the patterning of airway progenitors. The difference in Cut levels orchestrates distinct transcriptional expression programs necessary for patterning or growth. Furthermore, we show that the Cut gradient is established by the positive and negative actions of the Wnt/Wingless (Wg) and Notch signaling pathways respectively, which are activated in domains that overlap with the zone of highest cell proliferation. Thus, our study identifies the transcription factor Cut as a target of the Wg and Notch signals and highlights the importance of its graded expression in the transcriptional programming of patterning versus growth in a developing epithelial structure.

551B

The dynein motor complex and Whacked RabGAP/Rab35 regulate seamless tube morphogenesis in *Drosophila* terminal cells. Jodi Schottenfeld, Amin Ghabrial. Cell & Dev Biol, Univ Pennsylvania, Philadelphia, PA.

The *Drosophila* tracheal system is composed of a network of tubes that forms by specialized tip cells leading the migration of new branches and mediating their interconnection. Some of these tip cells become specialized “terminal cells” that go on to form branched seamless tubes, unicellular tubes that lack epithelial junctions, that ramify extensively on target tissues. In the mammalian vascular system, endothelial cells form seamless tubes during angiogenesis. Very little is known about the apical-basal properties of seamless tubes and the genes required to establish and maintain this polarity. We investigated terminal cell polarity and found that the lumenal membrane is “apical,” since puncta of the definitive apical membrane marker, Crumbs, decorate the seamless tube. Actin:GFP outlines this apical membrane and also highlights elaborate filopodia at the tips of growing branches. Microtubules also show striking polarity in seamless tubes: γ-tubulin, a primary component of the microtubule organizing center, lines the lumenal membrane and is enriched at the tube tip. Since γ-tubulin marks the site of microtubule nucleation, minus-ends of terminal cell microtubules are directed towards the lumen, thus leading us to hypothesize that minus-end directed transport along microtubules is required for tube formation. Stable microtubules appear in parallel arrays to the tube, with thickened microtubule bundles extending past the tube tip, likely laying the foundation for future membrane addition. In support of this hypothesis, terminal cells mutant for components of the dynein motor complex (*Dhc64c, dlic, GP^{150}*, and *lis-1*) generate extensions that fail to form seamless tubes. In the absence of tubes, microtubule organization remained intact, indicating a direct role for minus-end directed microtubule transport in seamless tube formation. In addition, we have identified the Rab35 GAP, *whacked*, as a regulator of polarized growth of seamless tubes and have evidence to suggest that the Rab35-Whacked pathway promotes apical membrane addition by way of the dynein motor complex.

552C

***Src42A*-dependent polarized cell shape changes mediate epithelial tube elongation.** Dominique Foerster, Stefan Luschnig. Institute of Molecular Life Sciences and PhD Program in Molecular Life Sciences, University of Zurich, 8057 Zurich, Switzerland.

Epithelial tubes are the basic unit of many organs, such as the mammalian lungs or kidneys. However, the cellular and molecular mechanisms of tube size control are poorly understood. We performed comprehensive EMS mutagenesis screens to identify new genes involved in tracheal tube development. Several new loci were found, which affect various morphological features (tube size and shape, branching, lumen fusion), as well as cellular processes, such as secretion and endocytosis. While many genes are required to limit tracheal tube elongation, we found only one gene to be required for tube elongation. We identified new mutations in the tyrosine kinase Src42A that result in tube elongation defects. Multicolor cell labelling and 3D image analysis revealed that this phenotype is caused by a change in epithelial organization and tracheal cell shapes. While wild-type tracheal cells form a simple columnar epithelium and expand along the tube axis during tube elongation, *Src42A* mutant cells show a pseudostratified organization, and cell bodies elongate perpendicular to the tube. In contrast, constitutive activation of Src42A induces tube overelongation and cell stretching, indicating that Src42A acts instructively in this process. E-Cadherin turnover is dramatically reduced in *Src42A* loss-of-function mutants, while constitutively active *Src42A* leads to E-Cadherin mislocalization. This suggests that Src42A-dependent remodelling of adherens junctions is limiting for the cell shape changes that mediate tube elongation. Strikingly, Src42A controls tube elongation independently of diametric expansion, which is driven by apical secretion. Thus we defined distinct cellular processes that independently regulate the dimensions of epithelial tubes.

553A

Orthogonal illumination microscopy live imaging of *Drosophila* embryo. Dmitri V Novikov[1], Gordon L Kindlmann[2], Kevin P White[1]. 1) Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL; 2) Department of Computer Science, University of Chicago, Chicago, IL.

The IGSB advanced imaging core is using a new, high speed, high resolution DSLM microscope (acquired from E. Stelzer, EMBL) for live imaging of *Drosophila* embryogenesis. We have generated over 50 fly lines with GFP and YFP tagged transcription factors and other developmental molecules that are expressed in their endogenous patterns. These reagents can be used for both imaging and for chromatin immunopreciptation (K.J. Venken et al., Nat. Methods, 2009; N. Nègre et al., Nature, 2011). Using orthogonal illumination microscopy and custom image processing software, we are generating a comprehensive database of 4D gene expression profiles in the fly embryo. To further increase resolution of the acquired images, we modified the protocol for mounting *Drosophila* embryo in the DSLM specimen chamber, using a new procedure whereby the embryo is attached to a clear polymer plate with an adhesive surface, eliminating the use of agarose embedding. Here we describe initial results obtained using this system.

554B

Investigating a mesenchymal to epithelial tranisition during development of the testis niche. Lindsey Wingert[1,2], Stephen DiNardo[2]. 1) Cell and Molecular Biology, University of Pennsylvania, Philadelphia, PA; 2) Cell and Developmental Biology, University of Pennsylvania, School of Medicine, Philadelphia, PA.

The 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 attachment and self-renewal, thus allowing them to maintain production of sperm for the lifetime of the animal. Much has been discovered about the key signaling pathways involved in steady state maintenance. However, less is known about how the architecture of the adult germline stem cell niche is achieved. During gonadogenesis, somatic gonadal precursors (SGPs) exhibit partial mesenchymal identity as they migrate and coalesce with germ cells (Jenkins et al., 2003, Development 130:4417-4428). Hub cells are specified from the pool of SGPs by Notch activation (Kitadate and Kobayashi, 2010, Proc Natl Acad Sci USA. 107(32):14241-6 and Okegbe and DiNardo, 2011, Development 138(7):1259-67). By the end of embryogenesis, hub cells form a true epithelium at the anterior pole of the gonad by remodeling cell-to-cell and cell-to-matrix adhesions (Tanentzapf et al., 2007, Nat Cell Biol 9:1413-1418, and Le Bras and Van Doren, 2006, Dev. Biol. 294:92-103). There are several markers expressed broadly in SGPs that become restricted to or repressed in hub cells during this transition. Both the morphological and genetic states adopted by hub cells during embryogenesis are maintained throughout the steady-state function of the testis. We are investigating the roles of the transcription factors Traffic jam (Tj) and Zfh-1 in the mesenchymal to epithelial transition (MET) using genetics and live imaging of hub formation. We suspect that the downregulation of these proteins is critical for refining gene expression associated with terminal differentiation in niche cells.

555C

Hoi polloi is a specific regulator of somatic muscle differentiation. Aaron N. Johnson, Eric N. Olson. Department of Molecular Biology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX.

A key interest in the field of muscle biology is to understand the molecular processes by which a specified muscle precursor undergoes the complex cellular and morphological changes to become a contractile, mature muscle. Using a forward genetic approach, we identified an essential function for the RNA binding protein Hoi Polloi (Hoip) during terminal muscle differentiation. Hoip belongs to an ancient family of RNA binding proteins that includes Snu13p in yeast and NHP2L1 in humans, both of which are key components of the spliceosome. *hoip* mutant embryos have a striking phenotype in which somatic muscle founder cells are correctly specified but the sarcomere component Myosin Heavy Chain (MHC) is not expressed. Surprisingly, in *hoip* embryos, F-actin assembles at wild type levels in the somatic musculature and MHC is expressed normally in the visceral musculature. Hoip therefore regulates the processing of a specific set of RNAs within the somatic mesoderm. Accordingly, we found that *hoip* is expressed solely in the mesoderm and the endoderm but, within the mesoderm, *hoip* expression is excluded from the visceral muscle lineage. We have identified a minimal enhancer that recapitulates *hoip* embryonic expression and found a highly conserved E-box sequence that is necessary for enhancer activity. To elucidate the mechanism by which Hoip regulates muscle differentiation, we have generated a series of distinct point mutations in the Hoip cDNA that are being used for functional in vivo rescue experiments. Expressing wild type Hoip in muscle founder cells rescues the *hoip* muscle phenotype, and we expect that mutant Hoip transgenic rescue experiments will clarify the function of Hoip during myogenesis. In addition, we are deep sequencing RNA from *hoip* embryos to evaluate the role of Hoip in regulating RNA expression and processing. This study has identified a novel, muscle-specific RNA regulatory network that directs terminal somatic muscle differentiation and will provide unique insights into the molecular events that direct muscle morphogenesis.

556A

JAK/Stat signaling regulates heart precursor diversification in *Drosophila*. Aaron N. Johnson, Mayssa H. Mokalled, Thomas N. Haden, Eric N. Olson. Department of Molecular Biology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX.

Intercellular signal transduction pathways regulate the NK-2 family of transcription factors in a conserved gene regulatory network that directs cardiogenesis in both flies and mammals. The Drosophila NK-2 protein Tinman (Tin) was recently shown to regulate Stat92E, the Janus Kinase (JAK) and Signal Transducer and Activator of Transcription (Stat) pathway effector, in the developing mesoderm. To understand if the JAK/Stat pathway also regulates cardiogenesis, we performed a systematic characterization of JAK/Stat signaling during mesoderm development. Drosophila embryos with mutations in the JAK/Stat ligand *upd* or in *Stat92E* have non-functional hearts with luminal defects and inappropriate cell aggregations. Using strong *Stat92E* loss-of-function alleles, we show that the JAK/Stat pathway regulates *tin* expression prior to heart precursor cell diversification. *tin* expression can be subdivided into four phases, and, in *Stat92E* mutant embryos, the broad Phase-2 expression pattern in the dorsal mesoderm does not restrict to the constrained Phase-3 pattern. These embryos also have an expanded pericardial cell domain. We show the E(spl)-C gene *HLHm5* is expressed in a pattern complementary to *tin* during Phase-3 and that this expression is JAK/Stat dependent. In addition, E(spl)-C mutant embryos phenocopy the cardiac defects of Stat92E embryos. Mechanistically, JAK/Stat signals activate E(spl)-C genes to restrict Tin expression and the subsequent expression of the T-box transcription factor H15 to direct heart precursor diversification. This study is the first to characterize a role for the JAK/Stat pathway during cardiogenesis and identifies an autoregulatory circuit in which *tin* limits its expression domain.

557B

A role for *Drosophila* Cyclin J in oogenesis is uncovered in piRNA pathway mutants. Paul Michael Albosta, Govindaraja Atikukke, Huamei Zhang, Russell Finley. Ctr Molecular Medicine & Genetics, Wayne State Univ Sch Medicine, Detroit, MI.

Cyclin J (*CycJ*) is a poorly characterized member of the cyclin superfamily of proteins, many of which regulate the cell division cycle. *CycJ* mRNA in *Drosophila* is limited to ovaries and early embryos, suggesting a role in one or both of these tissues. *CycJ* is adjacent to *armitage (armi)*, a gene involved in the piwi-associated RNA (piRNA) pathway. Mutants of *armi* and other piRNA pathway members are known to result in germline defects including transposon upregulation, DNA damage accumulation, and oocyte axis specification defects, but the function of *CycJ* during oogenesis has yet to be determined. We examined the roles for both *armi* and *CycJ* during oogenesis using null mutant flies created by deleting *CycJ* and *armi* and then complementing with transgenes for each gene individually. We demonstrate the previously defined role of *armi* in axis specification and further find that complete loss of *armi* leads to an apparent germline stem cell loss similar to *piwi* mutants. These *armi* null flies produce ovarioles with only two to three egg chambers, most of which contain the normal complement of 15 nurse cell nuclei and one oocyte. While *CycJ* null mutants display no obvious oogenesis

defects, *armi-CycJ* double null flies unexpectedly produce egg chambers with an excess of germline cells, and never generate stage 14 oocytes. Expression of *CycJ* from a transgene rescues the production of stage 14 oocytes. Similar phenotypic results were obtained with a double mutant of *CycJ* and another piRNA pathway member, *aubergin*e. The increase in germarium and egg chamber defects produced when *CycJ* is mutated along with either *armi* or *aubergine* reveals a role for *CycJ* during early oogenesis. These findings suggest that in the absence of the piRNA pathway, *CycJ* is required to limit germline cell numbers or to facilitate their proper packaging into egg chambers.

558C

Reorganization of germline P bodies and microtubules in response to nutrient stress. Katherine M. Burn, Lynn Cooley. Gen, Cooley Lab, Yale Sch Med I-359, New Haven, CT.

Drosophila females deprived of sufficient protein in their food (starved) have low fecundity, due to slowed stem cell divisions, slowed egg chamber development and apoptosis of egg chambers just prior to the onset of vitellogenesis (stage 8). However, little is known about the consequences of nutrient deprivation on polarized transport in previtellogenic (stage 7 and younger) egg chambers. We have found that under starvation conditions, large cytoplasmic foci appear in nurse cells that contain processing body (P body) components as well as members of the oskar mRNP. Starved chambers additionally show cortical enrichment of microtubules that may interfere with polarized transport. Importantly, both of these effects are rapidly reversed upon re-feeding or culturing egg chambers with insulin. We hypothesize that in the ovaries of starved flies, Insulin/TOR signaling is reduced, inducing germline P bodies. To examine the role that Insulin/TOR signaling in mediating the starvation response, we are manipulating the pathway in either the follicle cells or the germline by expressing RNAi, constitutively active or dominant negative proteins, as well as overexpressing wild-type proteins. Interestingly, depleting TOR signaling by overexpression of Tsc1 in the follicle cells can induce starvation response in the germline of well-nourished flies, suggesting that follicle cells may be modulating the starvation response. However, egg chambers expressing RNAi against TOR or the Insulin receptor in the germline show growth inhibition and do not respond to nutrient stress. Our current work is focused on further dissecting the starvation response and determining its physiological significance.

559A

Within the female germline, *ovo* promotes the expression of oogenesis genes while *otu* inhibits the expression of male-biased genes. Amy C. Cash, Justen Andrews. Department of Biology, Indiana University, Bloomington, IN.

The genes *ovo* and *otu* (*ovarian tumor*) are required for germline sex determination and proper oogenesis. *ovo* encodes a zinc finger transcription factor that directly regulates itself and *otu*. *otu* is a novel cytoplasmic protein thought to have RNA binding ability, and the two genes have very similar mutant phenotypes, suggesting that much of the effect of *ovo* may be mediated through its effects on *otu* transcription. We used microarray analysis on *ovo* and *otu* mutants to determine genes and pathways whose correct expression in the ovary is dependent on *ovo* and *otu* function. We found that *ovo* functions in the female germline to promote the expression of many genes known to function in oogenesis, including those involved in axis determination, meiosis, recombination, and egg shell formation. On the other hand, we found that *otu* functions in the female germline to prevent the expression of male-biased genes. Additional work will focus on identifying the genetic pathways by which *otu* normally represses male-biased genes.

560B

Female-sterile mutants in purity of essence. Paromita Gupta[1], Janet Rollins[2], Christopher Bazinet[1]. 1) Biological Sciences, St John's University, New York, NY; 2) College of Mount Saint Vincent,New York,NY.

Correct localization of a number of maternal gene products at the posterior pole of the egg is key to formation of cytoplasmic germline determinant called poleplasm. Defective poleplasm causes fertility issues in the fly. Previous studies in our lab on the Purity of essence (poe) gene focused on its role in male fertility. A poe mutant, poe[5970] caused by a PlacW insertion close to the 5' end had a weak female sterile phenotype with some polar vasa localization defects. Recent evidence provided by the Lasko and Lehmann laboratories has shown poe polypeptide to interact with vasa protein, a major poleplasm component. The 5970 mutant was backcrossed with the wild type for several generations to generate insertion carrying backcrossed lines which exhibit several distinct oogenesis phenotypes, demonstrating a complex dependence of the fs phenotype on genetic background. Mobilization of the PlacW yielded several stronger fs alleles among 4 different phenotypic classes of revertants: male,female-fertile ; male-sterile, female fertile; male-fertile, female-sterile, and male,female-sterile . fs mutants isolated after backcrossing or mobilization of the P element variously display fused egg chambers, bipolar egg chambers and fewer germ cells in the ovary. Some mutants produce defective eggs with disrupted localization of vasa-GFP. Sex-specific reversion of sterility, triggered by the mobilization of a single transposon, could be a result of differential splicing of the gene in male and female germlines, such that imprecise excisions of the P element can differentially effect male/female specific transcripts essential for gametogenesis. This is supported by a previous observation that several poe nonsense mutations resulting in male sterility have no apparent effect on female fertility. Primers flanking the insertion site of PlacW and the 2 nonsense mutations were designed to study the expression pattern in males and females in wild type and different mutant lines. Preliminary RT-PCR experiments reveal a consistent and complex pattern of alternative splicing of poe in males and females.

561C

Zfrp8, a conserved stem cell factor, interacts with the MAGUK family protein Dlg5. Eve Hardy, William Tan, Svetlana Minakhina, Ruth Steward. Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ.

Zfrp8, first identified as a regulator of hematopoietic stem cell maintenance in *Drosophila*, also functions in germline and follicle stem cells in the Drosophila ovary. By transgenic rescue of targeted proteins we have established that Zfrp8 most likely functions in the nucleus. Surprisingly we have identified Discs Large 5 (Dlg5) as an interactor of Zfrp8 by yeast-2 hybrid screening and TAP purification. Dlg5 is a member of the MAGUK family of proteins, which typically serve as molecular scaffolds. We have found that depletion of Dlg5 by expressing RNAi in follicle stem cells (FSCs) and follicle cells leads to a number of distinct phenotypes: ovaries contain a large number of fused and disorganized egg chambers, egg chambers with aberrant oocyte polarity are observed, and in some egg chambers more than one mislocalized oocytes are present. Interestingly, we have found that simultaneous ubiquitous overexpression of a GFP-tagged *Zfrp8* transgene and knockdown of *Dlg5* leads to a loss of Zfrp8 nuclear accumulation in the developing larval ovary, indicating that Dlg5 may be responsible for localization of Zfrp8 to the nucleus. We are currently investigating the implications of the interaction between

Zfrp8 and Dlg5.

562A

Investigation of Snail family proteins in cell death during *Drosophila* oogenesis. Victoria Kathryn Jenkins, Kim McCall. Department of Biology, Boston University, Boston, MA.

In organisms including *C. elegans* and mammals, members of the Snail family of transcription factors are responsible for aspects of cell death, the epithelial-to-mesenchymal transition, and cell cycle regulation; however, it is unknown whether they play a role in *Drosophila* cell death. During a screen for cell death genes in the ovary, we identified the Snail family member *escargot* as a possible regulator of cell death. The nurse cells (NCs) of egg chambers can undergo two naturally occurring death events. Starvation-induced death ("mid-stage") can be induced halfway through oogenesis, whereas developmental death ("late-stage") occurs near the end of egg maturation. Overexpression of the Snail family gene *escargot* prevented both mid- and late-stage death of the nurse cells, and disrupted other aspects of normal egg chamber development. This phenotype is suppressed by overexpression of the caspase gene *dcp-1* in mid-stage death, suggesting that *escargot* acts upstream of or in parallel to *dcp-1*. Additionally, since Escargot cannot suppress the phenotype of *dcp-1* driven by a UASp promoter, it may be a transcriptional repressor of *dcp-1*. The *escargot* overexpression phenotype mimics the effect of blocking caspases and of overexpressing the inhibitor of apoptosis protein DIAP-1. Therefore, either the Snail proteins are a part of normal nurse cell death, or when highly expressed, are capable of regulating a component of the cell death pathways which cause these phenotypes. In the future, we will analyze loss-of-function mutants to determine whether Snail family members have a role in normal cell death during oogenesis. Current progress will be presented.

563B

The Diverse Function of PAPI, a Tudor-Domain-Containing Interactor of PIWI Proteins, in Oogenesis and Embryogenesis. Li Liu, Na Liu, Sneha Mani, Haifan Lin. Yale University, New Haven, CT.

Drosophila Piwi proteins, Piwi, Aub (Aubergine) and Ago3 (Argonaute3), bind to Piwi-interacting RNAs (piRNAs) and function in epigenetic regulation and transposon control. We previously identified a novel Piwi-interacting protein, Papi, which binds symmetrically dimethylated arginine residues (DMAs) in Piwi proteins through its Tudor domain. Papi recruits Ago3 to the nauge and forms a complex with Ago3, Me31b and Trailer Hitch (Tral) to regulate transposition. Here we report a loss-of-function allele of papi that causes female sterility. papi, ago3 and tral mutants exhibit delays in oocyte determination and defects in oocyte polarity and embryonic axis formation, which are commonly observed in other nuage component mutants. The microtubule minus end-directed motor, dynein, is mislocalized in papi, ago3 and tral mutant ovaries, suggesting the microtubule-dependent transport is defective in these mutants. Moreover, ago3 and papi mutant embryos exhibit drastic defects in germline and somatic cell lineages, indicating that the Ago3 and Papi are involved in germline determination and mitosis during early embryogenesis. However, piRNA production is not significantly altered in papi mutant, suggesting that the Papi/Ago3 complex exerts its function in oogenesis and early embryogenesis independent of piRNA biogenesis pathway.

564C

Wnt4 regulates germline follicle formation. Lucy Morris, Joan Pulupa, Allan Spradling. Carnegie Institution, Baltimore, MD.

Germline follicle formation occurs when germ cells discard their covering of somatic escort cells and simultaneously recruit a wrapping of somatic follicle cells. Follicle cells are generated by division of follicle stem cells and subsequently direct formation of a mature egg. We found that Wnt4, a gene required for oogenesis in mouse, also regulates follicle formation in Drosophila. Wnt4 is produced by escort cells and acts both autonomously and on neighboring follicle cells. Using live imaging of follicle formation in mutant ovaries we have shown that Wnt4 regulates cell proliferation and not migration, its documented role in other tissues. The simple architecture of the Drosophila ovary combined with our ability to carry out live imaging with single cell resolution will enable us to use Wnt4 action on follicle formation to model how Wnts exert profound organizing activity on epithelial cells and during embryonic development.

565A

The Role of Translational Regulation in Meiotic Chromosome Segregation in Oocytes. James G. Ruggero, Sarah J. Radford, Kim S. McKim. Genetics, Waksman Institute of Microbiology, Rutgers, Piscataway, NJ.

A developing oocyte accumulates materials, such as nutrients, proteins and transcripts, from surrounding nurse cells. Concurrently, the oocyte undergoes meiosis. We performed a screen to find chromosome segregation-defective mutants that may be homozygous sterile or lethal, which are common for developmental and meiotic mutants. One of the proteins responsible for microtubule spindle formation during meiosis is Subito, a kinesin-6 protein required for bundling interpolar microtubules. Null mutants of *subito* (*sub*) are sterile due to a defect in pronuclear fusion. We identified a new mutation, sub^{4034}, that is fertile, but shows high levels of nondisjunction (11%, n=1160). In addition, nondisjunction is elevated in sub^{4034} heterozygous in combination with deficiencies in several regions. This evidence directed us to perform a screen to identify mutations that dominantly interact with sub^{4034} . We found nine mutants from this screen. Mapping, sequencing and successful rescue with a wild-type transgene identified one mutation in *maternal expression at 31B* (*me31B*) with 12% nondisjunction (n=620) in the sub^{4034} heterozygous background. Me31B mediates translational silencing. Thus, loss of *me31B* causes premature translation of oocyte-localizing proteins. This suggests that translational suppression may be important for chromosome segregation. Previous analysis of spindle formation in *me31B*-null mutants has not occurred because oocytes fail to develop to maturity. While $me31^{2212}$ mutants are mostly inviable, we were able to examine germline clones of $me31B^{2212}$ to test if the translation of meiotic proteins is affected. Immunocytochemistry of $me31B^{2212}$ germline clones showed wildtype spindle formation in both oocytes and embryos and expression of *sub*. We are lacking evidence that Me31B regulates *sub*. Testing for a more severe interaction is underway. We also plan to test other genes involved in translational suppression for an interaction with sub^{4034}. From the identification of the interaction of $me31B^{2212}$ with sub^{4034}, we have found a link between development and meiosis.

566B

PI4KIIIα is required for cortical integrity and cell polarity during *Drosophila* oogenesis. Julie Tan[1,2], Jason Burgess[1,2], Karen Oh[3,4], David Hipfner[3,4], Julie Brill[1,2]. 1) Program in Cell Biology, Hosp Sick Children, Toronto, ON, Canada; 2) Dept of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Epithelial Cell Biology, Institut de Recherches Cliniques de Montreal, Montreal, QC, Canada; 4) Department of Medicine, University of

Montreal, Montreal, QC, Canada.

Phosphoinositides are lipids that provide a molecular link for membrane interactions with cellular machinery. Phosphatidylinositol (PI) 4-kinases (PI4Ks) catalyze the conversion of PI to PI 4-phosphate (PI4P), a cellular effector that recruits trafficking proteins and serves as a precursor to other essential phosphoinositides. To investigate the function of phosphoinositides during development, we generated a deletion in the gene encoding the *Drosophila melanogaster* type IIIα PI4K (PI4KIIIα). We find that *PI4KIIIα* is required for production of plasma membrane phosphoinositides that are crucial for membrane trafficking, actin organization and cell polarity during oogenesis. Female germ cells mutant for *PI4KIIIα* lose cortical integrity and are impaired in activation of the PI 4,5-bisphosphate [$PI(4,5)P_2$]-binding cytoskeletal-membrane crosslinker Moesin. Titration of $PI(4,5)P_2$ using the pleckstrin homology domain of phospholipase C partially recapitulates *PI4KIIIα* phenotypes, indicating that $PI(4,5)P_2$ is the main phosphoinositide effector downstream of PI4KIIIα. These effects are specific to *PI4KIIIα*, as they are not produced in egg chambers mutant for either or both of the two remaining PI4Ks, *PI4KIIIβ/fwd* and *PI4KII*. Instead, *fwd* and *PI4KII* mutant germ cells exhibit altered Golgi distribution, whereas this is unaffected in *PI4KIIIα* mutant cells. Furthermore, membrane defects in *fwd*, *PI4KII*, and *fwd PI4KII* double mutant cells appears morphologically distinct from that in *PI4KIIIα* cells. Thus, as in yeast and mammalian cells, different *Drosophila* PI4Ks appear to have organelle-specific roles and produce functionally distinct pools of PI4P.

567C

The role of follicle cells in developmental nurse cell death and clearance in late oogenesis. Allison Timmons, Jon Iker Etchegaray, Claire Schenkel, 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 provide nutrients, proteins, mRNAs, and organelles for the developing oocyte. Beginning in stage 11, the nurse cells transfer their contents into the oocyte and undergo PCD. Interestingly, disruption of apoptosis or autophagy only partially inhibits PCD of the nurse cells, indicating that other mechanisms are contributing to the process. One possibility is that the follicle cells are contributing to the death and/or clearance of the nurse cells in late oogenesis. Using *UAS-mCD8-GFP* specifically expressed in the follicle cells, follicle cell membranes surround the nurse cells beginning in stage 10, and by stage 12 the follicle cells completely encompass each nurse cell. Disruption of *draper* or *basket* in the follicle cells results in a persisting nurse cell nuclei phenotype in late oogenesis, suggesting that these nurse cells are not dying or not being cleared properly. Furthermore, *puclacZ* expression indicates that JNK signaling is activated in the follicle cells during late oogenesis which previously has been attributed to the well known role for JNK in dorsal appendage formation. We have observed both *puclacZ* and Draper expression in the follicle cells that directly surround the nurse cells during PCD in late oogenesis. We hypothesize that in addition to dorsal appendage formation, JNK signaling in the follicle cells contributes to the death and clearance of the nurse cells. We also hypothesize that Draper and JNK in the follicle cells are interacting to promote the developmental PCD and clearance of nurse cells in late oogenesis. Further investigations are underway to characterize the involvement of the follicle cells in developmental nurse cell death.

568A

The bHLH protein, Sage, provides tissue specificity to FoxA/Fork head. Rebecca M. Fox, Aria Vaishnavi, Rika Maruyama, Deborah J. Andrew. Dept Cell Biol, Johns Hopkins Univ, Baltimore, MD.

Tissue morphogenesis is coordinated by the actions of transcription factors. In the salivary gland (SG), the homeotic genes *Sex combs reduced (Scr)*, *homothorax (hth)*, and *extradenticle (exd)* initiate SG specification by activating expression of the transcription factors Fork head (Fkh), Sage, CrebA and Huckebein (Hkb). Fkh is required for SG invagination as well as for maintaining its own expression and expression of many other SG genes. CrebA regulates the high-level secretory capacity required for SG function, and Hkb is required for SG tube elongation. Sage is the only SG specific transcription factor and has been implicated in the maintenance of the SG lumen through its regulation of two prolyl-4 hydroxylase genes, PH4αSG1 and PH4αSG2. We have generated Sage null mutants and discovered that Sage is required for SG survival in late embryos. To identify Sage target genes, we performed microarray analyses and discovered that Sage regulates genes encoding proteins secreted from the SG and the enzymes that modify these secreted products. Interestingly, whereas overexpression of either Fkh or Sage alone is not sufficient to induce ectopic SG target gene expression, coexpression of Fkh and Sage can activate SG gene expression in multiple ectopic locations. Consistent with this finding, we have discovered that Sage and Fkh protein localize to the same sites on SG polytene chromosomes, indicating that the two proteins act directly on the same sets of target genes. Thus, we have identified a SG specific transcripton factor, Sage, that functions with the FoxA factor, Fkh, to activate SG specific gene expression. These findings suggest a paradigm wherein a bHLH factor with very limited expression acts with the more widely expressed FoxA transcription factor to provide tissue specificity.

569B

oak gall and conjoined alter Branching Morphogenesis and Tube Formation in the Drosophila Tracheal System. Deanne M. Francis, Amin Ghabrial. Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA.

The Drosophila tracheal system is derived from 10 pairs of epithelial sacs, each comprised of approximately 80 cells. In response to an FGF (Branchless/Bnl) cue, new branches bud from the tracheal sacs. Each new branch migrates towards the source of the FGF cue, led by one or more "tip cells." Most, if not all, tracheal cells are capable of becoming tip cells, but tip cell number is restricted by a competition-based mechanism. We have identified 2 mutants, oak gall and conjoined, which confer a tip cell bias in genetic mosaic experiments. In addition, both oak gall and conjoined mutant cells have a rounded cell morphology, and terminal cells mutant for either gene show specific tube defects in the area between the terminal cell-stalk cell junction and the terminal cell nucleus. We have determined that oak gall and conjoined encode the E and G subunits of the vacuolar ATPase complex, respectively. Further experiments will identify the mechanism by which the vacuolar ATPase plays a role in tip cell specification, terminal cell morphology and tracheal tubulogenesis.

570C

The role of the exocyst in subcellular morphogenesis. Tiffani A. Jones, Mark M. Metzstein. Human Gen, Univ Utah, Salt Lake City, UT.

Branching morphogenesis and tubulogenesis are important morphological processes required for proper development of many organs and individual cells. However, the molecular mechanisms controlling these processes remain mostly unknown. To identify components required for branching and tubulogenesis,

we have analyzed genes required for these processes in tracheal terminal cells. Terminal cells, a component of the respiratory system, undergo two distinct morphogenetic processes: subcellular branching morphogenesis, and subcellular apical lumen formation. In previous work, we showed that both branching and lumen morphogenesis are regulated by components of the PAR-polarity complex, and that this complex is downstream of FGF signaling, a known regulator of terminal cell branching and outgrowth. Our recent work has turned to investigating the role of vesicle trafficking pathways in branching and tubulogenesis, focusing on the exocyst complex. The exocyst is an octomeric complex composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84, and is most commonly known for its role in directing vesicles to specific locations on the plasma membrane to facilitate exocytosis and cell growth. We find that in general all components of the exocyst complex are required for terminal cell branching, but only a subset are required for lumenongenesis. In particular, we find that exocyst members Exo84 and Sec15 are required for both branching and lumenogenesis in terminal cells. In contrast, we find Exo70 is required for subcellular branching but not lumen formation. These data suggest that individual members of the exocyst play two roles in terminal cell morphogenesis: one in which the exocyst is required for exocystosis and branching at the growing terminal tip, and another in which only specific complex members associated with vesicles are required for a tubulogenesis program.

571A

Tramtrack69 Regulates Gene Expression During Tube Morphogenesis in the *Drosophila* Ovary. Nathaniel C. Peters, Celeste A. Berg. Deptartment of Genome Sciences and the Molecular and Cellular Biology Program, University of Washington, Seattle, WA.

During late *Drosophila* oogenesis, the follicular epithelium that surrounds the oocyte instructs two dorsal groups of cells to undergo morphogenesis and form tubular molds for the respiratory filaments of the eggshell, the dorsal appendages (DAs). DA tubulogenesis is a simple and genetically accessible model for studying the poorly understood process of epithelial tube formation, providing insight into how an epithelium transitions from patterning to coordinated cell shape change. The Tramtrack69 transcription factor has essential roles throughout fly development; the *twin peaks* mutation is a *tramtrack* hypomorph that reduces TTK69 levels during late oogenesis. It causes shortened DAs by disrupting follicle cell shape and migration during DA tube expansion. Microarray analysis of wild type and *twin peaks* ovaries suggests that TTK69 influences a wide range of genes, from other transcription factors to regulators of the cytoskeleton, cell migration, axon guidance, and hormone processing. Array verification via *in situ* hybridization revealed that *paxillin*, a regulator of cell adhesion and migration in other contexts, is highly expressed in DA-forming cells throughout DA morphogenesis and is strongly reduced in *twin peaks*. We are testing whether *paxillin* is required for DA morphogenesis by follicle cell RNAi and by creating mutant alleles through P-element excision, perhaps linking *tramtrack* to the cytoskeletal regulators required for cell shape change and migration. RNAi against *mirror*, which encodes a transcription factor required for dorsal follicle cell patterning earlier in oogenesis, results in DA defects resembling *twin peaks*; *mirror* expresses in DA-forming cells during tubulogenesis but is absent at this time in *twin peaks*. The role of *mirror* may therefore extend past epithelial patterning into morphogenesis, and *tramtrack* may be required to maintain this expression. Identification and characterization of TTK69 targets, such as *paxillin* and *mirror*, will enhance our understanding of how regulatory factors and mechanical effectors interact to facilitate epithelial tubulogenesis.

572B

Characterization of $Pkn^{dlnΔ5}$, a derivative allele of the *delorean* mutation associated with the *Protein kinase N* gene in *Drosophila melanogaster*. Georgette Sass, Allison Burke, Sarah VanOeveren, Bruce Ostrow. Grand Valley State University, Allendale, MI.

The *delorean* mutation in *Drosophila melanogaster* was identified from a collection of mutants generated in a large-scale screen of *P[lacW]* transposon insertions on the second chromosome (Torok et al 1993 Genetics 135: 71-80). Wings of flies that are homozygous for the *delorean* mutation are held away from the body, noticeably curved downward and have additional defects of the wing margin. The *P[lacW]* insertion has been mapped to the first intron of the Drosophila *Protein kinase N* gene (*Pkn*) and the *delorean* mutation is thought to alter *Pkn* function (Ostrow and Momin 2001 A. Dros. Res. Conf. 42: 701B). The *delorean* wing phenotype is only seen when the *P[lacW]* insertion is homozygous (i.e Pkn^{dln}/Pkn^{dln}), yet is not due to a loss-of-function mutation as evidenced by the wild-type phenotype observed when Pkn^{dln} is heterozygous with a deficiency (*Df(2R)45C*) that removes the *Pkn* gene. This is in contrast to other *Pkn* alleles such as Pkn^{06736}; a null allele that results in dorsal closure defects during embryogenesis (Lu and Settleman 1999 Genes Dev. 13: 1168-1180). To understand the molecular basis of the *delorean* phenotype we have generated deletion derivatives of the Pkn^{dln} allele. One of these derivatives, $Pkn^{dlnΔ5}$, generates a less severe wing phenotype, but was found to have a profound effect on female fertility. In addition, $Pkn^{dlnΔ5}$ demonstrates that the *delorean* phenotype is transvection-dependent. We determined that the molecular lesion associated with the $Pkn^{dlnΔ5}$ allele was internal to the *P[lacW]* transposon, removing sequence from the *mini-white*$^{+}$ gene. We present our continued analysis of the $Pkn^{dlnΔ5}$ derivative with respect to the role of the *Pkn* gene in wing morphogenesis as well as its prospective role in oogenesis.

573C

***larval translucida* regulates growth and morphogenesis of the Malpighian tubules.** Milan Szuperak[1], Matthew Gibson[1,2]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Anatomy and Cell biology, KUMC, Kansas City, KS.

The processes of growth and morphogenesis must be tightly co-regulated to generate organs of appropriate size, shape and functionality. The renal tubules of *Drosophila melanogaster* provide an excellent model to understand organogenesis *in vivo*, although many aspects of their development remain poorly understood. Recently, we identified and described *larval translucida* (*ltl*), a novel feedback regulator of BMP signaling during wing development. Strikingly, homozygous null mutants of *ltl* exhibit a lethal bloated-larva phenotype characterized by excessive fluid retention, indicating a possible impairment of Malpighian tubule function. Consistent with this hypothesis, here we report that *ltl* mutant larvae exhibit disorganization of the Actin cytoskeleton in the tubules as well as enlarged ureters at the junction of the Malphigian tubules with the gut. These enlarged ureters appear to be blocked by gut contents, and consistent with this, water transport from the renal tubules into the guts is strongly reduced. We further show that Malphigian tubule cell numbers are significantly elevated in both embryos and larvae, with enlarged larval cell nuclei indicating defects in tissue growth control. We are currently investigating the function of *ltl* in embryonic renal development, with a focus on possible roles of BMP signaling in growth and morphogenesis of the Malpighian tubules.

574A

Investigating mechanisms of tubulogenesis using tandem mass spectrometry and FISH. Sandra G. Zimmerman, Celeste A. Berg. Department of Genome Sciences, University of Washington, Seattle, WA.

Tubulogenesis forms the neural tube, gut, and other organs. Errors in tubulogenesis lead to birth defects such as spina bifida. A Drosophila model that resembles neural tube formation is dorsal appendage (DA) formation in the egg chamber. The DAs are made by patches of follicle cells that reorganize into tubes, extend anteriorly over the squamous stretch cells (SCs), and secrete chorion into the tube lumens. In *bullwinkle* (*bwk*) mutants, the egg chamber forms moose-antler shaped DAs. Mutations in *shark*, which encodes a tyrosine kinase required in the SCs, strongly enhance the *bwk* phenotype. Other components of this pathway are unknown. Intriguingly, *shark* mRNA localizes in distinct foci at stage 10. We hypothesize that this pattern, which is transient and is lacking in *bwk* mutants, localizes Shark protein and is important for its function. To identify differences in protein expression and phosphorylation specifically in SCs from wild-type vs. *bwk* egg chambers, we optimized a magnetic bead cell-purification protocol for use with mass spectrometry (MS). An initial MS trial using this method revealed a number of potential differences in expression between wild-type and bwk SCs. We will choose candidates based on potential relevance and statistical significance over multiple MS runs, and test their function using phenotypic analysis of egg chambers in which candidate protein expression or phosphorylation state is altered. To investigate localization of endogenous or fluorescently-tagged Shark and Shark phosphorylation mutants relative to *shark* mRNA, we developed a method for simultaneous fluorescent *in situ* hybridization (FISH) and protein immunostaining for the ovary. Methods that work well in other tissues do not work in ovaries. Success of our protocol in ovaries depends critically on signal amplification, choice of blocking reagent, tissue permeabilization method, and RNase inactivation, factors that also influence FISH alone. These studies will increase our understanding of mechanisms governing tubulogenesis and may provide insight into the basis of human developmental defects.

575B

Patterns of molecular evolution of germ line specification genes in *Drosophila*. Abha Ahuja, Victor Zeng, Cassandra Extavour. Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Specification of germ cells during embryogenesis is critical for reproductive success and survival of sexually reproducing animals. In *Drosophila* molecular interactions between mRNA and protein products of different germ cell specification genes are required for the proper differentiation of germ cells. To gain insight into the molecular mechanisms underlying this process, here we assess the patterns of evolution of known germ line specification genes. For these genes, we estimate and compare the rates of synonymous (*dS*) and non-synonymous (*dN*) substitutions between sequenced *Drosophila* species, and identify genes that show signatures of positive selection. Next, we identify groups of genes that exhibit correlated rates of evolution. Co-evolving genes are expected to be functionally linked, and we discuss our findings in the context of known genetic and molecular interactions between these genes. Our goal is to identify conserved gene regulatory networks involved in germ cell specification. These results provide important insight into the modular nature of developmental processes underlying germ line specification, and will also help guide future biochemical and physical association studies of germ line genes.

576C

A surprising role for the Anaphase Promoting Complex in sex determination. Osamah Batiha, Varsha Padmanaban, Eric Fifield, Rami Mechael, Alison Petrie, Andrew Swan. Biological sciences, University of Windsor, Windsor.

Anaphase Promoting Complex (APC) is an E3 ubiquitin ligase that plays important roles in the progression from metaphase to anaphase in mitosis and meiosis by targeting cyclins and other proteins for destruction. In female meiosis, the APC requires a meiosis specific activator, Cort, in addition to the canonical mitotic activator, Fzy. Cort expression and degradation is highly regulated as Cort is expressed in late oogenesis and is degraded in early embryogenesis. To determine the significance of the tight control of Cort expression we mis-expressed Cort zygotically. Surprisingly, Cort mis- expression caused a female to male sex transformation. This sex transformation is APC dependent as it is suppressed by mutations in cort that disrupt APC interaction or by knockdown of APC genes. Based on these results we hypothesized that APC/Cort targets a protein in the sex determination pathway. Sex determination in Drosophila depends on a cascade of differential splicing starting with Sxl which is expressed initially only in females. Sxl acts to splice its pre-mRNA and the downstream target, tra. Tra is also a splicing factor and works with Tra-2 to splice dsx in the female mode. We used RT-PCR to show that Cort acts downstream of tra splicing and upstream of dsx splicing. Consistent with this conclusion our genetic experiments point toward Cort acting specifically on Tra protein. Interestingly, we found that Cort has the ability to affect Tra activity from a distant Diptera relative, which suggests that Cort plays a conserved role in sex determination. The main question we are trying to answer now is: how a germline- specific protein like Cort can affect sex determination, a process that does not happen until zygotic proteins are expressed in the embryo. An interesting paper by the Cline lab recently discovered a reciprocal activation loop between Tra and Sxl; in which maternal Tra can promote Sxl splicing. Currently, we are investigating the potential germline role of APC/Cort in keeping Tra levels low until sex determination is initiated zygotically.

577A

Hrp48 functions as a moderator of Sxl expression to allow for proper Notch expression and signaling in monomorphic organ development. Dvora Burshtein[1], Yaron Suissa[1], Yossi Kalifa[2], Tama Dinur[1], Patricia Graham[3], Girish Deshpande[3], Paul Schedl[3], Offer Gerlitz[1]. 1) Developmental Biology and Cancer Research, IMRIC, The Hebrew University-Hadassah Medical School, Jerusalem; 2) Department of Molecular Genetics, Weizmann Institute of Science, Rehovot Israel; 3) Department of Molecular Biology, Princeton University, Princeton, NJ 08540, USA.

Sex-determination genes specify the sexual identity of tissues, mainly by deploying the major signaling pathways that direct cells and organ primordia. The Notch (N) signaling pathway is central to a wide variety of contexts throughout development because of its ability to specify alternative cell fates. Recently, Sex-lethal (Sxl), the female determinant in Drosophila melanogaster was shown to downregulate N signaling to accomplish sex-specific patterning. With the exception of genital discs, most organ primordia follow identical developmental routes and develop in similar fashion in both sexes (monomorphic organs). Moreover, irrespective of the sex, N signaling plays a central role during organ development. Paradoxically however, Sxl activity is essential in every female cell to ensure the proper level of X-linked gene expression. This raises a key question as to how, during female development, N signaling escapes the negative impact of Sxl in monomorphic tissues. We have uncovered a novel mechanism where Hrp48, an abundant essential hnRNP, functions to restrict Sxl expression in monomorphic tissues and thus allows for proper development. Phenotypic consequences of the partial loss of hrp48 resemble that of N but are more pronounced in females than in males. Likewise, N levels are drastically diminished only in females. Interestingly, monomorphic female tissues including wing, eye and antennal discs display considerable increase in Sxl amounts. Lastly, female-specific attenuation of N signaling is rescued upon simultaneous removal of Sxl. Our findings bring into focus the critical role played by general homeostatic factors in specification of diverse cell fates and

morphogenesis.

578B
Jak-STAT regulation of Drosophila germ cell sex determination. Matthew J. Wawersik, Andrea Lin, Tigist Tamir, Rebecca Obniski. Biology Dept, Col William & Mary, Williamsburg, VA.

Germ cells are the only cells in the body that make sperm and eggs required for sexual reproduction. Proper germ cell development is, therefore, essential for survival of future generations. A critical step in this process is the decision made by germ cells to develop along male or female fates. In flies and mice, both cell autonomous and somatic signals control germline sexual identity. We have previously shown that Jak-STAT activation in Drosophila germ cells by male somatic gonad plays a critical role in establishment of male germ cell fate during embryogenesis (Wawersik et al, 2005). However, the extent to which this pathway promotes germ cell sex determination is not clear. Here, we show that hyperactivation of the Jak-STAT pathway during ovary development is sufficient to induce male germline gene expression in adult XX germ cells. Induction of these genes correlates both spatially and temporally with formation of germ cell tumors that fail to develop into functional sperm or eggs, a hallmark of altered germ cell sex determination. We also find that XX germ cells masculinized by mutations in snf and ovo induce STAT gene expression in a subset of ovarian germ cell tumors. Finally, we show that genetic interactions between snf and jak result in ovarian germ cell tumor formation. Together, these data suggests that the Jak-STAT pathway plays a direct role in germ cell sex determination and maintenance of sexual identity, and that there is significant cross talk between the pathways that control male vs. female germline development.

579C
microRNA miR-7 targets Tramtrack69 to regulate a developmental switch in Drosophila follicle cells. Yi-Chun Huang, Laila Smith, John Poulton, Wu-Min Deng. Dept Biological Sci, Florida State Univ, Tallahassee, FL.

Development in multicellular organisms consist both small incremental changes and major switches of cell differentiation and proliferation status. During Drosophila oogenesis, the follicular epithelial cells undergo two major developmental switches with global changes in the cell cycle program. One such switch, the switch from the endoreplication cycles to a gene amplification phase, during which special genomic regions undergo repeated site-specific replication, is attributed to Notch downregulation, Ecdysone signaling activation and upregulation of zinc finger protein Tramtrack69 (Ttk69) in follicle cells. Here we report that microRNA miR-7 exerts an additional layer of regulation in this developmental switch through targeting Ttk69 transcripts. miR-7 targets the 3' UTR of ttk69 transcripts and regulates Ttk69 expression in a dose dependent manner. Overexpression of miR-7 effectively blocks the switch from the endocycle to gene amplification through its regulation on ttk69. miR-7 also coordinates other cell differentiation events that lead to the formation of the mature egg. Our studies reveal the important role miR-7 plays in coordinating developmental switches in association with signal transduction pathways.

580A
Genetic Probing of Drosophila glycine requirements. Christopher W. Bazinet, Ujwala Gosavi, Debaki Sarkar. Dept Biological Sci, Saint John's Univ, Queens, NY.

Recent work from a number of laboratories has revealed that a posttranslational modification of microtubules via glycylation, the addition of glycine residues to one or more aspartate residues near the C-terminus of tubulin subunits, may be critical for regulating the stability and motility of microtubule-based structures. The extensive glycylation of axonemal microtubules in Drosophila's extraordinarily long sperm implies a significant requirement for glycine within the spermatogenic cyst. Observations from our laboratory indicate that the gene product of Neurotransmitter transporter-like (Ntl), expressed and required only in the testis, functions as a glycine transporter for supplementing the glycine stores in spermatogenic cysts. In its absence, glycylation levels of testis tubulin is reduced, and sperm fail to be transferred from the testis into the seminal vesicle. Thus, manipulation of glycine levels within the testis may offer a means for regulating male fertility in insects. As a first step towards manipulating glycine metabolism in Drosophila, we have mobilized a P element insertion at the 5' end of CG3011, encoding serine-glycine hydroxymethyl transferase (SGHM). Removal of the hydroxymethyl group from serine by SGHM is the primary or only means by which cells produce glycine. Mutations comprising two lethal complementation groups, one of which presumable represents the SGHM gene, have been recovered after this transposon mutagenesis. However, supplementation of Drosophila food with glycine fails to rescue mutants from either complementation group. This indicates that although glycine is not an essential amino acid, neither can it be provided to SGHM[-] cells in the diet. Instead, glycine synthesis appears to be cell autonomous.

581B
***tut* coordinates proliferation and differentiation of spermatogonia in Drosophila.** Di Chen[1,2], Bangxia Suo[1], Shaowei Zhao[1,2], Qing Geng[1,2], Yu Gao[1,2], Zhaohui Wang[1]. 1) Institute of Genetics and Developmental Biology, Beijing; 2) Graduate University of Chinese Academy of Sciences.

Drosophila spermatogenesis represents an excellent model system to study how proliferation and differentiation are coordinated. In order to identify more factors involved in this process, we conducted a large-scale EMS screen, through which we obtained an interesting mutant, homozygous viable but containing tiny testes full of un-differentiated germ cells. We mapped this mutation to a novel gene predicted to encode an RNA binding protein and designated this new gene as *tumorous testis* (*tut*). The over-proliferating germ cells in *tut* mutant testis are spermatogonia, the mitotic amplifying germ cells. Further analyses indicate that *tut* represents an intrinsic factor for regulating spermatogonial proliferation. We tried genetic interaction tests and found that *tut* and *bam* interact with each other to coordinate proliferation and differentiation of spermatogonia. We also found that Tut protein is strictly regulated and JAK-STAT signaling from somatic cells inhibits *tut* function in germline stem cells. Currently, we are studying the molecular mechanism by which *tut* coordinates spermatogonial proliferation and differentiation.

582C
Screening for dominant enhancers of Segregation distortion. Kaylie Church, Janna McLean. Olivet Nazarene University, Bourbonnais, IL.

Segregation distortion is a meiotic drive system that results in the favorable inheritance of the *SD* chromosome over the SD^{+} counterpart. It is understood that *SD* produces a malfunctioning form of RanGAP; instead of being able to move in and out of the nucleus this truncated RanGAP is unable to be exported and therefore aggregates within the nucleus. This appears to affect Rsp^{s} during the condensation phase of spermatogenesis; the exact mechanism for this is

unknown. In order to further understand the working components of this system, specific deletions within the second chromosome were studied. The study was conducted systematically by beginning with larger deletions that had previously shown distortion and slowly reducing the size of the deletion. When these deletion stocks were combined with *SD-5^{r7}*, with *Rsp^{s}* on the Y it was found that a deletion of the region 26C1;26D1 displayed distortion whereas a deletion of the region 26C3;26D1 did not. When a deletion of the 26C2;26C3 region was crossed with *SD-5^{r7}* distortion was observed. Within this region the genes *Cpr* and *Gef26* are of particular interest. Stocks containing a mutation in *Cpr* did not demonstrate distortion when combined with *SD-5^{r7}*. However, when a stock containing a mutation in the gene *Gef26* was combined with *SD-5^{r7}*, distortion was noted. These results suggest that better understanding of the gene *Gef26*, and its function within the cell during spermatogenesis, would shed some light as to how segregation distortion takes place on a molecular level.

583A

Phosphoinositides regulate nuclear morphogenesis in Drosophila. Lacramioara Fabian[1], Julie Brill[1,2]. 1) Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Molecular Genetics, University of Toronto, Ontario, Canada.

Spermatid nuclei undergo dramatic changes in shape and chromatin condensation during the late stages of sperm development. Histones that package the DNA in early spermatids are replaced by the smaller protamines, which further remodel and package the chromatin into the long, thin mature sperm nucleus. These changes are also associated with microtubule-dependent nuclear elongation, when the perinuclear microtubule cytoskeleton is reorganized to provide additional support to the elongating nucleus. Here, we show that levels of phosphatidylinositol phosphates (PIPs) are critical for shaping the sperm head and for chromatin condensation during Drosophila spermiogenesis. Spermatids in which levels of phosphatidylinositol 4,5-bisphosphate (PIP2) have been reduced show profound defects in nuclear shaping; the nuclei do not mature and the males are sterile. Posttranslational modification of histones is impaired and their removal is delayed. Protamines get incorporated into nuclei despite histones not being completely removed. Transition proteins are missing in these spermatids. Localization of inner nuclear membrane proteins is defective and repair of double-stranded DNA breaks is incomplete. Our present data suggest that normal levels of PIP2 are required to coordinate interactions between the nuclear membrane, chromatin and the cytoskeleton.

584B

A testis-enriched predicted ATP synthase subunit required for mitochondrial shaping during spermatogenesis. Yihharn Hwang, Lauren Ivey, Karen G. Hales. Department of Biology, Davidson College, Davidson, NC.

ms(2)1400 is a recessive mutation that causes sterility in male *Drosophila melanogaster* by affecting mitochondrial shaping in sperm cells. During spermatogenesis from the transition between the onion stage to the leaf blade stage, the Nebenkern in spermatids from homozygous *ms(2)1400* males is unable to properly unfurl into two distinct mitochondrial derivatives due to defects in the internal structure of the membranes of the Nebenkern. The mitochondria remain unelongated as the flagellar axoneme grows, leading to non-motility of mature sperm. Through deficiency mapping experiments and candidate gene analysis, the *ms(2)1400* mutation was suspected to be in gene *CG7813*. *CG7813* encodes a predicted paralog of ATP synthase subunit d that shows enriched expression in the *Drosophila* testis. ATP synthase dimerization in the mitochondrial inner membrane is known to affect cristae morphology in other organisms. We confirmed that *CG7813* represents *ms(2)1400* using RNAi via the GAL4-UAS system to trigger gene knockdown in the testis. Testis preparations from *CG7813* RNAi flies were compared to those from homozygous flies with the *ms(2)1400* mutation. We observed distinct phenotypic similarity and thus confirmed location of *ms(2)1400* in *CG7813*. A second technique, EMS mutagenesis, is currently in progress to generate a *ms(2)1400/CG7813* allelic series and thus to broaden our functional understanding of this gene product. Confirmation of *ms(2)1400* as *CG7813* provides new information of the role of ATP synthase variants in tissue-specific mitochondrial morphology. We suspect that the unusually large ATP synthase subunit d encoded by wild type *ms(2)1400/CG7813* may normally alter dimerization of the complex to enable the specialized Nebenkern internal structure. The functions of three other testis-enriched paralogs of ATP synthase subunits, b, g and F6, are being similarly explored.

585C

Loss of *Odysseus* function affects male fertility by decreasing germ cell numbers in *Drosophila melanogaster*. Chau-Ti Ting[1], Ya-Jen Cheng[2], Shun-Chern Tsaur[3], Shu Fang[4]. 1) Department of Life Science; Institute of Ecology and Evolutionary Biology; Institute of Zoology; Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taiwan, ROC; 2) Institute of Molecular and Cellular Biology, National Tsing Hua University, Taiwan, ROC; 3) Department of Life Sciences & Institute of Genome Sciences, National Yang-Ming University, Taiwan, ROC; 4) Biodiversity Research Center, Academia Sinica, Taiwan, ROC.

Loss of a testis-specific expression of *Odysseus* (*OdsH*) has been shown to cause male fertility defect in *Drosophila melanogaster*, but the underlying mechanisms at the cellular level is unknown. To address the possible mechanisms and functional roles of *OdsH* in *spermatogenesis*, we compared the cell numbers at different developmental stages during spermatogenesis between the *OdsH* null mutant and wild-type flies. Our results showed that the number of early developing germ cells, including spermatogonia and spermatocytes, is reduced in the *OdsH* mutant males. In addition, the number of germline stem cells in aged males is also reduced, presumably as a result of the disruption of germline stem cell maintenance, which leads to more severe fertility defect. These findings suggest that the function of the enhancement of sperm production by *OdsH* is to act across all the ages of males.

586A

Determining the molecular roles of *CG4701* and *nmd* in *Drosophila melanogaster* spermatogenesis through analysis of β-tubulin and anillin localization. Bethany L Wagner, Sarah C Pyfrom, Karen G Hales. Department of Biology, Davidson College, Davidson, NC.

Mutations in *CG4701* cause recessive male sterility with defects at particular stages of spermatogenesis such as meiosis, meiotic cytokinesis, and early post-meiotic spermatids. *nmd* is a broadly-expressed and essential paralog of *CG4701*; two different hypomorphic *nmd* alleles also affect spermatogenesis. In *CG4701* mutants, we observed vacuolated Nebenkerne, some of which were abnormally large, suggesting meiotic cytokinesis defects. In one of the hypomorphic male-sterile nmd alleles, mitochondria do not aggregate properly so the Nebenkern never forms; in the other strain, there is a malfunction during cytokinesis, so it is phenotypically very similar to *CG4701*. CG4701 and Nmd belong to the AAA ATPase family of proteins and are related to spastin and katanin, known microtubule-severing proteins. Nmd localizes to mitochondria and centrosomes/basal bodies, and attempts are underway to localize CG4701. β-tubulin and anillin (a contractile ring component) are proteins essential to spermatogenesis, particularly in the stages most affected by *CG4701* and *nmd*. Since we hypothesized β-tubulin and anillin function to be impaired in the mutants, we compared their localization in normal

spermatogenesis to those in *CG4701* and *nmd* mutants using green fluorescent protein-tagged versions of the proteins. Curiously, we found that mutant flies with the *β-tubulin-GFP* transgene had a partially rescued phenotype, consistent with the suspected functional connection between Nmd and CG4701 and microtubules. We are using chemical fluorescent tubulin stains as an alternative for studying true microtubule structure in the mutants. In addition, we are using RNAi to ask whether gene knockdowns phenocopy *CG4701* and *nmd* mutant alleles. Preliminary data show no effect on spermatogenesis, so we are examining whether our chosen GAL4 driver (expressed very early in spermatogenesis) in fact drives efficient gene knockdown.

587B

Transcriptional Pausing Orchestrates A Rapid Antiviral Immune Response in *Drosophila*. Jie Xu[1], Gregory Grant[1], Leah Sabin[3], Beth Gold[1], Rui Zhou[2], Gregory Hannon[3], Sara Cherry[1]. 1) School of Medicine, University of Pennsylvania, Philadelphia, PA; 2) Dept. of Genetics, Harvard Medical School, Howard Hughes Medical Institute, Boston, MA; 3) Watson School of Biological Sciences, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Virus-host interactions are a delicate interplay of opposing forces: the virus subverts cellular machinery to promote its replication while the host mounts an immune response to eliminate the infection. Innate immunity is the first and most ancient line of defense, yet little is known about the cellular factors that restrict viral infection. Our lab uses RNAi screening in cells of *Drosophila melanogaster*, a model organism that only has an innate immune system, to identify novel antiviral factors. Of particular interest are pan-antiviral factors that restrict arthropod-borne viruses, for which there is significant morbidity and mortality worldwide due to the lack of vaccines or therapeutics. Using this approach, we screened a panel of genes involved in various aspects of RNA metabolism against 4 RNA viruses, including 2 arthropod-borne pathogens. Of the genes whose depletion by RNAi leads to increased infection by at least 3 of the 4 viruses, we found that two independent subunits of the Negative Elongation Factor (NELF) complex had pan-antiviral effects. Additional directed RNAi screening identified that key components of the NELF-mediated transcriptional pausing pathway is broadly antiviral. These pausing factors also play a critical role in antiviral restriction *in vivo*. We are now characterizing the mechanism of antiviral restriction for this gene regulatory pathway. Funded by RO1AI074951 to SC.

588C

Food poisoning: *lam* larvae with melanotic masses are sensitive to frassfood. Mitchell S. Dushay, Yi Cui, Samiat Jinadu, Harshit Khasa, Neena Majumdar, Thomas Matthews, Monica Samelson. BCHS, Illinois Institute of Technology, Chicago, IL.

When 1st instar lam^{D395}/Df larvae were transferred to fresh food, 33% showed melanotic masses in the 3rd instar. However, when they were left to develop in normal culture vials with their faster-growing *lam*/+ siblings, only 66% of *lam* larvae survived, and none of these had melanotic masses. Similar results were seen when larvae were transferred to frassfood; frass and medium that other larvae had churned through. Wildtype larvae were fine, but *lam* larvae with melanotic masses died. This was not medium-limited: frassfood prepared from Nutrifly® or potato, yeast, agar medium gave similar results. Nor was lethality due to inability to get to nutrition, since *lam* larvae with melanotic masses moved as much as *lam* larvae without masses. Instead, microbes, presumably from larval gut were responsible. When frassfood was treated with antibiotics or autoclaved, *lam* survival and melanotic mass incidence were similar to fresh food. Intriguingly, the sensitivity to frassfood of *lam* larvae with melanotic masses was not caused by a general susceptibility to gut infection. Adding *Serratia marcescens* Db1140 to fresh food reduced the survival of controls and both *lam* mutant larvae with- and without melanotic masses. The oral susceptibility of larvae with melanotic masses appears limited to *lam* mutants. Fewer *cact* and hop^{TUM} mutant larvae survived on frassfood than fresh food, but melanotic mass incidence was similar in larvae on frassfood to those on fresh food. Thus, there is a three-way interaction between *lam*, melanotic masses, and frassfood that relates to immune system function and interactions with gut microflora.

589A

***Streptococcus gordonii* is virulent and enhances the virulence of *Porphyromonas gingivalis* in *Drosophila melanogaster*.** Christina Igboin[1], Ann Griffen[2], Eugene Leys[1]. 1) Division of Oral Biology; 2) Division of Pediatric Dentistry, Ohio State University College of Dentistry, Columbus, OH.

We previously developed a *D. melanogaster* killing model to study the interactions between the periodontal pathogen, *Porphyromonas gingivalis*, and the host, and identified bacterial and host factors that are involved in infection. Mitis group *Streptococci*, including *S. gord*, are typically associated with periodontal health, however the bacteria possess features that may promote the development of a pathogenic oral flora. We used the model to examine *S. gord-Drosophila* interactions, and as periodontitis is a polymicrobial infection we examined the interaction between a mixed *S. gordonii-P. gingivalis* infection and *Drosophila*. The *Drosophila* killing model was used to, determine the virulence of wt *S. gord* strains, screen *S. gord* putative virulence genes for a role in *Drosophila* killing, screen immune-response-defective flies to identify immune response players, and compare the virulence of mixed (*Sg/Pg*)- and mono (*Sg*, *Pg*)-infections. The bacteria were introduced into the thoraces of *Drosophila* using 30G needles, and the number of live flies was recorded every 12 hours for 4 days. Multiple wt *S. gordonii* strains are pathogenic in *Drosophila* although with different killing kinetics, and *S. gordonii* SrtA (sortase A), SspAB (antigen I/II family proteins) and AbpAB (amylase binding proteins) are important for *Drosophila* killing. Contrary to what was previously observed with the Gram- *P.ging*, the *Drosophila* Toll pathway is involved in fighting a *S. gord* (Gram+) infection. Eiger (JNK pathway ligand) is also involved in fighting a *S. gord* infection. Finally, although *S. gord* and *P. ging* monoinfections are virulent, a mixture of *S.gord* and *P. ging* is significantly more virulent in *Drosophila*. A previous study in a rodent model also showed that a *S. gord-P. ging* mixed infection is more virulent than either bacterium alone. These data demonstrate that *S. gord* can cause pathology in the host, and can enhance the virulence of other oral bacteria. Supported by DE10467.

590B

The anti-wasp immune response across the genus Drosophila. Balint Z. Kacsoh, Todd A. Schlenke. Department of Biology, Emory University, Atlanta, GA.

One of the most common parasites of Drosophila in nature are parasitic wasps, which lay their eggs in Drosophila larvae and pupae. Drosophila melanogaster mounts an immune response against wasp eggs and larvae termed melanotic encapsulation, whereby hemocytes form a multi-cellular, multi-layered capsule around the intruder before turning it black with melanin. We were interested in whether this melanotic encapsulation response is conserved across the genus Drosophila, and also whether the same hemocyte cell types used by D. melanogaster are used by other Drosophila species. Thus, we assayed fly immune mechanisms and immune success in a panel of 25 Drosophila host species using a diversity of parasitic wasp species. We found that different Drosophila species have unique hemocyte types not found in D. melanogaster, and that certain unique hemocyte lineages are involved in wasp egg encapsulation. Furthermore, there appear to be at least three distinct mechanisms Drosophila species use to kill wasp parasites: melanotic encapsulation, encapsulation without melanization, and non-encapsulation. Our study uncovers newfound complexity in the immune responses of Drosophila species against parasitic wasps.

591C

S-Nitrosylation in Immunity and Fertility: A Mechanism Conserved in Plants and Animals. Krieng Kanchanawatee[1], Gary Loake[1], David Finnegan[2]. 1) Institute of Molecular Plant Sciences , Univeristy of Edinburgh, Edinburgh, United Kingdom; 2) Institute of Cell Biology, Univeristy of Edinburgh, Edinburgh, United Kingdom.

Post-translational modification is an intracellular process that modifies the properties of proteins to extend the range of protein function without spending energy in de novo peptide synthesis. S-Nitrosylation is a post-translational modification which adds nitric oxide (NO) to sulfhydryl group at cysteine residue to form S-nitrosothiol (SNO), and is required for plant immunity and fertility. Cellular NO changes between a pool of free NO and bound SNO. During pathogen infection, nitrosative stress in plants is mainly controlled by S-nitrosothiolglutathione reductase (GSNOR) via the decomposition of GSNO. GSNOR is an alcohol dehydrogenase type 3 (ADH3) which has both GSNOR and formaldehyde dehydrogenase (FDH) activities. The roles of S-nitrosylation in mammals overlap with those in plants. This conservation led us to explore the relationship between S-nitrosylation, immune response, and fertility in Drosophila melanogaster as it might prove to be a good genetic model for further analysis of the role of S-nitrosylation in animals. I have identified Fdh as the likely GSNOR in D. melanogaster and have knocked this out using an overlapping deficiency technique in order to observe the effect on immunity and fertility. There are two main pathways in the Drosophila innate immune response, the Toll pathway for protecting against gram-positive bacteria and fungi, and the IMD pathway against gram-negative bacteria. I have investigated the effect of removing GSNOR on sensitivity to gram-negative bacteria (Escherichia coli and Erwinia carotovora) by septic and natural infection, and to fungi (Beauveria brassiana) by natural infection. Susceptibility to infection by the gram negative bacteria was similar to wild-type but susceptibility to B. brassiana was increased. This suggests that GSNOR is required for the normal activity of the Toll pathway. We also observed that GSNOR knockout impairs fertility and development of embryos.

592A

Characterization of a candidate immune receptor in Drosophila. Erin S. Keebaugh, Todd A. Schlenke. Dept Biol, Emory Univ, Altanta, GA.

To gain a better understanding of non-self recognition between eukaryotes we study the interaction between fruitflies and one of their natural metazoan pathogens, parasitic wasps. 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. Drosophila larvae can mount a robust cellular immune response against the wasp eggs termed melanotic encapsulation, where fly hemocytes form a capsule around and kill the entrapped wasp egg. As a first step in the encapsulation response, the host must be able to recognize the parasite as foreign. We are interested in identifying the immune receptors Drosophila use to identify parasitic wasps as non-self, and uncovering the evolutionary history of such genes as a first step towards understanding the selective forces parasites impose on their hosts in nature. Microarray analysis of Drosophila larvae post-wasp attack identified several promising candidate immune receptors including a C-type lectin, *lectin-24A*. Expression analysis of this candidate immune receptor shows enriched expression in immune tissue and a wasp-specific regulatory response. We are currently investigating the effects of mutant levels of *lectin-24A* on hemocyte viability, structure, and ability to form melanotic capsules, and have designed experiments to test Lectin-24A's binding specificity.

593B

Altered metabolism influences survival from infection. Karla L. Lightfield, David Schneider. Microgiology and Immunology, Stanford University, Stanford, CA.

The ability of a host to survive infection is dependent upon two main classes of responses. First, the host uses resistance mechanisms to isolate and kill the pathogen to limit pathogen burden. Secondly, the host must employ a wide variety of mechanisms in order to survive the infection long enough for the resistance mechanisms to work. The host must both prevent and heal from damage caused by the infection itself and the resultant immune response. The host must also regulate its energy balance and metabolism such that it can properly mount an efficient immune response while at the same time maintaining other key physiological processes. Here we use the well-established model of infecting Drosophila melanogaster with Listeria monocytogenes to probe the crosstalk between metabolism and the immune response. Drosophila mutants lacking adipokinetic hormone receptor are less able to mobilize energy stores after being stressed. We show that although these mutants can initially restrict the growth of Listeria they eventually succumb to infection. This death occurs much faster than that of their wild-type counterparts that are capable of efficiently accessing their energy stores. In conclusion, despite the fact that these mutants can mount an effective immune response initially, their inability to properly regulate energy metabolism renders them less able to survive the infection.

594C

Recovery 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. To this end, we developed a 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. The median survival of flies infected with 100 colony-forming units of Listeria monocytogenes is 13 days, and without support the infection is inevitably fatal. Ampicillin treatment of infected flies eliminates L. monocytogenes, and the cured flies live as long as control flies. As the number of bacteria decrease, the expression of antimicrobial peptides decreases and returns to baseline levels. We are currently analyzing gene expression changes across the full course of infection in ampicillin treated and untreated infected flies. This dataset will let us track transcription through high dimensional phase space along the full course of infection. Gene expression patterns that differ between flies that die and flies that recover will help to identify recovery mechanisms.

595A

Fat metabolic effects to immune responses in Drosophila melanogaster. Kyung Han SONG, David Schneider. Microbiology and Immunology, Stanford University, Stanford, CA.

Our goal is to understand whether metabolic disruptions that occur during infections aid survival or contribute to pathology. We use an infection model in which we challenge fruit flies with Listeria monocytogenes. Preliminary work from others in the lab suggested that fatty acid biosynthesis decreases during infection. We followed up on this work by testing mutants in this biosynthetic pathway for immune phenotypes. Triglyceride is synthesized by the addition of fatty acids to a glycerol backbone by fatty acyl transferases. We tested three of these enzymes and found a difference in their immune behavior. Normally

Drosophila melanogaster is able to control the extracellular growth of L. monocytogenes through two innate immune effectors, antimicrobial peptides and melanization. Flies mutated for the enzymes that convert monoacylglycerol to diacylglycerol (CG4729, CG4753) and diacylglycerol to triglyceride (midway) died faster than control flies. However expression level of antimicrobial peptides in midway mutant fly was not significantly different compared to controls. We monitored levels of triglyceride and glucose and found that they were significantly lower in mutant flies. Listeria infected flies lose fat stores. Our hypothesis is that infected midway mutants lost triglyceride and died more rapidly than wild type flies as the mutants reached the end of their energy stores more rapidly.

596B

***Spiroplasma* in *Drosophila melanogaster* populations: prevalence, male-killing, molecular identification and no association with *Wolbachia*.** Iuri M. Ventura[1], Ayana B. Martins[1,2], Mariana L. Lyra[1,3], Carlos C.A. Andrade[4], Klélia A. Carvalho[1], Louis B. Klaczko[1]. 1) Dept. Genética e Evolução, Universidade Estadual de Campinas, SP, Brazil; 2) Dept. de Ecologia, USP, SP; 3) Dept. de Zoologia, UNESP, SP; 4) Dept. de Biologia Marinha, UFF, RJ.

Spiroplasma endosymbionts are maternally transmitted bacteria that may kill infected sons resulting in the production of female-biased broods. The prevalence of male killers varies considerably both between and within species. Here, we evaluate the spatial and temporal status of male-killing and non-male-killing *Spiroplasma* infection in three Brazilian populations of *D. melanogaster*, nearly a decade after our lab reported the first world-wide occurrence for this species. The incidence of the male-killing *Spiroplasma* ranged from close to 0 to 17.7% (so far the highest estimate for a *Drosophila* species) with a suggestion of temporal decline in one of the studied populations. We also found non-male-killing *Spiroplasma* in lower prevalence (3% to 5%) in one population; and we did not detect it in the other two. This may be taken as a suggestion of a spreading advantage conferred by the male-killing strategy. Sequencing two loci, we identified the phylogenetic position of eleven *Spiroplasma* strains from the three localities, showing that all strains group closely in the *S. poulsonii* clade. We were able to test the association between *Spiroplasma* infections and another widespread endosymbiont, *Wolbachia*, whose prevalence ranged from 81.8% to 100%. The prevalence of *Wolbachia* did not differ between *Spiroplasma* infected and uninfected strains in our largest sample, nor were the two endosymbionts' prevalences associated across localities. We also assayed the male-killing effect of seven *Spiroplasma* strains in a standard Canton-S *D. melanogaster* strain. All strains induced a complete male-killing phenotype. In this scenario, a combination of other host and environmental conditions, as well as population historic factors, may be responsible for establishing the prevalence heterogeneity observed in the field.

597C

The *Drosophila* protein Mustard regulates targets of Relish. Paula Ivonne Watnick, Zhipeng Wang, Cristin Berkey. Division of Infectious Diseases, Children's Hospital Boston, Boston, MA. 02115.

As part of a genetic screen, we isolated a P-element insertion mutant with resistance to oral *Vibrio cholerae* infection. Here we identify the protein Mustard (Mtd) that is responsible for the resistance phenotype of the insertion mutant. Mtd contains a LysM domain, thought to be important for carbohydrate recognition, and a TLDc domain, whose function is unknown. We show that a short, nuclearly localized Mtd isoform comprised almost entirely of the TLDc domain inhibits multiple targets of the *Drosophila* NF-κB homolog Relish and provide genetic evidence for an interaction between these two proteins.

598A

Priming with S. pneumoniae infection causes changes in gene expression in Drosophila melanogaster. Junaid Ziauddin, David Schneider. Microbiology & Immunology, Stanford Univ SOM, Stanford, CA.

We found *Drosophila melanogaster* can raise a stronger specific immune response to *Streptococcus pneumoniae*, *Beauveria bassiana* or *Serratia marcescens* when the flies have been previously exposed to sublethal doses of each microbe. We call this phenomenon priming. Since flies lack B and T cells, to some it seems theoretically impossible that flies could have an adapting immune response. Data trumps theory. Still, we need to find a mechanistic explanation for this phenomenon, which has now been seen in a variety of insects. We injected *Drosophila* with a sublethal dose of dead *S. pneumoniae*, waited for 3 days, re- injected the flies, and measured fly survival, bacterial growth rates and gene expression. We performed a microarray analysis to identify genes modulated by priming during a *S. pneumoniae* infection. There are many changes in primed flies and we found multiple classes of genes that are modulated following priming: some whose expression rise in only early infection, some whose expression increase at later time points, and some whose expression are reduced post-infection. We followed 34 transcripts at a high level of resolution using QRT-PCR of tight timelines of infected primed and naïve flies. We conclude that environment changes the immune response of the fly and this includes past exposure to microbes. We hypothesize that these changes are evolved to be adaptive, where the fly's immunity changes in a fashion that allows it to respond more effectively to that infection in subsequent exposures. Most of these genes have not been implicated in immunity previously and we are exploring how the altered expression of these genes could lead to altered immunity.

599B

Modeling the effects of altered gravity on the immune response using Drosophila. D Kimbrell[1], C Fuller[1], L von Kalm[2], K Beckingham[3], M George[1], J Parker[4], M Thomson[5], D Hoshizaki[6], A Gibbs[6], J Alley[7], K Taylor[1], P Fuller[1], K Kleinhesselink[1], A Hammonds[8], R Morgan[2], T Smallwood[2], A Kloehn[1]. 1) Univ California, Davis; 2) Univ Central Florida, Orlando; 3) Rice Univ, Houston, TX; 4) Expression Analysis, Inc; 5) Vanderbilt Univ, TN; 6) Univ Nevada, Las Vegas; 7) Laverlam Inter Corp, MT; 8) Lawrence Berkeley Nat Lab, CA.

Astronauts have immune dysfunction during and after spaceflight that is a serious health concern for expanding the human exploration of space. A fundamental understanding of the relationship between altered gravitational fields (g) and the immune system is required in order to enable this exploration. Toward this understanding, we initiated study of the well-established Drosophila immunity model. First we analyzed hypergravity, and these results were fast-tracked to microgravity flight studies on the space shuttle Discovery STS-121. At hyper g vs. normal 1g, wild type and immune defective flies infected with B. bassiana fungus survived at significantly higher levels. Gravitaxis mutants were tested to begin discerning this as host and/or pathogen related. Infected gravitaxis yuri mutants were different, surviving the same at hyper and 1g. UAS-yuri rescue reverted survival back to higher at hyper g. These results indicate that hyper g has a positive effect on host post-infection survival. In microgravity-related experiments, flies that developed during flight on the space shuttle were returned to earth and infected with B. bassiana or E. coli. Gene expression was assessed by microarray analyses. Development in space altered many gene networks. For infection, antimicrobial protein gene induction was more normal in response to bacteria than fungus. However, the profiles

of transcriptional modulation to both types of infection for both protein-encoding and miRNA genes were significantly different in the space-raised flies compared to ground controls. Overall, the spaceflight data correlate with a differential effect based on the infecting microbe and the Imd and Toll pathways.

600C

Trade-offs and immune defense: the effect of mating and reproduction on immunity in female *D. melanogaster*. Sarah M. Short, Mariana F. Wolfner, Brian P. Lazzaro. Field of Genetics & Development, Cornell University, Ithaca, NY.

Immune defense is costly and is involved in multiple trade-offs with life history traits. A major barrier to understanding how immune defense functions at a whole-organism level is our lack of knowledge of the physiological bases of these trade-offs. In this work, we demonstrate that female *Drosophila melanogaster* suffer reduced immune defense after mating, an observation that is consistent with a trade-off between reproduction and immunity. We have determined that, while females are highly genetically variable for the degree of immunosuppression they experience after mating, males are not significantly variable in the level of immunosuppression they elicit in their mates. We also failed to detect a genetic interaction between males and females. These data are not consistent with an inter-sexual interaction such as sexual conflict, and instead suggest that the evolution of this trait is likely to involve an evolutionary trade-off between female reproductive traits and the humoral immune response. Were this to be the case, we would predict that lower immune defense after mating should give some benefit in terms of reproductive fitness. We are therefore currently testing for a negative genetic correlation between post-mating immune defense and short-term egg production. We are also investigating the mechanistic basis of post-mating immunosuppression. Using a series of reproductive mutants, we have shown that transfer of both sperm and sex peptide (a seminal fluid protein) are crucial to elicit post-mating immunosuppression in females. We have also found that females that fail to produce eggs demonstrate no effect of mating on immune defense, and that mated females have significantly lower levels of expression in multiple antimicrobial peptide genes after infection compared to virgin females. We are assaying changes in gene expression on a genome-wide basis in mated and unmated, infected and uninfected individuals to gain additional insight into the physiological and genetic intersections between reproduction and immune defense.

601A

Investigating the alleles responsible for immune natural variation of Drosophila melanogaster. Alejandra Guzman, David Schneider. Microbiology and Immunology, Stanford University, Stanford, CA.

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. This study aims to discover alleles responsible for natural variation in Drosophila melanogaster immunity. Until recently, immunity has largely been studied with non-natural mutations of essential immune genes (e.g. Toll and Imd) and little focus has been given to the genes and alleles that cause the natural variation of immunity. In 2004, Lazzaro et. al. (Science) showed that in D. melanogaster there are polymorphisms in the regions surrounding previously described immunity genes. Our work uses an unbiased approach to discover alleles responsible for the natural variation in D. menlaogaster immunity. In this study, we use a panel of 162 fully sequenced inbred Drosophila lines derived from a single population in North Carolina called the Drosophila Genetic Reference Panel. We infect these homozygous lines with Listeria monocytogenes and measure changes in fly survival and bacterial growth. Using this data we perform quantitative trait loci (QTL) analysis to find natural genetic polymorphisms responsible for variation in immunocompetance.

602B

Regulation of Hematopoietic Stem-Like Cell Multipotency in *Drosophila*. Hongjuan Gao, Xiaorong Wu, Nancy Fossett. Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine, Baltimore, MD.

Studies using *Drosophila* have contributed significantly to our understanding how stem cells are regulated *in vivo*. Work from our laboratory has shown that the Friend of GATA protein, U-shaped, regulates stem-like cell potency and differentiation. U-shaped expression is expressed in stem-like cells and downregulated in a sub-population of terminally differentiated blood cells. Our studies indicate that U-shaped maintains the stem cell population by blocking differentiation and that *u-shaped* expression is controlled by a complex regulatory network that includes the JAK/STAT and TGF-β signal transduction pathways. Collectively, these findings suggest that U-shaped is an important component of the stem cell regulatory machinery and may act as a key regulatory node that integrates multiple signals from the surrounding microenvironment to control stem-like cell potency and differentiation. To extend these studies, we are investigating the downstream effectors of the GATA:FOG complex that control stem-like cell multipotency. Given the functional conservation of Friend of GATA proteins and the role of the GATA:FOG complex in the control of cell-fate choice, our studies may identify novel interactions between conserved factors that control vertebrate stem cell fate choice.

603C

Steroid modulation of immune function in *Drosophila*. Jeanette E. Natzle, Patrick Finnegan, Damian Kuo, Phi Nguyen, Deborah Kimbrell. Dept Molec & Cellular Biol, Univ California, Davis, Davis, CA.

Several lines of evidence support a link between steroid hormones and immune cell regulation in *Drosophila*. A number of early studies implicated 20-hydroxyecdysone (20E) in regulation of blood cell development and function, however little is known about how the steroid response is integrated with other signaling pathways and what steps in control of blood cell growth and differentiation are controlled by steroids. Our preliminary studies are consistent with a requirement for a functional cell-autonomous 20E-response pathway within *Drosophila* immune cells. We have inhibited the activity of the Ecdysone Receptor (EcR) within immune cells by using several primarily immune-cell specific GAL4 drivers (Dorothy-Gal4, Hemese-Gal4, Hemolectin(HML)-Gal4) to express a UAS-regulated EcR-RNAi construct (UAS-CA104; C. Antoniewski) in hemocytes and/or lymph glands. Dorothy-Gal4 and Hemese-Gal4 in combination with UAS-CA104 lead to almost complete pupal lethality. A high proportion of adult escapers and arrested pupae show melanotic nodules, a hallmark of aberrant hemocyte proliferation, differentiation and function associated with a state similar to chronic inflammation. Immunostaining of Gal4-expressing hemocytes from pupae verified that EcR in the nucleus is absent or substantially depleted in the HemeseGal4; UAS-CA104 circulating hemocytes.In contrast, immunostaining of pupal UAS-CA104; HML-Gal4 hemocytes revealed a substantial level of nuclear EcR expression, presumably due to a lower level of RNAi expression produced by the Gal4 driver in this line. Consistent with the significant residual EcR function, the UAS-CA104; HML-Gal4 flies appear normal. Taken together, these results correlate loss of ecdysone signaling in the immune cells to aberrant function. In the absence of

steroid input, we observe a severe phenotype (melanotic nodule formation and reduced survival to adulthood) which may be related to defective immune function.

604A

Wolbachia show asymmetric localization to embryonic and larval neuroblasts and target specific neuronal cell bodies in the D. melanogaster adult brain. Roger Albertson[1], Rachel Leads[1], William Sullivan[2]. 1) Albion College, Albion, MI; 2) University of California at Santa Cruz, Santa Cruz, CA.

Wolbachia is a maternally transmitted bacterial endosymbiont that infects germline and somatic tissues of arthropods. We show that in both D. melanogaster and D. simulans, various strains of Wolbachia including wRiv, wMel, and wPop, asymmetrically localize to neuroblast stem cells during embryonic and larval development. Later in development, Wolbachia localize to specific areas of the adult brain, causing a high titer in those areas. Through confocal microscopy and fluorescent imaging of D. melanogaster adult brains, we show that wMel and wPop localize to neuronal cell bodies but not to axons, such as mushroom body axons. Elav and phalloidin staining suggests that wMel and wPop hyperproliferation within host cells leads to cell lysis. This is most striking in hosts infected with wPop. Relative to embryos and newly eclosed adult flies, hyperproliferation of wPop in the D. melanogaster CNS is most prominent after seven days of adult development.

605B

Evolutionary analysis of the *bag of marbles* gene reveals an interaction with *Wolbachia*. Heather A. Flores, Daniel A. Barbash, Charles F. Aquadro. Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY.

Drosophila germline stem cells (GSCs) can both self-renew and differentiate to give rise to oocytes or sperm thus making them the evolutionary target of mutations and pathogens trying to ensure their transmission. We have shown that multiple genes involved in GSC regulation are experiencing rapid, adaptive protein evolution in *Drosophila melanogaster* and the closely related species, *D. simulans*, suggesting that it is beneficial for these proteins to accumulate amino acid changes. We have focused on one of these adaptively evolving genes, *bag of marbles* (*bam*), to understand the functional consequences of this adaptive evolution. The best characterized function of *bam* is initiating GSC differentiation in ovaries. We are using interspecies complementation to test whether adaptive evolution of *bam* has caused detectable functional differences. We have assayed the ability of a *bam* ortholog from *D. simulans* to complement the male and female sterility associated with a *bam* mutation in *D. melanogaster*. We have found that the *D. simulans bam* ortholog can complement male sterility but fails to fully complement the female sterility in *D. melanogaster*. These data suggest that the evolutionary force driving the diversification of *bam* may be focused on the female germline, and we hypothesize this force may be conflict with bacterial endosymbionts due to their maternal inheritance and reproductive manipulation. The endosymbiont *Wolbachia pipientis* is an obligate, intracellular bacterium that has been shown to manipulate the germline in a variety of insects. To determine if any interaction existed between *bam* and *Wolbachia*, we tested the ability of *Wolbachia* to suppress *D. melanogaster bam* hypomorphic mutants and found that the presence of *Wolbachia* can suppress *bam* female sterility. We also found that *Wolbachia* can enhance the female fertility in flies with *D. simulans* transgenic *bam* in our complementation assay. We are currently examining the nature of the interaction between *bam* and *Wolbachia* to try and understand the mechanism of suppression.

606C

Density of *Wolbachia* in the host insect impacts antiviral protection. Karyn N Johnson[1], Sheree E Osborne[1], Jeremy C Brownlie[2]. 1) School of Biological Sciences, The University of Queensland, Brisbane, Queensland, Australia; 2) School of Biomolecular and Physical Sciences, Griffith University, Brisbane, Australia.

Recently the maternally inherited endosymbiotic bacteria *Wolbachia* has been shown to protect insects from a range of microbial and eukaryotic pathogens. *Wolbachia*-mediated antiviral protection has been demonstrated in *Drosophila* and mosquitoes against RNA viruses, including *Drosophila C virus* and *Dengue virus*. To explore the mechanism of antiviral protection, we screened five diverse strains of *Wolbachia* within their naturally associated *Drosophila simulans* hosts, to determine if antiviral protection occurred across all *Wolbachia* strains. Although three of the *Wolbachia* strains delayed viral induced mortality, the other two strains did not. The three strains that mediated antiviral protection were more closely related and more abundant in the host than the two non-protective strains. We investigated the importance of *Wolbachia* density within the insect host on antiviral protection. To do this, we used low doses of antibiotic to decrease the density of a protective *Wolbachia* strain in its natural host, to levels that were similar to those observed in the two non-protective *Wolbachia* strains. Flies were then challenged with the pathogenic *Drosophila C virus*. It was found that when the density of the previously protective *Wolbachia* strain *w*Au was decreased, *Wolbachia*-mediated antiviral protection was substantially decreased or lost completely. This data suggests that the density of *Wolbachia* in the insect host is important for the mechanism of antiviral mediated protection and suggests that insects carrying low load of *Wolbachia* will likely not be protected. These findings may facilitate prediction of *Wolbachia*-mediated protection in host-*Wolbachia* associations relevant for the biocontrol of insect-borne viruses.

Poster Full Abstracts - Neurophysiology and Behavior

Poster board number is above title. The first author is the presenter

607A

A new model of chronic social defeat in *Drosophila melanogaster*. David Popovic[1], Jill Penn[1], Justin Dalton[2], Michelle Arbeitman[2], Edward Kravitz[1]. 1) Neurobiology, Harvard Medical School, Boston, MA; 2) College of Medicine, Florida State University, Tallahassee, FL.

Behavioral changes following the loss of a fighting contest are exhibited in species throughout the animal kingdom. After experiencing a single loss *Drosophila* become less aggressive and lose subsequent fights. In mammals, chronic social defeat has been used as a model of depression- and anxiety-like behaviors. Here we have developed a model of chronic social defeat in *Drosophila*. In order to increase the likelihood of a loss, Canton S males fought against a larger fly from a hyper-aggressive strain, once a day for six consecutive days. Those males that lost all six fights were then paired in a test fight against an age-, size- and genetically-matched socially naïve male on the seventh day. Twenty-four hours later the same males were tested in a sucrose preference assay. Most flies displayed decreased aggression and subsequently lost their test fights, suggesting that they were susceptible to the experience of chronic social defeat. These same flies also had a decreased sucrose preference and a decrease in their overall food intake. Moreover, we found that these effects last at least one week after the six-day training period. We also found a resilient subpopulation of chronically defeated flies that behaved like control flies in both test fights and sucrose preference assays, suggesting that they are resistant to chronic defeat. In order to determine the genetic mechanisms underlying these two populations, we are now performing RNA-Sequencing analyses on susceptible and resilient chronically defeated flies (This work was supported by grants from NIGMS to EAK, and from The German National Merit Foundation and The Max-Weber-Program to DP).

608B

Effects of RNAi-Mediated Suppression of Odorant binding protein 56h on Aggression and Mating Behaviors. John R. Shorter[1], Kavita Sharma[2], Anandasankar Ray[2], Robert R. Anholt[3], Trudy F. Mackay[1]. 1) Genetics, North Carolina State, Raleigh, NC; 2) Entomology, University of California, Riverside, CA; 3) Biology, North Carolina State, Raleigh, NC.

Odorant binding proteins play an important role in chemosensation by transporting odorants to the receptor in the olfactory membrane. Previous research has shown that disruption of odorant reception will alter chemosensory perception and change behavior. Here, we knock down Odorant binding protein 56h (Obp56h) using RNA interference (RNAi). Gene expression of Obp56h is dramatically reduced in the RNAi line relative to the control. We performed a comprehensive analysis of behaviors in the Obp56h RNAi and the control. We found that that RNAi-mediated suppression of Obp56h reduces male-male aggression and increases the frequency of mating. The effects of Obp56h knock down on startle response, geotaxis, and phototaxis are very small compared to the effects on aggression and mating behavior. We are currently performing RNA-Seq and single-unit recordings from the trichoid sensilla of RNAi and control lines and will present these results. We hypothesize that Obp56h may be transporting an odorant for “maleness” or a signal for male quality.

609C

Drosophila olfactory-related preferences to diverse yeast volatiles profiles. J R. Arguello[1], Carolina Sellanes Parodi[2], Yann-Ru Lou[3], Andrew Clark[1], Robert Raguso[4]. 1) Mol Bio & Genetics, Cornell, Ithaca, NY; 2) Laboratorio de Ecología Química, Universidad de la República, Uruguay; 3) Dept. Of Plant Biology,Cornell, Ithaca, NY; 4) Dept. of Neurobio and Behavior,Cornell, Ithaca, NY.

Our understanding of the molecular and cellular basis of Drosophila chemosensation has been progressing rapidly. These advances enable evolutionary analyses aimed at understanding within and between species variation in these physiological pathways, and simultaneously provide motivation for furthering our understanding of the ecological contexts in which the pathways operate. We have combined chemical ecological and behavioral approaches to investigate olfactory-related fly preferences in response to a second model genetic system: yeast (S. cerevisiae), a known attractant and food source for Drosophila. Our experiments were designed to 1) test for population variation in yeast volatile production, 2) test for population variation in D. melanogaster preference towards differing yeast volatiles, and 3) test for preference differences between D. melanogaster and two closely related non-specialist species, D. simulans and D. yakuba. Our chemical approach relied on 14 worldwide yeast accessions within a randomized block-common garden design, and headspace sampling of volatiles using GC-MS. The results reveal extensive variation in volatile composition, particularly in 2-phenylethanol, ethyl esters of aliphatic acids, and acetoin; these compounds have previously been shown to be Drosophila olfactory ligands either directly or indirectly. To test for variation in fly preference to these varying volatile profiles, we have carried out single-fly choice experiments between the most variable yeast samples using a 4-armed olfactometer, and have quantified behavior throughout the experiments by video analyses of their tracks. Our results indicate both within and between species differences in responses to several of these profiles, and point to ecologically relevant compounds as modulators of these behavioral differences.

610A

The circadian neuropeptide PDF couples preferentially to a specific adenylate cyclase isoform. Laura B. Duvall, Paul H. Taghert. Department of Anatomy & Neurobiology, Washington University in St Louis, St Louis, MO.

The neuropeptide Pigment Dispersing Factor (PDF) coordinates *Drosophila* pacemaker cells and is essential for normal circadian function. However, specific signaling components downstream of the PDF receptor (PDFR) remain unknown. Using live imaging of intact fly brains and transgenic RNAi methods, we identify the particular adenylate cyclase (AC) isoform that is associated with PDF signaling in small LNv cells (also termed M cells). Genetic disruptions of Gsα60A including both RNAi knockdown and overexpression alter responses to both PDF and the related peptide dh31. In a screen of all *Drosophila* AC isoforms, knockdown of a specific adenylate cyclase (AC3) disrupted PDF responses specifically in small LNv cells. Manipulations of AC3 disrupt PDF, but not dh31 responses. Loss of PDF responsiveness was rescued by restoring appropriate levels of AC3. Flies with AC3 alterations show circadian dysfunction consistent with known roles of M cells. In addition, knockdown of the *Drosophila* AKAP-like scaffolding protein nervy reduces PDF responses and disrupts circadian behavior. Together these results indicate that within small LNv cells, the PDF receptor couples preferentially to a single AC, and that critical pathways of circadian synchronization are mediated by highly specific second messenger components.

611B

Extreme Light Sensitivity in *Drosophila*. P. Fozdar, J. Coupar, S. Hughes, P. Vinayak, W. Brasher, J. Kilby, J. Hirsh. Dept. of Biology, University of Virginia, Charlottesville, VA 22904.

We study the basis for the extreme light sensitivity of Drosophila melanogaster. Drosophila can entrain to LD cycles, and show positive behavioral masking, at light intensities below the limit of human perception. We find that both visual input via the R1-6 rhabdomeres and input from cryptochrome are

important for both circadian entrainment and behavioral masking, though visual input is more strongly involved in masking. To relate these studies to phase shifts resulting from light pulses during subjective night, we exposed flies to light pulses of equal numbers of photons given over different time intervals. Flies respond with graded phase changes to light pulses of graded intensity given over intervals from 0.1 to 360 min. Light sensitivity increases with time interval over this range when flies are exposed to equal numbers of photons, indicating that photon input to the circadian system can integrate and store photon information with high efficacy over many hours. We further study the dependence of this integration on visual versus cryptochrome photopigment input.

612C

Calcium and cAMP signaling in the prothoracic gland and its role in the circadian timing of Drosophila emergence. Angelina Palacios-Muñoz, John Ewer. Laboratory of Neurogenetics and Development, Interdisciplinary Center of Neuroscience of Valparaíso, University of Valparaíso, Chile.

In Drosophila melanogaster the circadian clock regulates the timing of behavior and physiology. The core mechanisms that control rhythmic behaviors consist of clock genes, which generate circadian molecular oscillations in pacemaker cells. Yet, how the activity of these genes causes outputs to be rhythmic is poorly understood. The brain contains pacemaker neurons that regulate the circadian rhythm of locomotor activity. The prothoracic gland (PG) is a peripheral endocrine tissue that synthesizes the molting hormones, which control metamorphosis. The PG also expresses clock genes and contributes, together with the brain clock, to the circadian timing of adult emergence (eclosion). We investigated the pathway through which the PG causes the pattern of Drosophila eclosion to be rhythmic by determining whether calcium or cAMP levels vary in this peripheral clock during the course of the day. To do so we monitored calcium levels by measuring the intensity of fluorescence of a genetically-encoded calcium-sensitive GFP (GCaMP) specifically expressed in the PG using the GAL4/UAS system. Similarly, we measured cAMP levels using the genetically-encoded cAMP sensor, Epac (Exchange protein directly activated by cAMP). Our results show that there are consistent changes in the levels of calcium and cAMP during the course of the day. Furthermore, our genetics studies show that the circadian rhythm of eclosion can be disrupted by interfering in the PG with the expression of clock genes and genes in the calcium and cAMP pathways,. Thus, ours results suggest that calcium and cAMP signaling expresses a circadian rhythm and may contribute to the circadian regulation of adult emergence.

613A

The transcription factor Mef2 is a key link between central clock, neuronal firing and the circadian regulation of axonal remodeling in Drosophila. Anna Sivachenko, Yue Li, Katherine Abruzzi, Michael Rosbash. Dept Biol, Brandeis Univ, Waltham, MA.

The transcription factor Mef2 is a key regulator of muscle development, and mammalian Mef2 homologues regulate synaptic plasticity and neuronal morphology. We identified Drosophila Mef2 mRNA in a screen for cycling transcripts enriched in the s-LNvs, key brain pacemaker neurons. Because these neurons have been shown to undergo a circadian cycle of axonal remodeling, we tested the role of Mef2 and showed that it is required within the s-LNvs for this fasciculation-defasciculation cycle as well as for activity-dependent remodeling. Moreover, the master circadian transcription complex CLK/CYC directly regulates Mef2 transcription. ChIP-Chip analysis identified circadian binding of Mef2 to the chromatin of numerous target genes implicated in neuronal plasticity, such as Fas2 and Fmr1. Genetic epistasis experiments showed that this regulatory hierarchy of rhythmic transcriptional regulation, CLK/CYC->Mef2->key output genes, governs the circadian fasciculation cycle of the s-LNvs. Lastly, we showed that Mef2 is the first transcription factor partner of the CLK/CYC complex; they synergize on a subset of CLK/CYC target genes, including the prominent circadian genes vri and Pdp1. Our results indicate that Mef2 serves a key role for circadian output functions and possibly for the core clock itself.

614B

Identification of sex-specific transcriptome differences by RNA-sequencing. Michelle N. Arbeitman[1], Simon Knott[3], Justin Fear[2], Lauren McIntyre[2], Justin Dalton[1]. 1) College of Medicine, Florida State Univ, Tallahassee, FL; 2) Molecular Genetics and Mircobiology, Genetics Institute, University of Florida, Gainesville, FL; 3) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

In *Drosophila melanogaster* the sex determination hierarchy specifies nearly all differences in somatic tissues. This hierarchy consists of a pre-mRNA splicing cascade that culminates in the production of sex-specific transcription factors, encoded by *doublesex* (*dsx*) and *fruitless* (*fru*). While there have been several genomic-scale efforts to identify the genes regulated by these transcription factors, the previous techniques lacked the sensitivity and resolution to identify many of the molecular events regulated by the sex hierarchy. RNA-sequencing (RNA-seq) technology provides an unprecedented opportunity to identify differences in overall transcript abundance, and isoform-specific transcript abundance differences with high resolution. We have used RNA-seq to analyze gene expression in tissues from animals mutant in the sex hierarchy genes and wild type animals. Analysis of the results identified hundreds of genes and their isoforms regulated by this hierarchy, which had not been previously identified. Additionally, we have been using systems level approaches to gain insight into the regulatory network downstream of *fru*, to understand how the genome is deployed to bring about sex-specific differences.

615C

Requirement of the Flightin Amino Terminal Sequence for Flight and Species-Specific Courtship Song in *Drosophila melanogaster*. Samya Chakravorty, Veronica Foelber, Bertrand Tanner, Jim Vigoreaux. Department of Biology, University of Vermont.

Flightin is a myosin binding protein that in *Drosophila melanogaster* is expressed exclusively in the indirect flight muscles (IFM). Flightin increases thick filament stiffness and is essential for sarcomere stability and flight. In addition to flight, IFM is activated during male courtship song, but its precise contribution has not been investigated. Here we show that courtship song is abolished in fln^{0}, a null mutation in flightin that also abolishes flight. Among Drosophilids, the N-terminal flightin sequence is poorly conserved (~15% identity) compared to the rest of the protein (>70% identity). Given the role of courtship song in mate selection and speciation, and the observation that many genes involved in sexual selection are positively selected, we hypothesize that hypervariable flightin N-terminus influences species-specific mating song parameters. To test this, we created transgenic *D. melanogaster* strains expressing a truncated flightin missing 62 N-terminal amino acids ($fln^{\Delta 2\text{-}63}$) and compared its flight and song properties to a wild-type rescued null control (fln^{+}). $fln^{\Delta 2\text{-}63}$ is slightly flight compromised compared to fln^{+} (flight index: 3.12±0.34 vs. 4.2±0.36, respectively), but has similar tethered wingbeat frequency (~ 200 Hz) and normal myofibrillar structure as determined by electron microscopy. Courtship song analysis showed that, compared to fln^{+} males, $fln^{\Delta 2\text{-}63}$ males produce songs with longer interpulse intervals (IPI, 60±6 ms vs. 40±1.7 ms) and higher sine song frequencies (220±3.2 Hz vs. 149±10.2 Hz). Other studies have

shown that IPI contributes to species recognition and con-specific mating. Our data shows that flightin N-terminal sequence has a stronger effect in song production than in flight ability. We propose that flightin fulfills a dual function, enhancing flight power output for survival and influencing song parameters important for pre-mating isolation, through separate protein domains that are under distinct evolutionary constraints. Positive or sexual selection acting on N-terminal flightin sequences may explain its hypervariability.

616A

***Drosophila* female precopulatory behavior is modulated by ecdysteroids.** Geoffrey Ganter[1], Joseph Desilets[1], Jessica Davis-Heim[1], Alexandra Panaitiu[1], Mark Sweezy[2], Joseph Sungail[1], Leonard Tan[1], Aurora Adams[1], Elizabeth Fisher[1], Joselle O'Brien[1], Kelsey Kincaid[1], Ralf Heinrich[3]. 1) Department of Biology, College of Arts and Sciences, University of New England, Biddeford, Maine, 04005, USA; 2) Department of Pharmaceutical Sciences, School of Pharmacy, Saint Joseph College, West Hartford, Connecticut, 06117, USA; 3) Department of Cellular Neurobiology, Institute for Zoology, Georg-August-University, Göttingen, Germany.

The effect of ecdysteroid signaling on *Drosophila melanogaster* female precopulatory behavior was interrogated using two types of mutants. The first featured reduced expression of ecdysone receptor in sex-specific fruitless neurons (Gal4-targeted RNAi allele). In the second, global ecdysteroid levels were reduced after successful development to adulthood using the temperature-sensitive allele *ecdysoneless^{1}*. While being courted by wild-type males, both types of mutant females performed significantly less full ovipositor extrusion behavior to reject male courtship progression. Wild-type levels of rejection behavior were partly restored in *ecdysoneless^{1}* females by feeding of 20-hydroxyecdysone. In addition, ecdysteroid depleted females (*ecdysoneless^{1}*) performed male-like courtship behaviors, including unilateral wing extension and song production with patterns very similar to male courtship song. Since ecdysteroid levels provided by this temperature sensitive allele were sufficient for successful larval and pupal development and were experimentally manipulated only after the females reached adulthood, the altered behavior observed may indicate acute effects of steroid level on an otherwise normal nervous system. These results support the hypothesis that ecdysteroids modulate female sexual behavior, perhaps acting as a regulator of sexual motivation, and as a component affecting the selection of sex specific behavior patterns.

617B

The seminal protein, ovulin, increases ovulation behavior through signaling of OA neurons. Clifford D. Rubinstein, Mariana F. Wolfner. Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY.

Broad behavioral changes are triggered in *Drosophila* females upon mating, and seminal proteins are important induces of these changes this process. However, little is understood about how these male proteins interact with female physiology to influence female behavior. The seminal fluid protein ovulin increases ovulation rates during the first 24 hours after mating. We tested whether it does so through neural signaling systems. Since the neuromodulator octopamine (OA) is known to regulate *Drosophila* ovulation, we hypothesized that ovulin may work through OA neuronal signaling. We found that increased OA neuronal activity in females could rescue the ovulation decrease normally observed in mates of ovulin null males, suggesting ovulin acts upstream of OA neuronal signaling. Consistent with ovulin acting through OA neurons, we found that the mating-dependant decrease in basal contraction state of oviduct muscle requires ovulin. Presumably, this relaxes the oviduct to facilitate movement of an egg from the ovary to the oviduct. Our results suggest that ovulin acts to increase OA signaling at the oviduct neuromuscular junction (NMJ). Kapelnikov et al. (2008 *BMC Dev Biol*) previously reported a mating-dependent increase in bouton number at the oviduct NMJ, a morphological measurement of synaptic strength. We are testing whether this synaptic response to mating is due to ovulin action. Together, our results suggest that ovulin acts as a neuromodulator between the male and the female to either increase OA neuronal activity or increase OA signaling at the oviduct NMJ.

618C

Behavioral Plasticity of Drosophila melanogaster in response to varying social experiences. Sehresh Saleem, Ginger Carney. Texas A&M University, College Station, TX.

Behaviors are multifaceted phenotypes mediated by a complex integration of genotypic and environmental information. Phenotypic plasticity is vital for increasing fitness of animals in the face of rapidly changing environments. We curretly have a poor understanding of how the environment affects gene expression to bring about these behavioral responses. We investigated *Drosophila melanogaster* reproductive behaviors to evaluate these complex interaction networks. Reproductive behaviors in *Drosophila melanogaster* are regulated by a tightly orchestrated set of genes and further modified by diverse environments. In order to determine environmental influence on these behaviors, we quantified the environmental influence on male copulation duration and female re-mating latency. We show that virgin, wild-type males aged in groups with other males modify their behavior towards females. Grouped males copulated with females for a significantly longer time compared to males raised in isolation. We found that female re-mating latency was affected by copulation duration. Females that experienced long initial copulations had longer latencies to re-mating. Therefore, longer mating duration may lead to increased offspring production by the first male. Together, these results suggest that males perceive high sperm competition when reared with in groups and modify their reproductive behaviors in order to increase their chances of siring more offspring. In order to identify the genes mediating these plastic responses in *Drosophila*, we evaluated candidate genes that are differentially expressed in the male head when males interact for 20 minutes (Ellis and Carney 2010). We tested the effects of mutations in these genes on copulation duration under environments where perceived sperm competition is high. Identification of genes which mediate phenotypic plasticity under varying environmental pressures, such as competition for mates, will be the first step towards delineating these complex genotype-to-phenotype maps.

619A

Investigating the role of a fourth chromosome mutation in courtship receptivity and decisionmaking. Joseph Schinaman, Rui Sousa-Neves. Department of Biology, Case Western Reserve University, Cleveland, OH.

In nature, during the process of mate selection, females often have to recognize males of their own species and select amongst them the individuals with the most effective courtship display. This process of decision-making relies on a multi-modal stimulation and results in either acceptance or rejection of the courting male. *dati^{1}* is a recessive mutation which causes females to be unresponsive to male courtship and respond actively with rejection. *dati^{1}* encodes a transcription factor located on the Drosophila 4th chromosome and its phenotype can be fully reverted by the excision of the P-element that causes the

mutation. We found that *dati*[1] is expressed in the nervous system, and began investigating specifically where in the nervous system the gene is required. To this end, we knocked down its expression in the entire CNS and in specific neuronal populations using RNA interference. We also analyzed the loss of *dati*[1] function in somatic clones in discrete brain regions using a novel technique we developed that allows the generation and analysis of fourth chromosome mutations. Here we show the status of the ongoing mapping of brain regions and present data that suggests that a subset of neurons is most affected by the loss of *dati*[1].

620B

The role of Juvenile Hormone in *Drosophila melanogaster* male courtship behavior. Thilini P Wijesekera, Brigitte Dauwalder. Biology and Biochemistry, University of Houston, Houston, Texas.

Juvenile hormone binding proteins (JHBPs) of many insects have a similarity to *takeout*, a male preferentially expressed protein, shown to influence male courtship behavior. This raises the possibility for a role for Juvenile Hormone in male courtship behavior. To test this hypothesis, we reduced the amount of Juvenile Hormone Acid Methyl Transferase (JHAMT), a key enzyme involved in the synthesis of Juvenile hormone, specifically in the *corpora allata* and examined its effect on the behavior.

To test whether Juvenile Hormone has a role in male courtship behavior in *Drosophila*, we reduced the amount of JHAMT by RNA interference. We created transgenic *JHAMT-GAL4* lines that express Gal4 in the *corpora allata*. Expression of JHAMT-RNAi by *hsp-GAL4* and *JHAMT-GAL4* reduced male courtship. Juvenile hormone is a vital hormone strongly influencing the development of insects. Therefore, the possibility of the above mutant phenotype being a developmental defect arises. Therefore, JHAMT levels were reduced conditionally in the adult flies using the GAL80ts system. When the resulting flies were studied for courtship defects, they also indicated a reduced courtship index. These results demonstrate that the reduction of Juvenile hormone levels in adult males leads to a mutant courtship phenotype. This strongly suggests that normal Juvenile hormone levels are vital for courtship behavior in *Drosophila melanogaster.*

This work was funded by NSF grant No. IOS-0919697.

621C

***Drosophila* sNPF regulates feeding through the dFOXO post-translational modification.** Kyu-Sun Lee[1,2], Seung-Hyun Hong[1], Su-Jin Kwak[1], Ae-Kyeong Kim[1], Hua Bai[3], Marc Tatar[3], Kweon Yu[1]. 1) Aging Res Ctr, KRIBB, Daejeon; 2) Functional Genomics Program, University of Science and Technology, Daejeon; 3) Department of Ecology and Evolutionary Biology, Brown University, Providence, RI.

Feeding behavior is one of the most essential activities in animals, which is tightly regulated by neuroendocrine factors. *Drosophila* short neuropeptide F (sNPF) is an orexigenic neural hormone. Understanding the regulative mechanism of sNPF signaling is critical for elucidating feeding regulation. Here, we found that *minibrain* (*mnb*), which is a target gene of sNPF signaling, regulated feeding in *Drosophila* and fasting-feeding cycles are controlled by the post-translational modification of the dFOXO transcription factor. During fasting, increased Mnb kinase phosphorylated and activated NAD+ deacetylase Sir2, which in turn deacetylated and activated the dFOXO transcription factor. This activated dFOXO turned on the *sNPF* expression and increased feeding. Conversely, during feeding, insulin signaling activated the Akt kinase, which phosphorylated and inhibited dFOXO. This inactivated dFOXO suppressed the *sNPF* expression and decreased feeding. These findings demonstrate that the post-translational modification of dFOXO play a critical role in the regulation of sNPF-mediated feeding in *Drosophila*.

622A

The Role of Odorant Binding Proteins in Aversive Taste Perception in *Drosophila melanogaster*. Sruthipriya Sridhar[1,2], Michael Nokes[1,2,4], Shilpa Swarup[2,3], Tatiana V. Morova[1,2], Robert R.H. Anholt[1,2,3]. 1) Department of Biology; 2) W. M. Keck Center for Behavioral Biology; 3) Department of Genetics, NCSU, Raleigh NC; 4) University of Notre Dame, Notre Dame IN.

Chemosensation in *Drosophila* is mediated by large multigene families of chemoreceptors, including olfactory receptors, gustatory receptors, and odorant binding proteins (OBPs). Although the contribution of OBPs to olfaction is well documented, their role in mediating taste remains largely unknown. We used the "CAFE" (capillary feeder) assay to quantify intake of "bitter" (i.e. aversive) compounds. We used a tubulin-GAL4 driver to express UAS-RNAi constructs targeting 17 *Obp* genes and their co-isogenic control to systematically dissect the functions of OBPs in mediating aversive taste perception. Single sex groups of eight flies were food deprived for 24h and placed in a vial with three capillaries filled with either 50mM sucrose solution alone or a sucrose solution supplemented with denatonium benzoate, berberine chloride, N-phenylthiourea, papaverine hydrochloride, quinine hydrochloride, escin, theophyline, coumarin, or caffeine. After allowing flies to feed 24h, the amount of each solution consumed compared to the control was calculated, while accounting for evaporation. Targeted knockdown of several OBPs resulted in either reduced or increased consumption of aversive tastants, presumably due either to reduced removal of tastants or reduced transport of tastants to the chemoreceptors, respectively. To distinguish post-ingestive effects from gustation, we also measured proboscis extension responses in tubulin-GAL4/UAS-RNAi-Obp hybrids that showed altered intake of tastants in the CAFE assay. Our results implicate a function for OBPs in aversive taste avoidance. Supported by NIH grant GM059469.

623B

Identification of regulatory elements impacting AKH signaling. Jason T Braco, Greg E Alberto, Emily L Gillespie, Erik C Johnson. Biology, Wake Forest University, Winston-Salem, NC.

The mechanisms of how organisms maintain metabolic homeostasis in light of significant environmental variation in food availability are not completely understood. We are particularly interested in the starvation stress response and the mechanisms that form homeostatic responses to starvation. The Adipokinetic Hormone (AKH) has been identified as a principal component in maintaining metabolic homeostasis. AKH is responsible for increasing lipid mobilization, energy availability, and starvation induced hyperactivity. We have previously identified that the energy sensor, AMP-activated kinase (AMPK) is a critical element that regulates AKH signaling. Reduced AMPK function partially phenocopies AKH null alleles, suggesting additional, and AMPK - independent mechanisms which also regulate AKH secretion. To identify these additional AKH regulatory mechanisms, we initiated a candidate gene screen employing RNAi elements targeting hormone receptors. We hypothesized that the loss of excitatory transmitters would lead to a reduction in secreted AKH

manifesting as a longer lived fly. Conversely, the loss of inhibitory transmitters should lead to an excess of AKH release and a shorter lived fly during starvation. We identified the receptor for the proctolin, octopamine, allatostatin c (ASTc), and pyrokinin as candidate molecules that participate in regulation of AKH signaling. We further validated these findings, by employing single cell PCR and found specific expression of these genes in AKH cells. To directly assess hormonal impact on AKH cell activation we employed GCaMP imaging. ASTc addition increased AKH cell excitability; this response was, in part, dependent on AMPK. The significance of this finding might suggest an additional target of AMPK modulation and we speculate that this may be a conserved feature of metabolic homeostasis.

624C

The Role of Hormones in the Stress Response. Kathryn J. Argue, Wendi S. Neckameyer. Pharmacological and Physiological Sciences, Saint Louis University School of Medicine, St. Louis, MO.

We have predicted that through complex neuroendocrine interactions, gonadotropic hormones will modulate the recruitment of neurons into the stress response circuitry and therefore alter behavioral parameters. We assayed both heart rate and locomotion, since these have been shown to be altered in response to stress, and are modulated, at least in part, by dopamine (DA). In flies, juvenile hormone (JH) is one of the major gonadotropic hormones shown to be important for reproductive competence and mature sexual signaling. We have data showing that JH interacts directly with DA in vivo and that manipulation of the levels of JH can have long-term effects on the animals' behavior. Ecdysone is another important gonadotropic hormone in Drosophila; it is the only steroid hormone in flies and has been shown to be important for generation and growth of adult-specific DA circuits (Mesce, 2002). Using pharmacological and transgenic manipulations to investigate the role of both JH and ecdysone in modulation of the stress response in Drosophila, we have demonstrated that the actions of these hormones on the stress response circuitry are both temporally and sexually dimorphic. This work has been funded by NIMH 1RO1MN083771 and NSF 0616062.

625A

The membrane-bound ecdysteroid receptor DopEcR plays a unique role in the regulation of behavioral response to ethanol in Drosophila. Emily Petruccelli, Toshihiro Kitamoto. 51 Newton Rd, University of Iowa, Iowa City, IA.

Steroid hormones are critical modulators of various biological processes across phyla. Although actions of steroids are primarily mediated by nuclear hormone receptors, some steroid responses cannot be explained by this classical "genomic" mechanism because they occur rapidly and independent of mRNA synthesis. Despite the potential importance of such "non-genomic" steroid actions, their physiological functions and underlying mechanisms are still poorly understood. Drosophila DopEcR is a G-protein coupled receptor that has been implicated in non-genomic actions of ecdysteroids (ecdysteroid and 20-hydroxyecdysone), the major steroid hormones in insects. In heterologous culture systems, DopEcR responds to both ecdysteroids and the catecholamine dopamine to rapidly activate MAPK and cAMP signaling pathways, respectively (Srivastava et al., 2005). The role of DopEcR in vivo, however, still remains largely elusive. We have recently identified a hypomorphic allele of Drosophila *DopEcR* (*DopEcR*^{PB1}) and found that *DopEcR* mutants are defective in both associative and non-associative learning (Ishimoto et al., submitted). Here we further discover that *DopEcR* mutants show various aberrant behaviors in response to ethanol. First, *DopEcR*^{PB1} flies are more sensitive to the effect of ethanol on postural control, similar to amnesiac mutants. Second, as seen in flies with enhanced EGFR signaling, *DopEcR*^{PB1} flies display increased resistance to ethanol-induced sedation. Third, resistance to ethanol-induced sedation is drastically enhanced in *DopEcR*^{PB1} mutant males, but not females, following heat shock. Our results suggest the DopEcR can be used to study the in vivo functions and molecular mechanisms for non-genomic actions of steroids while also offering important insights into the molecular and cellular underpinnings of behavioral response to alcohol. Srivastava et al. (2005) J Neurosci 25, 6145-6155.

626B

A genetic RNAi screen for G-protein coupled receptors regulating *Drosophila* flight. Tarjani Agrawal, Gaiti Hasan. Natinal Centre for Biological Sciences, TIFR, Bangalore, INDIA.

IP_3 is a second messenger that activates IP_3 receptor and leads to release of calcium from endoplasmic reticulum to cytosol. Pan-neuronal down regulation of the IP_3 receptor in *Drosophila* results in wing posture and flight physiology defects *(1)*. Binding of ligands to G-protein coupled receptors (GPCRs) can lead to IP_3 formation in the cytosol. I am interested in identifying the neuronal GPCRs which function through IP_3 mediated calcium release and regulate flight in *Drosophila*.

In order to down-regulate the expression of GPCRs in neurons, a tissue specific expression technique of UAS-GAL4 was used. RNAi strains to all the predicted GPCRs in *Drosophila* genome are available at the Vienna Drosophila RNAi Centre. Flight phenotype by pan neuronal down regulation of all GPCRs was checked. Flight assays were done by recording single flight movies or by measuring firing activity from the dorsal longitudinal muscles, for 30 sec after giving an air puff.

I have screened 110 non-gustatory and non-olfactory GPCRs present in the *Drosophila* genome. This helped me to identify 20 receptors which affect flight. Most of these receptors are activated by neuropeptides or neurotransmitters. Down regulation by RNAi expression for 7 GPCRs resulted in phenotypes other than flight. These receptors were tested in sub-neuronal domains for flight defects. To investigate if these receptors function through IP_3 mediated calcium release, a modifier screen using an activated form of the G-protein (Ac-Gq) or over-expression of an intracellular calcium signalling molecule (dSTIM) is in progress. I have identified a putative neuropeptide receptor that appears to regulate flight through IP_3 mediated calcium release in glutamatergic neurons. Identification of receptors that regulate complex motor behaviors like flight will help understand neuromodulation of rhythmic motor behaviors.

References: 1. Agrawal,et al.(2010), J Neurosci 30, 1301-1313.

627C

Characterization of three ligand gated ion channel subunits - Potential pesticide targets? Daniel Feingold, Stephanie Bourque, Patrick Janukavicius, Saima Sidik, Laura Nilson, Joseph Dent. Biol, McGill Univ, Montreal, QC, Canada.

Cys-loop ligand gated ion channels (LGICs) are pentameric neurotransmitter receptors that are ubiquitous in both vertebrate and invertebrate nervous systems. Their large diversity as well as their central role in mediating rapid synaptic transmission has made these channels attractive molecular targets for

various pesticides. Despite the widespread use of such pesticides, issues regarding drug specificity and resistance continue to pose serious problems in regions that rely on pesticides for crop protection and prevention against disease. We are characterizing three novel Cys-loop LGIC subunits; CG7589, CG6927 and CG11340 in *Drosophila melanogaster* to determine their potential as pesticide targets. These genes are of particular interest because they are specific to arthropods and do not possess any orthologs in vertebrate systems (Dent, 2006). Consequently, pesticides that target channels formed by these genes are predicted to be safe and have low risk for off-target effects. Electrophysiological tests indicate that CG11340 can form a functional homomeric channel while CG7589 and CG6927 can form a heteromeric channel. We also generated loss of function alleles for all three genes and data suggest that mutations in CG7589 and CG11340 exhibit lethal phenotypes. The expression profiles of all three channel subunits are unconventional in that they are seemingly absent from neural and muscular tissue and instead, appear to be localized in secretory tissues. CG7589 and CG11340 are expressed in the midgut and Malpighian tubules - tissues involved in ion regulation and renal function - and CG6927 appears to be expressed in tracheal tissue and salivary glands. Furthermore, consistent with the CG11340 expression data, preliminary findings indicate that CG11340 mutants are sensitive to osmotic stress. Based on the divergence of these genes from other Cys-loop LGIC subunits as well as the lethal phenotypes associated with the corresponding mutants, these putative subunits may provide a promising target for a novel class of highly selective and efficient pesticides.

628A

The role of glia in axonal degeneration. Bibhudatta Mishra, Catherine A Collins. Molecular,Cellular and Developmental Biology, University of Michigan, Ann Abor, MI.

Purpose of statement Axonal degeneration occurs after injury, as well as in neuropathies and neurodegenerative disorders. However the cellular mechanism for axonal degeneration is poorly understood. The aim of our study is to understand the role of glia in axonal degeneration after injury, particularly the role of glia which function in insulating axons in peripheral nerves, and the role of electrical activity in degeneration. **Methods used** We used a nerve crush assay (Xiong X et al., 2010) to study Wallerian degeneration of Drosophila motoneurons after injury, and screened mutations known to disrupt specific subtypes and functions of glia. We genetically manipulated the electrical activity of a small subset of motoneurons by the m12-Gal4 driver line (Ritzenthaler et al., 2000) to over-express either a dominant negative mutation in the shaker K channel to hyperexcite neurons, or an inward rectifying K channel (Kir2.1) to silence neurons. We also used a conditional allele of the voltage gated Na channel, para-ts, to silence neurons acutely at different time points either before, during, or after injury. **Summary of results** We found that disruption of the moody gene cause a dramatic delay in the initiation of axonal degeneration after injury. moody encodes an orphan GPCR whose function is required for regulation of the blood brain barrier (BBB). One function of this barrier is to insulate neurons from high concentrations of potassium in the hemolymph. Consistent with an important role for this insulation, electrically silenced neurons do not degenerate, while hyperexcitable neurons degenerate faster. Timing of action studies with para-ts suggest that electrical activity in the distal stump is an important factor in the initiation of the degeneration process. **Conclusion** Our results suggest that the disrupted septate junction leads to delayed axon degeneration, and altered electrical activity in neurons affects their resiliency to axonal degeneration. We propose that the electrical activity of neurons is an important factor in the axonal degeneration process.

629B

Probing the regulatory mechanisms of AKH cell excitability. Rebecca J Perry, Jason T Braco, Erik C Johnson. Department of Biology, Wake Forest University, Winston-Salem, NC.

The mechanisms of how organimsms 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 may 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 TASK6 potassium channel as a candidate AKH regulatory element. Expression of the TASK6 RNAi in AKH cells leads to lengthened lifespan during starvation. Additionally, there were observable changes in starvation-induced hyperactivity. We are in the process of confirming TASK6 expression in AKH neuroendocrine cells through single-cell RT-PCR. We will also report preliminary experiments on AKH cell activation in a TASK6 mutant background and report other findings from the genome-wide RNAi screen.

630C

CREB results in memory enhancement for a conditioned place preference & courtship suppression task in Drosophila melanogaster. Eugenia Friedman[1], Toshihiro Kitamoto[2], Jerry Yin[3]. 1) Neuroscience Training Program, University of Wisconsin-Madison, Madison, WI; 2) Dept. of Anesthesiology, University of Iowa, Iowa City, Iowa; 3) Dept. of Genetics, University of Wisconsin-Madison, Madison, WI.

It is generally agreed that activation of cAMP-responsive element binding protein (CREB) is required for long-term memory (LTM) formation. There are however conflicting results regarding memory enhancement produced from the dCREB2a “activator” isoform. Using a transgenic N-terminally truncated dCREB2a (807), we show that dCREB2a enhances memory in both conditioned courtship suppression and conditioned place preference, a novel paradigm developed in the lab. Conditioned courtship suppression is a well-established learning and memory task that exploits natural mating behaviors. Briefly, male 807 and matching controls were paired with premated females for 5 hours. On the following day only 807 flies exhibited courtship suppression compared to the controls indicating that 807 enhanced memory. To date there is no behavioral paradigm designed for flies measuring a reward based associative memory at the single animal level. We have developed an assay for Drosophila, comparable to the conditioned place preference used in rodents. In this protocol a single fly is exposed to visual stimuli differentiating one arm of a t-maze from another. Ethanol is presented in one arm over 3 trials inducing associative memory formation between a visual cue and ethanol. One day following training individual flies are tested for place preference (time in arm previously associated with ethanol versus the non-ethanol arm), indicative of a reward based memory. We tested this paradigm using 807 and an 807 null mutant, ATG2. Similarly to courtship suppression results, 807 enhances place preference for the ethanol associated arm 20 hours following training compared to ATG2 controls. These results suggest overlapping roles for dCREB2 in the genetic pathways of both types of associative memories, and supports prior evidence for the role of CREB in Drosophila LTM.

631A

Dopamine neurons signal reward for odour memory in Drosophila. Chang Liu[1,2], Anja Friedrich[1], Igor Siwanovicz[1], Hiromu Tanimoto[1]. 1) Max-Plank Institute of Neurobiology, Martinsried, Germany; 2) Kunming Institute of Zoology, Chinese Academy of Science, Kunming, China.

Animals tend to approach stimuli that predict pleasant outcome. Following the paired presentation of an odour and a reward, Drosophila melanogaster can develop a conditioned approach towards that odour. Despite recent advances in understanding the neural circuits for associative memory and appetitive motivation, the cellular mechanisms for reward processing in the fly brain are unknown. Dopamine (DA) plays important roles in variety of behaviors in both vertebrates and invertebrates. However, unlike in mammals, DA is widely accepted as the critical neurotransmitter for signaling the punishment in aversive associative learning in insects. Recent studies suggested that DA may also contribute to appetitive learning in adult flies and larvae. In this study, we tried to evaluate the role of DA neurons in appetitive learning and memory in Drosophila. By transient activation and inactivation of DA neurons in behavioral fly, we found that a specific group of DA neurons are required for the formation of appetitive memory. Activation of these neurons could induce appetitive memory. These neurons convey reward information to the medial lobes of the mushroom bodies. Our results demonstrate that a specific group of DA neurons are responsible for appetitive learning and memory in Drosophila.

632B

Effect of LIMK1 isoform ratio on Drosophila melanogaster courtship behavior. Ekatherina Nikitina, Alena Kaminskaya, Dmitry Molotkov, Gennady Zakharov, Tatyana Payalina, Elena Savvateeva-Popova. Dept Neurogenetics, Pavlov Inst Physiology, St Petersburg, Russian Federation.

Motile processes at the origin of cell migration, cell division, morphogenesis, synaptic plasticity and endocytosis are governed by spatially and temporally controlled assembly of actin filaments. Increased attention of neurobiologists to the signal cascade of actin remodeling, integration of different neurodegenerative disorders under the name «cophilinopathies» pointed to wide spectrum of inner adaptive processes related to this cascade. The signal cascade of actin remodeling: receptors of neurotransmitters - small Rho GTPases (RhoA, Cdc42 and Rac1) - LIM kinase 1 (LIMK1) - cofilin - actin - is believed to play the main role in dendrite- and synaptogenesis. LIMK1 - is the key enzyme of actin remodeling which controls dendritic spine morphology necessary for synaptic plasticity during learning and memory formation. Conditioned courtship suppression paradigm was used to asses learning acquisition and memory formation in four Drosophila strains polymorphic for the limk1 gene harbored by the agnostic locus: the wild type strains Canton-S, Berlin, Oregon-R and the mutant agnts3. Wild type strains Canton-S and Berlin are characterized by normal learning acquisition and 3-h (intermediate) memory formation. Oregon-R proved to be disabled in learning; we observed a failure of 3-h memory formation in this strain too. Also agnts3 mutants showed 3-h (intermediate) memory and learning ability that were three-fold lower than those in Canton S flies. Behavioral performances were compared to the ratio of two LIMK1 isoforms in these Drosophila strains. Quantity of D- and C-isoforms of LIMK1 in agnts3 multiplied thrice as compared to the same level in Canton-S, but their ration was identical. Quantity of D-isoform in Berlin doubled the same in Canton-S, but in Oregon-R decreased twice as compared to Canton-S. Apparently, observed disturbances of learning acquisition and memory formation in flies may be linked to the alteration in ratio of LIMK1 D/C isoforms.

633C

Tip60 HAT activity regulates synaptic plasticity: Implications for epigenetics in learning and memory. Jessica Sarthi, Felice Elefant. Biology, Drexel Univ, Philadelphia, PA.

Age-associated cognitive decline and neurodegenerative disorders such as Alzheimer's disease (AD) are associated with misregulation of synaptic plasticity linked genes; however the mechanisms underlying decline of such gene control during aging are unknown. Histone acetylation of chromatin promotes dynamic transcriptional responses in neurons that influence neuroplasticity critical for cognitive ability. Accordingly, aberrant changes to histone acetylation patterns in the aging brain epigenome are linked to memory loss. It is therefore critical to identify and study the histone acetyltransferases (HAT) that create such marks. One promising candidate is Tip60, a HAT implicated in AD and shown by our laboratory to be critical in regulating neuronal processes linked to cognition (Genetics, 2007; PLoS ONE, 2010; PLoS ONE, 2011).To explore a direct role for Tip60 in synaptic plasticity, here we explore the consequences of misregulating Tip60 HAT activity in the Drosophila neuromuscular junction (NMJ). We show that the HAT dTip60 is concentrated both pre and post-synaptically within the NMJ. Presynaptic targeted reduction of dTip60 HAT activity significantly increases synaptic bouton number that specifically affects type Is boutons while postsynaptic reduction results in significant loss of these boutons. The excess boutons show a suppression of the active zone synaptic function marker bruchpilot, suggesting defects in neurotransmission function. Analysis using immunohistochemical staining to the MAP, futsch reveals a significant increase in the rearrangement of microtubule loop architecture that is required for bouton division. Moreover, α-tubulin acetylation levels of microtubules are also reduced in response to dTip60 HAT reduction. Our results are the first to demonstrate a causative role for the HAT dTip60 in the control of synaptic plasticity that is achieved, at least in part, via regulation of the synaptic microtubule cytoskeleton. These findings have implications for dTip60 HAT dependant epigenetic mechanisms underlying cognitive function. NIH grant HD045292-01 to F.E.

634A

An epigenetic role for dTip60 in locomotion and axonal vesicle transport. Ashley Zervos, William Reube, Felice Elefant. Dept Biol, Drexel Univ, Philadelphia, PA.

Histone acetyltransferases (HATs) are a key class of enzymes that control chromatin accessibility to regulate gene expression profiles critical for diverse cellular processes. Tip60 is one such HAT that has been shown by our laboratory to play a critical role in regulating neuronal genes linked to neurodevelopment and cognition (Genetics, 2007; PLoS ONE, 2010; PLoS ONE, 2011). Consistent with our findings, Tip60 has been implicated in the age-related neurodegenerative disorder Alzheimer's disease (AD) via its interaction with the AD linked amyloid precursor protein intracellular domain (AICD). This complex is essential for the epigenetic regulation of certain genes critical for neuronal function. Inappropriate complex formation may contribute to pre-clinical AD-related pathology by misregulation of target genes involved in neurogenesis; however a direct epigenetic based role for Tip60 in this process remains unclear. Here, we investigate a causative role for Tip60 in axonal vesicle transport, a process affected in the pre-clinical stages of AD. We show that reduction of Tip60 HAT activity specifically in the nervous system of the fly leads to locomotor defects and a distinctive tail flipping phenotype. These phenotypes are reminiscent of nervous system defects linked to mutations in genes required for axonal vesicle transport machinery. Confocal imaging of

third-instar larval motor axons reveals abnormal vesicle aggregation and clogging in response to Tip60 HAT reduction. These defects are exacerbated by APP overexpression and dependent upon AICD, the region of APP that interacts with Tip60. Importantly, treatment of larvae with ms-275, a nervous system specific class 1 HDAC inhibitor, rescues both vesicle clogging in motor axons as well as locomotion defects. In addition to providing new biological insight into epigenetic gene control mechanisms underlying neurodegeneration in AD, these studies will be fundamental in exploring the utility of novel epigenetic-based therapeutics to improve healthcare and quality of life in the elderly. NIH grant HD045292-01 to F.E.

635B

ROS-mediated detection of epidermal mechanical stress by larval peripheral nociceptors. Wayne A. Johnson, Justin Carder. Dept Molec Physiol/Biophysics, Univ of Iowa Carver College of Medicine, Iowa City, IA.

Wandering stage larvae are particularly susceptible to dessication displaying a strong aversion to locomotion on dry surfaces to prevent movement into areas of potentially lethal low humidity. This aversion is manifested by the humidity-dependent height of pupation within a culture vial. This high friction aversion is mediated by the class IV multiple dendritic(mdIV) nociceptor neurons expressing the DEG/ENaC subunit, Pickpocket1(PPK1), within complex dendritic arbors tiling the larval body wall. Direct electrophysiological recordings showed that the mdIV neurons are activated by nanomolar levels of the reactive oxygen species(ROS), H_2O_2. Both the aversion behavior and ROS-mediated mdIV nociceptor activation are dependent upon the PPK1 protein. We have further investigated the source and role of an endogenous ROS signal by genetic and transgenic manipulation of various components of the cellular redox machinery resulting in modifications of larval aversion behavior. Transgenic overexpression of catalase in epidermal cells to increase breakdown of endogenous H_2O_2 duplicated phenotypes associated with mdIV nociceptor inactivation or *ppk1* loss-of-function. Knockdown of endogenous epidermal catalase using transgenic RNAi, to increase ROS levels, caused a reciprocal mdIV nociceptor hypersensitization. Similar opposite phenotypes were observed due to either overexpression or RNAi-based knockdown of superoxide dismutase(SOD) which catalyzes the conversion of superoxide to H_2O_2. A potential source of endogenous H_2O_2 may be the Duox(NADPH oxidase dual peroxidase) protein capable of producing H_2O_2 in response to a variety of chemical and/or mechanical stimuli. Transgenic RNAi-based knockdown of Duox in larval epidermis caused striking effects upon mdIV nociceptor and PPK1-dependent larval behaviors. These results support a model describing ROS-mediated signaling from the larval epidermis undergoing high friction mechanical stress to activate the mdIV nociceptors and mediate a larval aversion behavior to move away from potentially lethal low humidity environments.

636C

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, CHILE.

The insect molt culminates with ecdysis, an innate 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 ETH targets in wild-type animals as well as 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.

637A

Differential Recruitment of Dopamine Neurons into the Stress Response Circuitry. Kathryn J. Argue, Wendi S. Neckameyer. Pharmacological and Physiological Sciences, Saint Louis University School of Medicine, St. Louis, MO.

Sex, sexual maturity, and reproductive status have been shown to affect whether a mutant Drosophila strain with specific anatomical defects limited to critical brain regions modifies its response to stress relative to wild-type flies (Neckameyer and Matsuo, 2008). Our results suggest that for each population (sexually immature and mature males and females), unique subsets of neurons are recruited into the stress response circuitry and differentially affect behavior. By knocking down dopamine (DA) synthesis in subsets of DA neurons and assaying for behavioral changes in response to starvation and oxidative stress in these lines, we will be able to identify DA neurons that are important for a given behavioral response to stress within a given population. This work has been funded by NIMH 1RO1MN083771 and NSF 0616062.

638B

Dissection of the Dopaminergic Circuitry Regulating Sleep/Wake in *Drosophila*. Qili Liu[1], Sha Liu[1], Lay Kodama[1], Maria Driscoll[1], Shahnaz Lone[1], Mark Wu[1,2]. 1) Department of Neurology, Johns Hopkins University, Baltimore, MD; 2) Department of Neuroscience, Johns Hopkins University, Baltimore, MD.

Dopamine (DA) has been shown to regulate a wide variety of behaviors, including arousal and locomotion, in animals ranging from worms to mammals. There are around 200 dopaminergic neurons, divided into 13 subgroups in the adult *Drosophila* brain. To identify specific DA neurons involved in sleep/wake regulation, we generated novel transgenic Gal4 lines labeling subsets of DA neurons. Analysis of the activation of these specific DA subsets using *UAS-dTrpA* suggests that 1 subgroup in particular is important for promoting wakefulness, by reducing the arousal threshold. We are now carrying out MARCM analysis using these drivers to identify the few DA cells that are most critical for arousal. We have also carried out loss-of-function experiments with these restricted DA drivers, by using *UAS-Shi^{TS}*. These studies suggest that several DA neurons in the thoracic ganglion may promote locomotion specifically. These findings are now being confirmed by the concomitant use of *Tsh-Gal80*, which blocks Gal4 function specifically in the thoracic ganglion. On the postsynaptic side, there are 4 DA receptors in Drosophila. We find that the dramatic decrease of sleep seen when activating all DA cells using dTrpA

is completely blocked by a mutation in the *DopR* gene, suggesting that DopR is the main DA receptor involved in sleep/wake regulation. Similar results were obtained by feeding L-dopa to *DopR* mutants. We are now performing tissue-specific rescue experiments with DopR to determine the specific DopR-expressing cells that are the downstream targets of the DA wake-promoting neurons. In summary, we have dissected the circuitry underlying the wake-promoting and locomotion-promoting functions of DA. Current studies include further narrowing down the wake-promoting cells and identifying their downstream DopR+ targets. Finally, our novel restricted DA Gal4 drivers will be useful tools for those studying the role of DA in behavior.

639C

Using natural variation to investigate *Drosophila*- yeast interactions. Kelly M. Schiabor. Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

Environments are teeming with microbes that actively contribute to the environment's chemical makeup. We are interested in understanding the molecular details of the animal-microbe symbiosis between *Drosophila* and fungi. Specifically, fungi are an essential component of the Drosophila larval diet, and both adult and larvae Drosophilids have evolved sensory mechanisms to detect and locate fungal species within the environment. Fungi also benefit from this physical association, as their interaction with *Drosophila* help to disperse these non-motile microbes to fresh, sugary substrates. We hypothesize that small molecules produced by fungi help to manage this symbiosis by acting as both attractants and behavioral cues for Drosophilids. We have developed and conducted behavioral assays to determine if *D. melanogaster* show a preference for particular natural *S.cerevisiae* isolates. We have also used gas chromatography coupled to mass spectroscopy (GC-MS) to characterize differences in the volatile profiles of each *S. cerevisiae* strain.

640A

Structural evidence supporting a conserved role for sleep in synaptic homeostasis. Daniel B. Bushey, Giulio Tononi, Chiara Cirelli. Dept Psychiatry, Univ Wisconsin, Madison, Madison, WI.

Sleep is a conserved behavior among divergent metazoans. Although conserved, the functions being performed during sleep remain contested. Evidence in mammals indicates that sleep is necessary for synaptic homeostasis. During wake, electrophysiological, molecular, and structural studies show that there is a net increase in synaptic strength in many brain regions that is balanced by a net synaptic downscaling during sleep. Previous studies in *Drosophila* show that pre and post-synaptic proteins accumulate during wake as compared to sleep in several regions of the fly brain, consistent with the results in mammals. Recently, we sought to rigorously test the effect that wake and sleep have on the structure of three different neuronal types: small LN_vs (s-LN_vs), mushroom bodies gamma neurons, and visual system interneurons. These circuits are necessary for arousal and circadian rhythm, olfactory learning and memory, and flight orientation, respectively. Individual neurons expressing unique epitopes that localized either pre or post-synaptically were compared in fixed brains harvested from sleeping, awake, or sleep deprived (SD) flies. During wake and SD, synaptotagmin-eGFP expression accumulated in larger volumes at pre-synaptic terminals of the s-LN_vs and gamma neurons as compared to sleep. Since synaptic volume correlates with synaptic strength, the results are consistent with increased global synaptic potentiation during wake as compared to sleep. Post-synaptically, visual interneurons had more complex dendritic branches and more spines in flies that remained awake in an enriched environment compared to flies allowed to sleep or kept awake in small tubes where flight was not possible. Structural complexity (branch points and spine number) decreased with the time spent asleep. Together, these structural results suggest that synaptic downscaling occurs during sleep in *Drosophila*. Future studies with calcium imaging will determine whether functional measures of synaptic strength also change with sleep and wake in flies.

641B

Virtual Fly Brain. Marta Costa[1], David Osumi-Sutherland[1], Simon Reeve[1], Nestor Milyaev[2], Cahir O'Kane[1], J. Douglas Armstrong[2]. 1) Department of Genetics, University of Cambridge, Cambridge, United Kingdom; 2) University of Edinburgh, School of Informatics, Institute for Adaptive and Neural Computation, Edinburgh, United Kingdom.

Navigating the Drosophila neurobiology literature and related databases is challenging. For example, it can be a daunting task to find details of the connectivity between two brain regions, the properties of the neurons involved, and the genes and GAL4 drivers that they express. This problem is rapidly growing worse as yet larger datasets are produced. One way to tie neuroanatomical data together is in an atlas. Google Earth, with its ability to rotate, zoom, overlay data and link any feature to additional information is an obvious template. Inspired by this approach, we have developed the Virtual Fly Brain (VFB), a web-based tool that allows users to browse a 3D confocal stack of a *Drosophila* brain at any angle and various scales. For any brain region down to the level of individual glomeruli and layers, users can run point-and-click queries for neuron classes based on innervation patterns, for alleles based on phenotype and for markers and GAL4 drivers based on expression. Subdivision of the brain on VFB is defined using names, boundaries and textual definitions agreed by the BrainName project [Ito *et al*., in preparation]. Annotations are stored in the FlyBase *Drosophila* anatomy ontology, which also stores detailed information from the literature about neuron classes, including their lineage, innervation patterns and neurotransmitters. This information can be searched on VFB via simple template-based queries for neuron classes. Phenotype and expression data is pulled directly from FlyBase, who use this ontology extensively in their curation. We are currently extending the data sets annotated with our ontology to other neuroanatomical resources. We are also incorporating alignment tools that allow users to register their stacks to our painted atlas stack and to annotate them using our ontology. With this approach, we aim to make VFB a hub for querying across multiple neuroanatomical resources and integrating them with genomic and literature resources.

642C

Walking parameters in adult wild type and sensory impaired *Drosophila melanogaster*. César S. Mendes[1], Imre Bartos[2], Turgay Akay[1], Szabolcs Márka[2], Richard S. Mann[1]. 1) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY; 2) Department of Physics, Columbia University, 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 (CPG's) - a premotor network of interneurons - and sensory neurons. CPGs produce rhythmic outbursts, without input from the central brain that target leg motor neurons. Sensory neurons constantly report the position and load of each of the leg segments and the terrain conditions. This allows precise coordination, stability and a permanent adaptation of the behavior to the environment. A central question for neuroscientists is the identification of the circuits and cellular mechanisms that govern walking behavior. The fruit fly is an attractive model to

address these questions due to its relative neuronal simplicity and increasingly sophisticated genetic tools. Nevertheless, the lack of a reliable and accurate gait analysis method limits insights into the circuits and mechanisms that regulate coordinated walking. In order to address this challenge, we developed an optical method coupled with high-speed imaging that allows us to unambiguously identify footprints and simultaneously track the fly's body as it walks freely on a flat surface. A custom analysis software allows us to track and quantify many parameters exhibited by walking flies, such as step timings, footprint positions and left-right coordination. With this method we have characterized the walking behavior of wild-type animals and begun to carry out loss and gain of function studies in a subset of leg sensory neurons. For this, we established a combinatorial expression system to specifically manipulate neuronal function in different regions of the adult leg and to target specific components of the sensory system. Our results reveal how the interaction between the CPGs and the sensory system modify the walking behavior in fruit flies.

643A

Egg laying decisions in Drosophila depend on the size of the oviposition substrate and are consistent with optimal larval foraging strategies. Nicholas U Schwartz[1], Lixian Zhong[2], Andrew Bellemer[3], W. Daniel Tracey[1,2,3,4,5]. 1) Neuroscience Program, Duke University, Durham, NC; 2) Pharmacology Science Training Program, Duke University Medical Center, Durham, NC; 3) Department of Anesthesiology, Duke University Medical Center, Durham, NC; 4) Department of Cell Biology, Duke University Medical Center, Durham, NC; 5) Department of Neurobiology, Duke University Medical Center, Durham, NC.

Decision-making is defined as selection amongst options based on their utility, in a flexible and context-dependent manner. Oviposition site selection by the female fly, Drosophila melanogaster, has been suggested to be a simple and genetically tractable model for understanding the biological mechanisms that implement decisions. Here we show that egg-laying behavior in female Drosophila is sensitive to the potential foraging success of larval offspring in different environments. With larger experimental substrates, females preferred to lay eggs directly on sugar containing media over other media. This was in contrast to smaller substrates with closely spaced choices where females preferred non-sweetened media. However, the avoidance of sucrose on the smaller substrates occurred only when a diffusion gradient from the sugar source was present. In the absence of diffusion, sugar was always preferred. Since a diffusion gradient can be used by the larval progeny to locate the sucrose food on smaller substrates, we propose that the female choices have evolved to be consistent with the search capabilities of their larval progeny. This represents evidence for egg-laying choices of female Drosophila that may relate to optimal foraging strategies. Our findings offer a powerful model for the biology of decision-making. We propose that egg laying strategies may comply with the marginal value theorem when viewed from the perspective of the larval progeny.

644B

The "secondary cells" of the D. malanogaster male accessory gland make products that prolong the female's post mating response. Jessica L Sitnik[1], Dragan Gligorov[2], Robert K Maeda[2], François Karch[2], Mariana Wolfner[1]. 1) Molecular Biology and Genetics, Cornell, Ithaca, NY; 2) Department of Genetics & Evolution and NCCR Frontiers in Genetics, University of Geneva, Geneva, Switzerland.

In *Drosophila melanogaster*, products of the accessory gland (AG) of the male reproductive tract are essential for initiating and maintaining the female postmating response (PMR) including changes in egg laying, receptivity to courting males, and sperm storage. All of these changes have been shown to be mediated by the receipt of accessory gland proteins "ACPs" from her mate. The two lobes of the AG are composed of two major cell types that are morphologically and biochemically distinct: the flat, hexagonally-shaped main cells which make up 96% of the gland and the large, spherical, vacuole-filled secondary cells that are dispersed at the distal tip. While studies have determined that the main cells of the AG are necessary for these processes, no tools were available to explore the role of the secondary cells. An enhancer deletion identified in the Hox gene *Abdominal-B (Abd-B)*, *iab-6*^{cocu}, results in a morphologically uniform AG that lacks large vacuole filled secondary cells. By testing the impacts of the *iab-6* deletion on female post mating response, we determined that products of the secondary cells are required for long-term changes in egg laying and receptivity in post-mated females, and are influential during sperm competition. Further, the secondary cells contribute to regulating the glycosylation of at least three Acps; ovulin (Acp26Aa), CG1656, and CG1652. Our results show that the secondary cells play an essential role in male fertility, likely through affecting the storage and gradual release of sex peptide. To identify secondary cell products necessary for the PMR, we have used RNA-seq to identify genes whose expression is down regulated in *iab-6*^{cocu} mutants. Using a secondary cell-specific driver derived from our enhancer mutant, we are driving RNAi in these cells to test our candidate genes for roles in regulating the PMR.

645C

Identification of interneurons involved in Drosophila larval reactions to distinct somatosensory stimuli. Marta Zlatic, Tomoko Ohyama, Tihana Jovanic. HHMI Janelia Farm Research Campus, Ashburn, VA.

The principles by which sensory information is processed and used to generate motor responses by the underlying neural circuits are still poorly understood. Most neural circuits are composed of large numbers of interconnected neuron classes and the roles of most of the neuron classes in most circuits are poorly understood. We use the somatosensory circuitry of Drosophila larvae to study the role of distinct neuron classes in sensorimotor transformations. This system offers numerous advantages. All somatosensory neurons and motor neurons have been anatomically identified and are known to project to the ventral nerve cord (VNC). The VNC contains a relatively small number of interneuron classes (several hundred), and the Truman lab at Janelia HHMI has identified a GAL4 line for a large number of these (generated by the Rubin lab). Thus, the basic sensorimotor pathways are genetically tractable and a variety of genetic tools can be applied to test the contributions of individual neuron classes in reactions to sensory stimuli. We have developed a platform for high-throughput and high-resolution analysis of larval reactions to a number of distinct somatosensory stimuli including pain, vibration and air currents. Using this system, we found that each stimulus induces not just a single action, but a stereotyped and characteristic sequence of actions. We used Rubin GAL4 lines to identify sensory and interneuron classes required and sufficient for various aspects of larval reactions to pain, vibration and air currents. We are currently performing calcium imaging experiments to further characterize the roles of identified interneurons in these somatosensory guided behaviors.

646A

Neural representations of courtship song in the *Drosophila* brain. Philip Coen[1], Sina Tootoonian[2,3], Mala Murthy[1]. 1) Molecular Biology and Princeton Neuroscience Institute, Princeton University, Princeton, NJ; 2) Computation and Neural Systems Program and Division of Biology, California Institute of

Technology, Pasadena, CA; 3) Max Planck Institute for Brain Research, Frankfurt, Germany.

The *Drosophila* mating ritual is an extremely robust example of an auditory social interaction. Each male of the roughly 2000 *Drosophila* species sings a unique song using wing vibration and females typically 'listen' to many minutes of song before accepting a mate. Despite decades of research on courtship songs and behavior in *Drosophila*, central auditory responses have remained uncharacterized. We report on intracellular recordings from central neurons that innervate the *Drosophila* AMMC (antennal mechanosensory and motor center), the first relay for auditory information in the fly brain. These neurons produce graded-potential (non-spiking) responses to sound; we compare recordings from AMMC neurons to extracellular recordings of the receptor neuron population (Johnston's Organ neurons or JONs). We discover that while steady-state response profiles for tonal and broadband stimuli are significantly transformed between the JON population in the antenna and AMMC neurons in the brain, transient responses to pulses present in natural stimuli (courtship song) are not. For pulse stimuli in particular, AMMC neurons simply low-pass filter the receptor population response, thus preserving temporal features (such as the spacing of song pulses) for analysis by postsynaptic neurons. We also compare responses in two closely related *Drosophila* species, *D. melanogaster* and *D. simulans*, and find that pulse song responses are largely similar, despite differences in the spectral content of their songs. Our recordings inform how downstream circuits may read out behaviorally-relevant information from central neurons in the AMMC.

647B

Screening of Central Pain Circuits. Wijeong Jang, Sunwoo Kim, Changsoo Kim. Sch Biological Sci, Chonnam National Univ, Gwangju-Si, South Korea.

In Drosophila, multi-dendritic (MD) neurons represent the peripheral nociceptive neural circuits sending nociceptive signals to the brain. Little is known about the central nociceptive neural circuits that process these nociceptive signals from peripheral noxious stimuli. Here, we describe both the screening and identification of the potential central nociceptive neural circuits that mediate pain processing in the brain. Drosophila does not carry capsaicin receptors, so capsaicin can selectively activate neural circuits that do express capsaicin receptors. The mammalian vanilloid receptor TRP channel (TRPV1) is a capsaicin receptor that responds to noxious heat and capsaicin. We crossed UAS-hTRPV1 flies with "brain-Gal4" lines known to express Gal4 in the subsets of the brain. Progenies from the cross were then reared with capsaicin-laced food to stimulate neural circuits when they were eating. The logic behind this experiment was that flies experiencing nociception through stimulation of their central nociceptive neural circuits will avoid ingestion of food and subsequently starve to death. From the screening, we identified several lines that exhibited such starvation- induced death. We focused on one line of the "brain-Gal4" whose expression was limited in the subset of the brain and thus could represent the central nociceptive neural circuits.

648C

Analgesic Drugs Relive Pain in Drosophila. Sunwoo Kim, Myungsuk Oh, Eunhee Cho, Wijeong Chang, Changsoo Kim. Chonnam National University, Gwangju, South Korea.

Analgesic drugs relieve pain in vertebrates. We were interested in knowing whether analgesic drugs also reduce nociception in Drosophila. We generated transgenic flies that expressed the human capsaicin receptor (or TRPV1) in nociceptive multidendritic (MD) neurons, using the Gal4UAS binary expression system to induce nocicpetive neural circuits by capsaicin. We found that capsaicin-induced nociceptive behaviour appeared in the transgenic larva and adult flies. Of note, we found that the flies avoided ingestion of food if the food contained capsaicin, leading to death by starvation. This death rate increased as temperature, capsaicin concentration, and the dose of the UAS-hTRPV1 increased, suggesting that the death rate represented the level of nociception that the flies experienced when they ingested capsaicin-laced food. We also found that when analgesic drugs were included in the capsaicin food, reduced death rate, suggesting that analgesic drugs are effective in relieving nocicpeiton in Drosophila. We propose that this simple assay system can be useful for assessing potential compounds for in vivo efficacy for pain reduction.

649A

Drosophila exhibit active avoidance behavior in response to a predator. Claire J. Manson-Bishop[1,2], Gregg W. Roman[1,2]. 1) Biology and Biochemistry, University of Houston, Houston, TX; 2) Biology of Behavior Institute, University of Houston, Houston, TX.

Understanding the response of *Drosophila melanogaster* to predators is both relevant ethologically and important for 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. The behavioral response of Drosophila to predators has not been previously characterized; it is the goal of these experiments to characterize how the presence of predators changes the behaviors of Drosophila and to elucidate the sensory mechanisms responsible for these responses to a predator. In order to address this fundamental question, we studied Drosophila within the circular open field paradigm. For these experiments, we used 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. We show that Drosophila avoid the predators that are caged within the center of a circular open-field arena. Furthermore, in the presence of a caged predator Drosophila also exhibit a preference for a recessed alcove. This preference may represent a shelter-seeking response that is enhanced by the proximal danger of the predator. To begin to assess the sensory modalities required for this active avoidance behavior, we investigated the response of blind $norpA^{7}$ flies and broadly anosmic $or83b^{2}$ flies, independently, to a caged Pantropical jumping spider. Both $norpA^{7}$ and $or83b^{2}$ flies demonstrate avoidance of the predator, and a preference for the recessed alcove. The significant responses of both these genotypes to these predators may suggest that Drosophila uses redundant sensory modalities to detect and avoid predators.

650B

A functional genomic screen for phototransduction genes in *Tribolium* . Arun K Sasikala-Appukuttan[1], Matthew Kulpa[1], Zahabiya Husain[1], Magdalena Jackowska[1], Bryce Daines[2], Jason Caravas[1], Rui Chen[2], Heinrich Jasper[3], Markus Friedrich[1,4]. 1) Department of Biological Sciences, Wayne State University, 5047 Gullen Mall, Detroit, MI 48202, USA; 2) Human Genome Sequencing Center, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA; 3) Department of Biological Sciences, University of Rochester, River Campus Rochester, New York 14627, USA; 4) Department of Anatomy and Cell Biology, Wayne State University, School of Medicine, 540 East Canfield Avenue, Detroit, MI 48201, USA.

The visual system of higher Diptera like *Drosophila* is characterized by a complex pattern of differential photoreceptor sensitivities across the retina and an exceptionally fast photoreceptor response. Previous research in our lab has shown that the latter is associated with the derived duplication and

modification of phototransduction genes, implying differences in the usage and organization of visual genes in other insects. We have therefore begun to explore the molecular organization of photoresponse in the simple visual system of the red flour beetle *Tribolium castaneum*. Comparing the adult head transcriptome of wild-type and eye-depleted animals, we identified over 1000 retinal candidate genes. These include validation genes previously characterized in *Tribolium* (opsins, *cinnabar* and *glass*) but also candidate validation genes based on presumed evolutionary conservation of *Tribolium* with *Drosophila* (*Pph13*, *chaoptin*, *white* and many members of the phototransduction network). However, approximately 95% of the *Tribolium* retinal candidate genes have so far not yet been associated with vision related gene ontologies. These loci are now being investigated by whole mount in situ hybdridization and gene knockdown, testing for photoreceptor-specific expression and possible effects on the photonegative behavior of the *Tribolium* larva. Interestingly, homolog analysis revealed that 54 of the *Tribolium* candidate genes are shared with vertebrates, but absent in *Drosophila*.

651C

The Prion Protein Binds to Synapsin and Syntaxin in the Presynaptic Neuromuscular Junction. Jose E Herrera[1,2], Diego Rincon-Limas[2], Pedro Fernandez-Funez[2,3]. 1) éMaster's Program in Translational Biotechnology, University of Florida, Gainesville, FL; 2) Neurology, University of Florida, Gainesville, FL; 3) Neuroscience, University of Florida, Gainesville, FL.

The prion protein (PrP) is a GPI-anchored glycoprotein located in the extracellular membrane of neuronal and glial cells of the developing and mature nervous system. Although PrP misfolding and aggregation are the causative factors in several neurodegenerative disorders, the physiological role of PrP is unknown. Previous studies have described the localization of PrP in the synapse, where it interacts with synaptic proteins; however, the physiological relevance thereof remains unknown. Thus, my goal is to ascertain the function of PrP in the synapse, based on its interaction with known synaptic proteins. Using transgenic flies that express wild-type PrP, I first tested the co-localization of PrP with candidate synaptic proteins by immunofluorescence. Then, I utilized co-immunoprecipitation to confirm the direct interaction of positive candidates. I found that the presynaptic proteins, Synapsin and Syntaxin, co-localize and interact directly with the wild-type PrP, suggesting a role in neurotransmission for PrP. Moreover, I studied two mutant PrP fly strains, M206, 213S and Y145stop, to characterize phenotypic changes in synaptic morphology and protein distribution. The distribution of Syntaxin also changed in flies expressing PrP-M206, 213S, as did the number and size of synaptic boutons. To further elucidate its role in the synapse, I plan to use a proteomics approach involving synaptosomal separation, co-immunoprecipitation, and mass spectrometry, which will provide a more unbiased, complete list of binding partners for PrP. A comprehensive identification of all the PrP-binding proteins at the synapse will allow us to better understand the role of PrP in synapse function and/or architecture.

652A

Myosin VI contributes to synaptic transmission and development at the Drosophila neuromuscular junction. Marta Kisiel[1], Bryan Stewart[1,2]. 1) Cell and System Biology, University of Toronto, Toronto, Canada; 2) Department of Biology, University of Toronto Mississauga, Mississauga, Canada.

Myosin VI, encoded by jaguar (jar) in Drosophila melanogaster, is the only member of the myosin superfamily of actin-based motor proteins known to move towards the minus ends of actin filaments. In vitro studies demonstrate that Myosin VI has the ability to perform distinct functions as a cargo transporter and anchor in the cell, however which of these roles Myosin VI plays in the nervous system has yet to be determined. A locomotor defect, observed as sluggish movement in severe jar mutant larvae, was confirmed by behavioural assays. As this can indicate problems at the neuromuscular synapse, microscopy and electrophysiology were used to investigate neuromuscular junction (NMJ) structure and function in jar loss of function mutants of varying severity. Confocal imaging studies revealed a decrease in NMJ length, a reduction in bouton number per NMJ and mislocalization of synaptotagmin in jar mutant boutons. Electrophysiological experiments revealed a role for Myosin VI in basal synaptic transmission, with a reduction in low frequency nerve-evoked responses and spontaneous release in severe jar mutants. Changes in short-term synaptic plasticity were also observed in Myosin VI mutants using high frequency stimulation paradigms to recruit vesicles from different functional pools. In addition, a decrease in the number of active zones, which are the sites of vesicle release, was observed by staining against Bruchpilot at jar mutant synapses. As Bruchpilot loss of function is associated with a reduction in evoked nerve response, this is consistent with impaired synaptic function in jar mutants. Taken together, the data suggest that Myosin VI may function as an anchor to maintain proper peripheral vesicle localization at the bouton. Accompanied by the reduction in active zones, vesicles could be displaced from areas of higher probability release at jar mutant synapses. This study aims to elucidate the mechanism of Myosin VI function in neural communication.

653B

Rugose, a Drosophila homologue of the mammalian Neurobeachin, is involved in larval locomotion, adult habituation, learning and activity patterns. Emma Schatoff[1], Julian Flores[1], Alexandria Wise[2], Tadmiri Venkatesh[1]. 1) Biology, City University of New York The City College, New York, NY; 2) The Graduate Center, City University of New York, New York, NY.

Rugose is the Drosophila homologue of the mammalian and human Neurobeachin. Recent studies have shown that the Neurobeachin gene is disrupted in human patients with idiopathic Autism (Castermans et al., 2003) and the Neurobeachin gene spans the common Fragile site FRA 13A (Savelyeva et al., 2006). Our previous genetic and molecular analyses have shown that *rugose* (*rg*) encodes a Drosophila A kinase anchor protein (DAKAP 550) which interacts with the components of the EGFR- and Notch-mediated signaling pathways and facilitates cross-talk between multiple signaling pathways (Shamloulaet al., 2002). Genetic studies in *Drosophila melanogaster* have shown that cAMP and PKA (A kinase or protein kinase A) mediated signaling is required in a variety of processes, such as embryogenesis, pattern formation and synaptic function (Lane and Kalderon, 1993; LANE and Kalderon, 1995; DAVIS et al. 1996; Davis et al., 1998). A kinase anchor proteins (AKAPs) modulate the specificity of PKA function by targeting and localizing PKA to specific subcellular structures (Scott and Pawson, 2000). We will present our recent behavioral and electrophysiological studies, which show that *rugose* mutants exhibit defective learning, habituation, aberrant locomotion and hyperactivity. Our cell biological studies on the larval neuromuscular junction show abnormal synaptic architecture.

654C

Dube3a differentially regulates mEJPs in a ubiquitin dependent manner. Reese Scroggs[1], Rachel Chassen[2], Lawrence Reiter[1,3]. 1) Anatomy and Neurobiology, UTHSC, Memphis, TN; 2) IPBS Program, UTHSC, Memphis, TN; 3) Neurology, UTHSC, Memphis, TN.

Angelman syndrome is caused by maternal loss of the ubiquitin ligase gene *UBE3A*, while maternally derived duplications encompassing *UBE3A* can cause autism. We studied the elecrophysiological effects of *Drosophila UBE3A* (*Dube3a*) loss and over-expression using the larval neuromuscular junction (NMJ). We measured spontaneous, mini-excitatory junction potentials (mEJPs) and evoked excitatory junction potentials (EJPs) using the A3 region of third instar larvae. Over-expression of Dube3a using the neuronal driver *elav*-GAL4 increased mEJP amplitude, while flies deficient for *Dube3a* (i.e. *Dube3a*^{15b} homozygotes) had fewer mEJPs of a lower amplitude when compared to wild type. In addition, we found that a Dube3a construct that was not able to ubiquitinate substrates (*Dube3a*-C/A) did not result in high amplitude mEJPs and the number again approached wild type levels indicating that the ubiquitin ligase function of Dube3a is required for the high amplitude mEJP phenotype. The total number of synaptic active zones per bouton and number of boutons per muscle area were not affected by increased Dube3a levels, and an analysis of presynaptic terminal vesicle size suggests that the increased mEJPs may result from increased synaptic vesicle diameter. The average amplitude of rapidly evoked EJPs (15 Hz) decreased more rapidly after the beginning of stimulation in Dube3a over-expressing larvae compared to controls. The significant rapid decline in average EJP amplitude primarily resulted from an increase in the failure rate of evoked responses. Occasionally in control animals, large spontaneous depolarizations similar in amplitude to evoked EJPs were observed. These were blocked by TTX and thus may have resulted from spontaneous motor neuron firing. In Dube3a over expressing larvae, there was a significant increase in this putative spontaneous neuronal firing. Our findings suggest that Dube3a may regulate neurotransmitter release and neuronal excitability in a ubiquitin dependent manner.

655A

The synaptic vesicle-associated Ca^{2+} channel Flower couples synaptic exo-endocytosis cycle and regulates synaptic growth. Chi-Kuang Yao[1,2,3], Yong Qi Lin[2,3], Claire Haueter[2,3], Hugo J Bellen[2,3]. 1) Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan; 2) Department of Molecular and Human genetics, Baylor college of medicine, Houston, TX, USA; 3) HHMI.

Synaptic function and growth are two key components for synaptic plasticity that has been implicated in learning and memory. Synaptic function is achieved by synaptic vesicle (SV) exocytosis that, in response to action potentials, elicits a fusion of SV with the presynaptic membrane, leading to the release of neurotransmitters to provoke postsynaptic responses. SVs must then be properly endocytosed to sustain repeated transmission. Hence, a tight coupling of exo- to endocytosis is critical for synaptic function. Yet how this coupling is controlled remains poorly understood. Our previous work (Yao et al., Cell 2009, 138(5):947-60) has shown that the SV-associated Ca^{2+} channel Flower (Fwe) promotes synaptic endocytosis and thereby couples exo- to endocytosis. Intriguingly, loss of *fwe* also leads to synaptic outgrowth at larval neuromuscular junctions (NMJs), characterized as a increased number of satellite boutons, similar to those observed in many endocytic mutants, including *shi*, *synj*, *endo*, *dap160*, and *lap*. The similarity in phenotype suggests that these endocytic proteins may govern a common cellular machinery underlying SV endocytosis and synaptic growth. However, what these cellular mechanisms are is still unclear. More recently, we have been focusing on understanding the mechanisms underlying Fwe-mediated synaptic growth. Our results indicate that the Ca^{2+} influx triggered by Fwe is required for synaptic endocytosis but not growth, suggesting that Fwe has two distinct cellular functions. We are attempting to explore mechanisms underlying these two processes.

656B

Characterization of the neural expression and roles of the Ret tyrosine kinase receptor. Daniel Perea, Irene Miguel-Aliaga. Zoology, Cambridge University, Cambridge, Cambridge, United Kingdom.

In mammals, the RET tyrosine kinase receptor plays an important role in the regulation of kidney development and the specification of peripheral neurons, including sympathetic, parasympathetic and enteric neurons. In the nervous system, absence of RET has been shown to have a crucial role in the migration of sympathetic precursors and the specification of parasympathetic neurons. RET mutation also leads to defects in the survival, migration and proliferation of enteric neurons: a condition known as Hirschsprung's disease in humans. The Drosophila homologue of RET (dRet or Ret) has been cloned, and has been shown to be expressed in multiple tissues during embryogenesis, including the yolk sac, the stomatogastric nervous system and the developing Malpighian tubules (Hahn and Bishop, PNAS, 2000). Ret mRNA is also apparent in the central and peripheral nervous system (Sugaya et al, Mechanism of Development, 1993). This conserved neuronal expression in flies has prompted us to investigate whether, like its mammalian counterpart, Ret controls the specification of Drosophila intestinal neurons. As a first step towards investigating its functions, we have characterized Ret expression in embryonic, larval and adult enteric neurons. Our expression analysis indicated that Ret is expressed in different subsets of hindgut-innervating neurons throughout development. Experiments are currently underway to establish its significance in the specification and function of these neuronal subpopulations.

657C

Disassembling F-actin Networks Through Manipulations of Mical and Actin Bundling Proteins. Jimok Yoon, Heng Wu, Jonathan Terman. Center for Basic Neuroscience, U.T.Southwestern Medical Center at Dallas, Dallas, TX.

Cells continually interact with their environment and change their morphology in response to extracellular cues. Semaphorins are one of the largest families of these extracellular guidance cues and play critical roles in neurobiology, immunology, cardiovascular health, and cancer. Semaphorins are best known for their ability to disassemble actin filaments (F-actin) and we recently found that Mical, a protein that directly associates with the Semaphorin cell-surface receptor Plexin, is a novel F-actin disassembly factor that mediates Semaphorin/Plexin F-actin rearrangements. Herein, we use genetic approaches in the Drosophila model system and in vitro actin biochemical approaches with purified proteins to further investigate Mical-mediated F-actin alterations. We find that Mical and F-actin stabilizing/bundling proteins such as fascin and espin play antagonistic roles in regulating the F-actin cytoskeleton during development in vivo. Consistent with our in vivo data, we find that purified Mical protein disassembles fascin and espin bundled actin filaments in vitro. Our results go on to support a hypothesis that Semaphorin/Plexin/Mical directly disassembles the F-actin cytoskeleton and by so doing, triggers other actin regulatory proteins to reorganize a more complex F-actin network.

658A

The fate of identified dHb9-positive larval motor neurons during metamorphosis. Soumya Banerjee, Marcus Toral, Matthew Siefert, Joyce Fernandes. Zoology, Miami Univ, Oxford, OH.

During metamorphosis the nervous system is remodeled- a process which involves generation, re-specification, and elimination of specific neurons to form new adult-specific neural circuits. Many of the larval muscle fibers and motor neurons are eliminated during this time period to allow for the development of an adult motor system. Interestingly, some larval muscle fibers persist into the adult and function in eclosion. The focus of this study is to observe the fate of dHb9 expressing larval motor neurons which innervate a pair of ventral larval muscles (MF12 and MF13) which persist to the adult stage. These muscles selectively persist in the A1 and A2 segments. Since these muscle fibers are required for eclosion and then die, we propose that the cognate motor neurons will persist and then be eliminated after eclosion. To visualize individual motor neurons, we employ the 'flip-out' approach in conjunction with live imaging. Our preliminary studies have determined that in A1 and A2, larval MN12-1b maintains its larval pre-synaptic target, MF12, through metamorphosis and into the adult stage (n=14). However, we find that larval MN13-Ib changes its target from MF13 to adult specific ventral muscles in A1 and A2 during metamorphosis (n=4). Additionally, we have observed that the persistent MF13 is innervated in the adult stage by one of three dHb9-positive neurons; neurons belonging to a dorsal cluster of identified neurons in the ventral ganglion (n=14). Finally, we have also shown through TUNEL assays that the motor neuron innervating adult muscle fiber 13 shows signs of cell death around 4-6 hours post eclosion (n=10), much earlier than the time-point when the muscle fiber itself is eliminated. Our current work is aimed at identifying which member of the dorsally located neural cluster innervates persistent larval MF 13 in the adult. These studies will contribute to our lab's goal in following the re-specification of identified larval motor neurons to their adult fates.

659B

Neurotrophic actions of dopamine on the development of a serotonergic feeding circuit in *Drosophila melanogaster*. Parag Bhatt, Wendi Neckameyer. Pharmcological and Physiological Science, Saint Louis University School of Medicine, St Louis, MO.

In the fruit fly, *Drosophila melanogaster*, serotonin functions both as a neurotransmitter to regulate larval feeding, and in the development of the stomatogastric feeding circuit. There is an inverse relationship between neuronal serotonin levels during late embryogenesis and the complexity of the serotonergic axonal fibers projecting from the larval brain to the foregut, which correlate with perturbations in feeding, the functional output of the circuit. Dopamine does not modulate larval feeding, and dopaminergic fibers do not innervate the larval foregut. However, both decreased and increased neuronal dopamine levels during late embryogenesis result in depressed levels of larval feeding and hypersensitive feeding responses to the neurotransmitter actions of serotonin. Perturbations in neuronal dopamine during development also result in greater branch complexity of the serotonergic axonal fibers innervating the gut, as well as increased size and number of the serotonergic presynaptic vesicles along the neurite length. This neurotrophic action for dopamine is modulated by the dopamine D2 receptor expressed during late embryogenesis in central 5-HT neurons. Animals carrying transgenic RNAi constructs to knock down both dopamine and serotonin synthesis in the central nervous system display normal feeding and fiber architecture. However, disparate levels of neuronal dopamine and serotonin during development result in abnormal gut fiber architecture and feeding behavior. These results suggest that DA can exert a direct trophic influence on the development of a specific neural circuit, and that the actions of both dopamine and serotonin are critical for its development. National Science Foundation Grant No. 0616062 and The President's Fund, Saint Louis University.

660C

RNA-seq reveals diverse neurosecretory properties of the CNS-midline cells in *Drosophila*. Joseph R. Fontana[1], Stephen T. Crews[1,2]. 1) Molecular

Biology, Univ North Carolina, Chapel Hill, Chapel Hill, NC; 2) Dept. of Biochemistry and Biophysics, Univ North Carolina, Chapel Hill, Chapel Hill, NC.

The generation of cellular diversity in the central nervous system (CNS) is a critical process in the development of complex metazoans. The CNS midline of *Drosophila* is a segmentally repeated cluster of ~20 interneurons, motoneurons, and glia. With well-defined lineages and a common origin from the mesectoderm, these cells provide an isolated system that facilitates studying the establishment of this diversity. Using fluorescence-activated cell sorting (FACS), we have purified embryonic CNS-midline cells at two developmental time-points and performed RNA-seq to study gene expression. A comparison with information on our lab's publicly available database of midline expressed genes, MidExDB, revealed a 100% correlation between the RNA-seq data and the 77 genes previously examined via confocal microscopy. Additional validation of the data using fluorescent in situ hybridization also indicates an accurate representation of gene expression. Data analyses have led to the discovery that 8 neuropeptide precursor genes and 12 neuropeptide receptor genes produce expression in subsets of CNS-midline cells, including the glia. The characterization of this gene expression has provided an increased understanding of both the segmental variation and cellular diversity that exists in these cells. One example of this is *Myoinhibiting peptide precursor* (*Mip*) which only shows CNS midline expression in a single cell of segments S1 and S3. As an initial step in characterizing the transcriptional programs involved in setting up this diversity, we find *short neuropeptide F precursor* (*sNPF*) expression in ventral unpaired median interneuron 5 (iVUM5) to be downstream of the transcription factor Castor. Future work will involve identifying additional factors responsible for the regulation of these neural function genes, as well as analyzing RNA-seq data collected from isolated ventral unpaired median motoneurons (mVUM), a subset of the CNS-midline cells.

661A

Genome-wide expression profiling identifies genes regulated by JAK/STAT in the *Drosophila* optic lobe. Hong Luo, Hongbin Wang. School of Life Sciences, Tsinghua University, Beijing, China.

The JAK/STAT pathway is evolutionarily conserved from invertebrates to vertebrates, and plays important roles in animal development and human disease. Although the signaling pathway has been well established, we know relatively little about what are the relevant target genes that mediate the effects of JAK/STAT activation. Here, we have used DNA microarrays to identify JAK/STAT targets in the *Drosophila* larval brain and identified 45 genes that are positively regulated by JAK/STAT; many of these genes contain clustered STAT92E binding sites in short conserved genomic sequences suggesting that they may be direct target genes. More than two thirds of the genes identified encode proteins that have orthologs in humans. Analyses of *Nop56*, which encodes a conserved protein involved in ribosome biogenesis and cell growth, reveal an essential role of *Nop56* in optic lobe development, as loss of *Nop56* activity prevented neuroepithelial growth and expansion. RNAi knockdown of *Nop56* in clones of cells caused premature differentiation of neuroepithelial cells into neuroblast-like cells. Interestingly, *Nop56* knockdown in the lamina precursor cells accelerated lamina neurogenesis while ectopic expression of *Nop56* inhibited lamina neuron differentiation. Thus, Nop56 promotes neuroepithelial cell growth and division and suppresses their differentiation into both medulla neuroblasts and lamina progenitor cells. These results provide a novel insight into the control of lamina neurogenesis in the *Drosophila* brain.

662B

Identification of novel maternal neurogenic genes that are potential components of Notch signaling in *Drosophila*. Kenjiroo Matsumoto[1], Naoki Aoyama[1], Takahiro Seto[1], Ryo Hatori[1], Akira Ishio[1], Takahiro Maeda[1], Tamiko Itou[1], Syusuke Shimaoka[1], Hironao Iida[1], Takuma Gushiken[1], Yuu Atsumi[1], Tomoko Yamakawa[1], Takeshi Sasamura[1], Kenji Matsuno[1,2]. 1) Dept. Biol. Sci./Tec., Tokyo Univ of Science; 2) Res. Inst. Sci./Tec., Tokyo Univ of Science.

Notch signaling regulates many cell-fate specifications through local cell-cell interaction in *Drosophila* development. Notch signaling is involved in "lateral inhibition" that prevent 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 results in the hyperplasia of neuronal cells in *Drosophila* embryos, which is referred to as the "neurogenic phenotype". Because most of the genes that encode Notch-signaling components are essential for lateral inhibition, these genes were first identified by the neurogenic phenotype resulting from their disruption. 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 address this problem, we screened for mutants that showed neurogenic phenotype in their homozygous embryos 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. We screened the left arm of the second chromosome, which covers about 20% of the *Drosophila* genomes. We identified 5 mutants that showed maternal neurogenic phenotype. Currently, we are mapping the genetic loci of these mutants. The summary of this screen and molecular genetics analyses of these maternal neurogenic genes will be presented. Our analyses of these maternal neurogenic genes will contribute to the understanding the molecular mechanism of Notch signaling.

663C

The Role of Dscam in Dendrite Development of an identified Drosophila Motoneuron. Katie M Hutchinson, Carsten Duch. Arizona State University, Interdisciplinary Graduate Program in Neuroscience, Tempe, AZ 85287-4501.

Correct dendritic architecture development requires precise control over various dendritic arborization characterstics in order to ensure that dendrites cover a specific input territory non-redundantly. We have previously analyzed the complex dendritic tree of an identified adult Drosophila flight motoneuron, MN5, by means of quantitative geometric dendritic arbor reconstruction. The complex MN5 dendritic trees develop during pupal life, comprise more than 4000 dendritic branches which add to 6500 microns of total dendritic length, and cover a well-defined input territory according to specific rules. First, self dendrites avoid each other. Further, different sub-trees cover different non-interdigitating areas of the input space (intra-neuronal tiling), which is predicted to have consequences for the flight motor network and computation of synaptic input. This study aims to unravel the developmental mechanisms underlying intra-neuronal tiling of complex dendritic fields in the CNS. We hypothesize a combination of activity dependent competition for synaptic partners and dendritic self-avoidance. Based on findings in sensory dendritic arborization neurons that do not receive input synapses, we propose that Down syndrome cell adhesion molecule (Dscam) underlies dendritic self-avoidance in central neurons. In fact, targeted expression of UAS-Dscam-RNAi in MN5 causes increased fine dendrite branching and self-dendrite contacts. Inclusion of extra DICER (UAS-DICER) in MN5 causes a range of phenotypes. The most severe is a loss of all mature dendrites with only intermingled filopodia. To circumvent unspecific network or RNAi effects we created mutant MN5 in an otherwise wildtype background by employing the MARCM technique. Preliminary MARCM and RNAi data suggest dual functions for Dscam in adult

neuron central dendrite development; a Dscam function in self-recognition and avoidance, and second, a Dscam function in stabilizing immature filopodia so they may mature into stable dendrites. Mechanisms and functional consequences for flight behavior are currently under investigation.

664A

NMNAT protects against hypoxia-induced dendrite degeneration. Yuhui Wen, Grace Zhai, Michael Kim. Molecular and Cellular Pharmacology, University of Miami, Miller School of Medicine, Miami, FL.

The proper maintenance of dendritic arbors is important for neuronal connectivity and function. Loss of dendrites induced by hypoxia is one of the pathological hallmarks of brain injury after stroke. Dendritic fields of Drosophila class IV dendritic arborization (da) sensory neurons provide a unique system to investigate the mechanisms important for dendrite maintenance. We previously found that the NAD synthase Nicotinamide mononucleotide adenylyl transferase (NMNAT) is required for the proper maintenance of class IV dendrites during larval development. Here, we show that NMNAT is also important for maintaining dendritic integrity under hypoxia. We found that wild-type flies under anoxic conditions (extreme condition of hypoxia, 0.1% O2) exhibited slightly reduced dendritic branching, but no signs of degeneration. However, flies heterozygous for a loss-of-function mutation in nmnat showed reduced dendritic branching along with severe dendrite degeneration under the same anoxic conditions. These results suggest that NMNAT maintains dendritic integrity under anoxia in a dose-dependent manner. We further found that knockdown of genes with important roles in autophagy suppress dendrite degeneration phenotypes in nmnat heterozygotes under anoxia. Our findings suggest that NMNAT protects against autophagy-related processes that contribute to dendrite degeneration in response to hypoxic conditions.

665B

RNAi screen to identify genes involved in retinal basal glia (RBG) cells in Drosophila. Yen-Ching Chang[1,2], Y. Henry Sun[1,2]. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, Republic of China; 2) Department of Life sciences and Institute of Genome sciences, National Yang-Ming University, Taipei, Taipei, Taiwan.

During the process of the eye development, elaborate photoreceptor-glia interaction is required for the formation of mature visual system. Retinal basal glial (RBG) cells proliferate and migrate from optic stalk to the eye disc after the differentiation of photoreceptor cells. When they migrate into the eye disc, they provide a guidance cues for photoreceptor axon projection, and they differentiate into wrapping glial cells responsible for axonal insulation. Several important genes have been discovered to elucidate the relationship between glia cells and photoreceptor cells. These genes can be separated into two groups: one is the intrinsic factor (acting within RBG) like Ras, the other are the extrinsic factors (derived from other cells but affecting RBG) such as FGF ligands for migration and differentiation. However, the mechanism regulating the cellular development and interaction with cells in eye disc remains unclear. Additional molecular players need to be identified. We will screen for genes functioning in RBGs to affect their migration, distribution, differentiation and proliferation. We drove UAS-RNAi expression in RBG using *repo*-Gal4, and monitored the fluorescent-labeled cellular morphology and pattern of RBG. Among 139 RNAi lines screened, we found 21 genes with morphological defects. Some of them are cell cycle regulated genes played roles in RBG development. The genes affecting proliferation/migration/differentiation have been further analyzed by immunostaining and other genetic approaches.

666C

Study of Cell Lineage in Drosophila Retinal Basal Glia. Yu Fen Huang[1,2], Y. Henry Sun[1,2]. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Department of Life Science and Institute of Genome Science, National Yang-Ming University, Taipei, Taiwan.

Glial cells play important roles in neuronal development and function. The retinal basal glia (RBG) is a subset of glial cell that originates in the optic stalk in second instar of *Drosophila*. RBG cells start to migrate into the eye disc as photoreceptor cells (R cell) begin to differentiate. The presence of RBG cells in eye discs is essential for R cell axons to enter the optic stalk. Three main classes of RBG cells have been identified including carpet glia (CG), surface glia (SG), and wrapping glia (WG). According to the “sequential differentiation model” proposed by Silies et al. (*J. Neurosci.* 27:13130-9), surface glia located at the basal side migrates forward along the carpet glia. Once they reach the anterior margin of carpet glia and contact the neuron, these migratory glia starts to differentiate into wrapping glia and wrap around R cell axons. The two large nuclei CG cells have extensive membrane and are regard as an insulator between SG and WG. To test this model, I am using Twin-spot MARCM (Yu et al., *Nat. Neurosci* 12, 947-953) to trace the cell lineage of RBG. My results suggested that there are independent lineage decisions for SG and WG.

667A

Degeneration of optic lamina caused by defective endocytic function in glial cells. Yuan-Ming Lee[1,2], Y. Henry Sun[1,2]. 1) Inst Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Inst Genomic Science, National Yang-Ming University, Taipei, Taiwan.

The visual system is composed of neurons and glial cells. In the optic lamina and medulla, there are also several distinct groups of glial cells. These are known to play a role in photoreceptor axonal projection and maintain the physiological function of the lamina monopolar neurons. We are interested in the function of glias in the adult visual system. By blocking the endocytic pathway by expressing the temperature-sensitive dominant-negative dynamin (shits1) in glia, the degenerative vacuoles formed in the optic lamina. Our analyses also suggest that the vacuoles are formed cell-autonomously by the epithelial and marginal glia in the optic lamina in MARCM experiment. The visual synaptic transmission was abolished in phototaxis and in ERG assays. The vacuolized lamina was rescued by the coexpression of dTOR but not anti-apoptotic p35. Therefore the endocytic function may be required for the glia to survive by inhibit caspase-independent or autophagy. Molecular definition of the autophagy features in degeneration neuropile is still studying.

668B

Number Matching Between Ommatidia and Retinal Basal Glial Cells (RBGs) during *Drosophila* Eye Development. Par B. Pun[1,2], Yi Henry Sun[1,2]. 1) Molecular and Cell Biology, Taiwan International Graduate Program, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Graduate Institute of Life Science, National Defense Medical Center, Taipei, Taiwan.

Glial cells are important for nervous system in maintaining homeostasis of neurons, guiding neuronal axon projection and regulating axon fasciculation. The compound eye of *Drosophila* consists of around 750 ommatidia along with glial cells. These glial cells, located at the basal part of eye disc, called the retinal basal glia (RBG) cells,originate from optic stalk in second instar of larva and migrate into eye disc along with photoreceptor cells differentiation.

RBG cells are involved in guiding the axon of photoreceptor cells toward the optic stalk and wrapping the axon. Previous studies have reported that the number of ommatidia and RBGs in eye disc have a relatively fixed ratio.However, the actual ratio differs in different reports. We still do not know whether number of ommatidia and RBG cells match during development of *Drosophila* eye. Here, we closely monitored the number of ommatidia and RBGs in different developmental stages ranging from 2nd instar larvae to 4hrs after puparium formation (APF) of wild type flies. We observed that the ommatidia/RBGs ratio is one at early stage. Along with the development, the number of ommatidia increases; however, number of RBG cells remains plateau after mid late 3rd instar or after 15 rows of ommatidia development. The ommatidia/RBG ratio becomes around two at 4hrs APF. In genetically manipulated flies with increased number of ommatidia, more RBGs cells were found in the eye disc and the ommatidia/RBG ratio remained around two similar to wild type flies.Further,In small eye mutants, where the number of RBGs reduced, the ommatidia/RBGs ratio also remained around two. These findings suggest a mechanism to match the number between ommatidia and RBG cells; possibly, photoreceptor regulates number of RBG cells for final adjustment of ommatidia/RBGs ratio. Possible mechanisms involve in such number matching may be migration, proliferation or survival/apoptosis of the RBG in response to the changes in ommatidia number.

669C

Glial remodeling during reorganization of the peripheral nervous system. Matthew Siefert, Soumya Banerjee, Bridget Hartman, Tara Fallah, Todd Simmons, John Wilber, Dorothy Lakis. Zoology, Miami University, Oxford, OH.

During metamorphosis of *Drosophila*, the nervous system is remodeled to execute developed adult specific behaviors. One of the morphological changes is the fusion of posterior abdominal nerves (A4-A8) to form a terminal nerve trunk (TNT). The objective of our study is to understand how glial ensheathment of individual nerves in the larva is rearranged to form the TNT. Four layers are known to ensheath individual nerves (Stork et al 2008) the outer layer, the neural lamella (NL) which is an extra cellular matrix, the perineurial glia (PG) present just below the lamella, the subperineurial glia (SPG), and lastly the wrapping glia (WG). Using layer specific markers, we have seen that the NL is absent just before TNT formation and remains absent until the 72h after puparium formation (APF) stage. This has been confirmed using electron microscopy at 28h APF, a time when the transition to the TNT begins. Using anti-repo, we observe a 3 fold increase in the total number of glial cells at 24h APF, and roughly 80% of these cells are PG. Because this layer is prevalent throughout metamorphosis, our current research is focused on the contribution of PG to the formation of the TNT. We plan to target the cell death gene *reaper* to PG at time points that coincide with proliferation and differentiation of glial cells. We anticipate that this manipulation will lead to abnormalities in the TNT formtaion. Another aspect of the project is to manipulate glial process outgrowth to test its role in TNT formation.

670A

Dissecting the regulation of a novel gene cg11910 in the longitudinal glial cells of drosophila embryos. Pavithra Vivekanand[1], Jaclyn Malat[2]. 1) Biology, Dickinson College, Carlise, PA; 2) Franklin and Marshall College, Biology Department, Lancaster, PA 17604.

The long-term goal of this research project is to understand the molecular mechanisms that regulate the differentiation of glial cells within the central nervous system (CNS) of Drosophila. Within the NS of Drosophila, the glial cells are broadly divided into two classes, the midline and lateral glia, based on their cellular origin and location within the VNC. The midline glial (MG) cells develop from mesectodermal tissue, while the lateral glial cells arise from the neuroectoderm. The differentiation of the lateral glial cells is regulated by the transient expression of the transcription factor, Glial cells missing (Gcm). Gcm initiates lateral glial cell differentiation by inducing the expression of downstream transcription factors such as Reversed polarity (Repo), Pointed (Pnt) and Tramtrack (Ttk). While the initial events of lateral glial cell determination have been well characterized, the generation of diversity in the sub-types of lateral glial cells is poorly understood. In order to understand how cell fate specification of the different types of lateral glial cells is established, we are investigating the regulation of a novel gene cg11910 that is expressed in longtidudinal glial cells. The regulatory region of cg11910 has predicted binding sites for Repo and Pnt, which suggests that Repo and Pnt might regulate its expression. Consistent with this hypothesis cg11910 mRNA expression is drastically reduced in gcm mutants and completely abolished in both repo and pnt mutant embryos. To determine whether cg11910 expression is directly regulated by Repo and Pnt we will perform transcription assays in Drosophila cultured cells and generate transgenic flies expressing GFP reporter constructs under the control of the identified upstream regulatory region.

671B

The actions of gonadotropic hormones on the development and mature function of a defined neural circuit in *Drosophila melanogaster*. Selma Avdagic, Bhatt Parag, Neckameyer Wendi. Pharmacological 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 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 both 5-HT innervation of the gut as well as larval feeding behavior. Using transgenic and pharmacological approaches, we have manipulated levels of the critical gonadotropic hormones (juvenile hormone and ecdysteroids) in the brain and the fat body (a sexually dimorphic tissue that secrete hormones) during CNS development. Our preliminary studies have established that, as for mammals, gonadotropic hormones affect the development of neural circuitry, which is sensitive to the sexual identity of the tissue. Funded by National Science Foundation Grant No. 061606.

672C

Sexual identity affects the development and mature function of a defined neural circuit in *Drosophila* melanogaster. Parag Bhatt, Selma Avdagic, Wendi Neckameyer. 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 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 both 5-HT innervation of the gut as well as larval feeding behavior. In addition, although feeding does not differ in male and female larvae, it is differentially sensitive to the trophic, or developmental, actions of neuronal 5-HT and DA, evidence that the developing brain is sensitive to the hormonal environment. Using transgenic approaches, we have manipulated the sexual identity of the brain and the fat body (a sexually dimorphic tissue that secrete hormones) during CNS development. Our preliminary studies have established that the sexual identity of these tissues are sensitive to the actions of DA and 5-HT function during CNS development. Funded by National Science Foundation Grant No. 0616062 and The President's Fund, Saint Louis University.

673A

jim lovell, a BTB-POZ domain protein implicated in neural differentiation and embryonic pattern formation. Kathleen M. Beckingham, Sonia Bjorum, Rebecca A. Simonette, William J. Deery, Raul Alanis, Benjamin Lewis. Dept Biochem & Cell Biol, Rice Univ, Houston, TX.

jim lovell (*lov*), CG16778, encodes a transcription factor with a BTB (*Broad*, *tramtrack*, *bric-a brac*) dimerization domain. *lov* mutations *lov47*and *lov66* delete adjacent DNA in the 5' flank of three of the *lov* transcripts and produce different phenotypes. *lov47* produces enhanced sensitivity to larval crowding and defects in male courtship. In contrast, *lov66* shows no behavioral defects but has reduced female fertility associated with dorsal appendage abnormalities. All four *lov* transcripts encode the same protein. Thus we have used a Lov antibody and RT-PCR to investigate *lov* expression and determine the effects of *lov47* and *lov66* on *lov* transcripts. The *lov* transcripts show highly individual expression patterns. Transcript A is testis-specific, but our antibody gives no distinctive expression pattern in the testis. Transcript C is expressed in early embryogenesis. Our antibody shows that this expression results in a "stripey" pattern of Lov protein, like the pair rule patterns, and later, in accumulation of Lov in the amnioserosa. Transcripts B and D are responsible for neural-specific expression that begins late in embryogenesis. In the PNS, Lov is found in subsets of chordotonal and external sensory neurons, suggesting that Lov acts to differentiate individual neurons within a given class. *lov66* results in ectopic expression of *lov* transcripts in the ovary and disrupted expression of *Broad*, a determinant of dorsal appendage fate, in ovarian follicle cells. Given that Broad is a *lov*-related BTB transcription factor, we hypothesize that *lov66* disrupts chorion development by interfering with Broad activity. In contrast, *lov47* results in loss of expression of the neural transcript D. The behavioral phenotypes associated with *lov47* presumably reflect loss of this transcript. *lov47* and *lov66* probably delete different regulatory elements associated with the gene. Supported by NASA Grant NNX09AH43G.

674B

Identification of a novel suppressor of Crumbs and its role in growth regulation. Eunbyul Yeom, Kwang-Wook Choi. Department of Biological Sciences, Graduate School of Nanoscience and Technology, KAIST, Daejeon, South Korea.

Crumbs (Crb) is a transmembrane protein which regulates the apical-basal polarity in *Drosophila*. Recent studies have shown that Crb is also involved in growth control by regulating the Hippo signaling pathway. To gain further insights into the intracellular function of Crb, we screened a set of RNAi lines for genetic modifiers of the eye phenotype caused by overexpression of the Crb intracellular domain (Crb^{intra}). RNAi knockdown of one of these modifiers labeled as Su(Crb) strongly suppressed the Crb^{intra} phenotype. The C-terminal half of this gene is highly conserved in metazoan species, but no specific function has been identified yet. To investigate the biological function of Su(Crb), we generated loss-of-function mutant lines by imprecise excision of an adjacent P-element insert. Two mutant alleles $Su(Crb)^{\Delta 52}$ and $Su(Crb)^{\Delta 182}$, are likely to be null, as they are deficient in almost entire coding sequence. Su(Crb) homozygotes also suppressed the Crb^{intra} eye phenotypes and they have physical interaction in vitro. These data suggest that the genetic interaction between Crb and Su(Crb) is specific. Su(Crb) mutants are semi-lethal, as they die during different stages while adult escapers show low fecundity and reduced lifespan. Dying mutant animals show severe developmental defects. Taken together, our study suggests that Su(Crb) is a novel component that functions in the Crb pathway necessary for growth regulation.

675C

scalloped expression during Drosophila embryogenesis. Michael Benson[1], Nicholas Gubitosi[1], Elizabeth Norris[1], Chelsea Gurvis[1], Elena Brandano[1], Karrie Brondell[1], Rachel Yonker[1], James Skeath[2], Kirsten Guss[1]. 1) Biology, Dickinson College, Carlisle, PA; 2) Genetics, Washington University School of Medicine, St. Louis, MO.

scalloped (sd) functions with *vestigial (vg)* to control wing development in Drosophila melanogaster. To characterize the expression of *sd* in other tissues during fly embryogenesis, we employed whole mount immunodetection of an antibody that we developed for the corresponding protein. We show that SD expression becomes localized to subsets of cells in the central and peripheral nervous systems and limb primordia as embryogenesis proceeds. In the ventral nerve cord, SD expression overlaps with that of VG in neuroblast 1-2 descendants and the ventral unpaired median motorneurons. This work provides the necessary descriptive foundation for functional studies regarding the role of *sd* during development of the Drosophila central nervous system.

676A

CHARACTERIZATION OF *aaquetzalli (aqz)*, A GENE REQUIRED FOR DEVELOPMENT OF THE NERVOUS SYSTEM DURING *Drosophila melanogaster* EMBRYOGENESIS. Miguel Mendoza-Ortiz, Juan Riesgo-Escovar. Dep. de Neurobiología del Desarrollo y Neurofisiología, Inst. Neurobiología, UNAM, Campus Juriquilla, Querétaro, México.

The gene *aqz locus* encodes pioneer proteins with limited homology. *aqz* is requiered for nervous system formation, since *aqz* mutants die as embryos with malformed nervous system. The name *aaquetzalli* derives from the fan-shaped appearance of mutant cuticles, also affected, as *aaquetzalli* is the Nahuatl word for fan. *aqz* expression occurs at all stages of the *Drosophila melanogaster* life cycle, and is dynamic. Mutant analysis of *aqz* in epidermal and nervous tissues reveal early defects implying a function in early ectoderm differentiation into epidermis and nervous system: *aqz* mutant alleles have anterior, dorsal and ventral holes in the embryonic epidermis, as well as central nervous system malformations and faulty axons and commisures, plus loss of peripheral nervous system. We analyzed if these mutants exhibit deficits during neural stem cell formation. Using neural stem cell markers like Deadpan (Dpn) for neuroblasts and Even Skipped (Eve) for ganglion mother cells (GMC), we showed that *aqz* mutant embryos present severe neural stem cell defects in the ventral and procephalic regions. In most cases, we document spotty hyperplasia of neural stem cells and a parallel loss of epithelium. This neural stem cell phenotype resembles the Notch-Delta neurogenic phenotype, so we have conducted genetic interactions with this pathway. Results imply that *aqz* interacts with this pathway.

677B

Dissecting the *cis*-regulatory enhancers that control the POU-domain transcription factor genes, *pdm-1 & pdm-2*. Jermaine Ross, Thomas Brody, Mukta Kundu, Alexander Kuzin, Ward F Odenwald. NINDS, NIH, Bethesda, MD.

While neuroblast (NB) lineage studies have identified transcription factor (TF) genes important to cell identity decisions, we currently have only an incomplete understanding of the cis-regulatory elements that control their expression. To identify, compare, and functionally test these regulatory sequences, we have employed transgenic reporter assays along with our comparative genomic programs, *EvoPrinter* and *cis*-Decoder (also see Kundu et. al and Brody et al. abstracts). Here, we describe the enhancers that regulate the *pdm-1 & pdm-2* genes. These POU-domain TF paralogs carry out overlapping functions during CNS lineage development. Thus far, we have identified over 20 enhancers that are located within a 125 kb region that spans the *pdm-1 & -2* locus on the second chromosome. Our studies have shown that these enhancers are functionally autonomous and control different subsets of the *pdm* expression patterns during embryonic, larval and/or adult CNS development. Among these, we have identified *pdm-1 & -2* enhancers that activate expression in overlapping subsets of NBs. For example, enhancers that drive transgene activity in overlapping but nonidentical expression patterns during intermediate stages of NB lineage development. Further, *cis*-Decoder analysis of the conserved DNA within the NB enhancers identifies shared and unique conserved sequences. Site-directed mutagenesis of these conserved sequences reveal that they are important for cis-regulation. One of the tested sequences includes a highly conserved 9-mer sequence, 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 absence 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 identified through *EvoPrinter* and *cis*-Decoder analysis, and will report these findings.

678C

Molecular basis of the production of neuronal diversity in the Drosphipha visual center. Takumi Suzuki, Masako Kaido, Rie Takayama, Makoto Sato. Frontier Science Organization, Kanazawa University, Kanazawa, Japan.

The medulla, the primary region of the optic lobe, shares structural features with a mammalian brain such as layer and columnar structures and contains 60 types of 40,000 neurons. So far, the developmental mechanisms that produce such neuronal diversity are largely unknown. Our recent study revealed that the medulla is subdivided into concentric zones that are characterized by the expression of four conserved transcription factors (Homothorax (Hth), Brain-specific homeobox (Bsh), Runt (Run), Drifter (Drf); collectively called concentric genes) during larval development (Hasegawa et al., Development 138, 983- (2011)). Although these concentric genes contribute to defining each neuronal type, regulatory mechanisms underlying specification of their expression are unclear at all despite their functional importance. In the embryonic central nervous system, the neuronal type is defined by transcription factors that are sequentially expressed in the neuroblasts (NBs: Isshiki et al., Cell 106, 511- (2001)). In the medulla, NBs situated on the cortical surface generate neurons and birth order of the neurons correlates with the concentric gene expression. This suggests that types of the medulla neurons are specified in a birth order-dependent manner. We therefore searched for genes that are temporally expressed in the medulla NBs and found a group of candidate genes. Among them, sloppy-paired (slp) is expressed in the newly produced NBs while Dicheate (D) is expressed in the older ones. To investigate roles of these genes, we generated gain-of-function and loss-of-function clones for each gene. In clones expressing slp, ectopic Bsh expression was observed. In slp mutant clones, Run and Drf expression was induced ectopically. In clones expressing D, ectopic Run expression was observed. By contrast, knock-down of D resulted in disruption of Run domain. These results suggest that sequential expression of slp and D in NBs is involved in specification of concentric gene expression, and that types of medulla neurons are specified in a birth order-dependent manner.

679A

***Drosophila* motor neuron retraction mediated by inputs from TGF-β/BMP signaling and orphan nuclear receptors.** Jean-Maurice Dura[1], Ana Boulanger[1], Morgane Farge[1], Christophe Ramanoudjame[1], Kristi Wharton[1,2]. 1) Inst of Human Genetics, CNRS/UPR 1142, Montpellier , France; 2) Dept of Molecular Biology, Brown University, Providence, RI, USA.

Larval motor neurons remodel during *Drosophila* neuro-muscular junction (NMJ) dismantling at metamorphosis. In this study, we describe the motor neuron retraction as opposed to degeneration based on the early disappearance of β-Spectrin and the continuing presence of Tubulin. Importantly, we show the presence of peripheral glial cells close to the neuro-muscular junction that retracts before the motor neuron. By blocking cell dynamics with a dominant-negative form of the Dynamin, we show that glial cell and macrophages have key role in this process. We show also that expression of *EcR-B1*, encoding the steroid hormone receptor required for muscle dismantling, is under the control of the *ftz-f1/Hr39* orphan nuclear receptor pathway. In the motor neuron, activation of *EcR-B1* expression by the two parallel pathways (TGF-β signaling and nuclear receptor) triggers axon retraction. We propose a model for the sequential events that are occurring during NMJ dismantling at early metamorphosis. First, *EcR-B1* is expressed in the muscle under the control of FTZ-F1. FTZ-F1 activates *EcR-B1* and represses *Hr39*. This repression is compulsory for *EcR-B1* activation. Importantly, TGF-β/BMP signaling does not appear to be required for *EcR-B1* activation in this tissue, however, a result of *EcR-B1* activation in the muscle would be the production of a secreted TGF-β family ligand. Then, this secreted TGF-β family ligand reaches the appropriate receptors and activates the TGF-β signaling in the motor neuron. Finally, TGF-β signaling in association with the nuclear receptor pathway activates *EcR-B1* expression resulting in motor neuron retraction. The requirement of the two pathways in the motor neuron provides a simple molecular explanation of the instructive role of postsynapse degradation on motor neuron retraction. This mechanism insures the temporality of the two processes and prevents motor neuron pruning before postsynaptic degradation.

680B

Axonal transport and synaptic function are linked in two Drosophila disease models of neurodegeneration. Shermali Gunawardena[1], Min Jung Kang[1], Monique Michiewicz[2], Hong Bao[1], Samantha Fye[1], Tadeusz J. Kaczynski[2], Bing Zhang[1], Shermali Gunawardena[1]. 1) Biological Sciences, SUNY at Buffalo, Buffalo, NY; 2) Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019.

Formation of new synapses or maintenance of existing synapses requires the delivery of synaptic components from the soma to axodendritic sites via axonal transport. However, the link between transport and synaptic function is poorly understood. Previously we found that expression of disease proteins; pathogenic polyQ repeat protein or human amyloid precursor protein with an FAD mutation (SWE) caused axonal transport defects. Here we show that

larval synapses expressing pathogenic polyQ protein or SWE also show defects in the total number of boutons, in the ratio of the pre and post synaptic size of boutons and in the synapse length. Similar defects are observed in kinesin and dynein mutants. Abnormalities in synaptic transmission are also observed in dynein mutants and in larvae expressing pathogenic polyQ proteins. In contrast, a synaptic protein mutant that has severe synaptic defects does not show axonal transport defects. Together our findings suggest that problems in transport can propagate defects in synapse maturation and function; however, synaptic problems have no direct consequence on transport. Further, proper maintenance and function of synapses requires the efficient transport of BMP signaling components, which genetically interact with kinesin and dynein motors. Collectively our data suggest that perturbations in axonal transport can directly contribute to synaptic abnormalities observed is degenerative diseases by affecting the transport of BMP signaling proteins.

681C

Spatial and Temporal Analysis of Axonal Transport Defects in Primary Neuronal Cultures from Drosophila Larvae. Gary Iacobucci, 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 of 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 larvae to 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 these movement behaviors at each time point. In contrast, motor protein mutant cultures show reduced movement of cargos with increased numbers of stalled blocks. Over time, these mutant cultures also show defects in neuronal growth. Strikingly, we find that axonal blockages are not fixed, permanent blocks that impede transport as previously thought, but are instead dynamic and can resolve over time. Under some mutant conditions blocks resolve while under others they do not. Taken together, our results propose that non-resolving blocks may initiate deleterious pathways leading to death and degeneration while resolving blocks are benign.

682A

The memory gene nalyot (Adf-1) functions downstream of CaMKII to regulate activity-dependent dendritic plasticity. Christina Timmerman, Subhabrata Sanyal. Cell Biology, Emory University, Atlanta, GA.

Understanding molecular mechanisms that underlie the complex processes of adaptive neuronal plasticity and memory is a central question in neuroscience. In Drosophila, genetic screens have isolated several “memory mutants”, among them nalyot, a hypomorphic mutation in the transcription factor Adf-1. Although nalyot mutants exhibit a dramatic defect in long-term memory formation, cellular phenotypes that results from a loss of Adf-1 activity remain largely unknown. Here, we show that Adf-1 plays a prominent role in the regulation of dendrite development and plasticity. Using the RP2 motor neuron as a model and in vivo 3D reconstruction techniques, we find that loss of Adf-1 inhibits basal dendrite development and precludes activity-dependent plasticity. Adf-1 also functions downstream of CaMKII signaling since a “phospho-null” variant of Adf-1 inhibits, while a “phospho-mimic” form of Adf-1 promotes dendrite growth. These results, together with a series of epistasis experiments, provide biological relevance for previously suspected CaMKII-dependent phosphorylation sites in Adf-1. We also report that behavioral consequences of Adf-1 perturbation in motor neurons are severe. Furthermore, similar manipulations in mushroom body neurons (sites of associative learning and memory) affect memory formation. Finally, to identify novel transcriptional targets of Adf-1, we have carried out ChIP-Seq analysis from brain tissue using our anti-Adf-1 antibodies. These experiments, while confirming the few previously known targets of Adf-1 (e.g. Alcohol dehydrogenase), have revealed high confidence binding sites upstream of neuronally enriched genes with known functions in axon/dendrite guidance. A summary of these findings and investigations into several of these candidates will be discussed.

683B

Comparative Analysis of larval Locomotion Activity and neuromuscular junction formation among Drosophilids. Yunyi Yang, Mirela Belu, Claudia Mizutani. Department of Biology, Case Western Reserve University, Cleveland, OH.

Comparative studies among related Drosophilids have the potential to reveal how functional structures, such as somatic muscles and Neuromuscular Junctions (NMJs), may evolve across species and provide a framework to study the evolutionary basis of locomotor patterns. Results from our lab indicate that the larval somatic body wall musculature of four Drosophila species, *D. busckii*, *D. pseudoobscura*, *D. simulans* and *D. sechellia*, display the same stereotyped pattern of 30 muscle fibers per segment, as previously described for *D. melanogaster*. However, the number of myoblasts per individual fiber is highly variable, resulting in fibers of small size in *D. busckii* and *D. pseudoobscura*, and large size in *D. simulans* and *D. sechellia*, in comparison to *D. melanogaster*. Here we asked whether the increase in muscle size may have modified the development of NMJs in these species, and consequently their locomotor patterns. By using a MatLab-based video-tracking motion analysis software, we found species-specific patterns of larval crawling movements with significantly different rates of contractions, speed and displacement efficiency. The locomotion patterns are not directly influenced by muscle fiber size, since species with similar muscle fiber sizes may display distinct locomotor patterns. We next analyzed the types and numbers of synaptic boutons of the NMJ of muscle fibers 6 and 7. Our data indicate that each analyzed species has characteristic size and patterns of the NMJs, which might be responsible for the species-specific locomotion activities. More interestingly, we have identified the presence of type II synaptic boutons in *D. sechellia*, which are not present in fiber 6/7 of *D. melanogaster*. We are currently using this novel phenotypic variation of locomotion patterns to screen for candidate genes involved in NMJ development based on sequence divergence within the melanogaster sub-group.

684C

The Microtubule Regulatory Protein Stathmin is Essential for Axonal Transport. Alfredo Zuniga, Tori Pagel, Jason Duncan. Department of Biology, Willamette University, Salem, OR, 97301.

Neurons employ a microtubule-based transport system to bidirectionally transport proteins, vesicles, and organelles between the cell body and the synaptic terminal through the axoplasm. We have identified the protein stathmin (stai), which regulates the dynamics of the microtubule cytoskeleton, as a component required for axonal transport in Drosophila. We have isolated several hypomorphic mutations in the stai gene that cause neuronal dysfunction resulting in phenotypes consistent with severe defects in axonal transport. Mutant third instar larvae exhibit a posterior paralysis, or 'tail flip' phenotype, after each

peristaltic contraction of the body wall musculature. Immunostaining of the axons of the longitudinal segmental nerves identify focal swellings and accumulations of transported components, reduced density of microtubules, and altered axonal morphology. Despite this neurological dysfunction, a small percentage of stai mutants survive to the adult stage but exhibit severe movement defects, often dragging their hind limbs as they walk. In addition, viable adult stai mutants have a significantly reduced lifespan. Unexpectedly, adult stai mutants also exhibit a progressive, age-dependent seizure phenotype characteristic of the class of 'bang-sensitive' mutants that have altered neuronal excitability. We demonstrate directly that all observed phenotypes are due to loss of stai function. First, mobilization of a mutagenic transposable element in one of the mutant stai alleles reverts all phenotypes to wildtype. Second, genetic rescue of the phenotypes by introduction of an exogenous copy of a Drosophila stai transgene ameliorates all observed phenotypes. Interestingly, we are also able to rescue the observed phenotypes in our Drosophila stai mutants with an exogenous copy of the human stathmin gene STMN1, indicating that the Drosophila and human stathmin proteins are functional homologues. Collectively, our data identifies a novel, evolutionarily conserved role for stai in the regulation of microtubule-based axonal transport.

685A

The T-box transcription factor *midline* collaborates with the insulin-regulated *dFOXO* transcription factor to regulate cell-fate specification in the developing eye of *Drosophila melanogaster*. Sudeshna Das[1], Deepak Kumar[1], Yan Zong[2], Brandon Drescher[1], Sarah Morgan[2], Sandra Leal[1]. 1) Biological Sciences, University Of Southern Mississippi, Hattiesburg, MS; 2) School of Polymers and High Performance Materials, University of Southern Mississippi, Hattiesburg, MS.

The *Drosophila midline* (*mid*) gene encodes a highly conserved invertebrate ortholog of the mammalian Tbx20 transcription factor gene family and regulates critical aspects of embryonic central nervous system (CNS) development. Embryos homozygous mutant for *mid* exhibit severe CNS defects due to the misspecification of neuronal subtypes and axon guidance defects within the ventral nerve cord (VNC). To understand the molecular-genetic mechanisms by which *mid* regulates neuronal specification and axon guidance, it is essential to decode the complex *mid* transcriptional networks that mediate these functionally integrated processes. For this reason, we are using the *Drosophila* eye as a practical model system to combine a classical genetic modifier screen with both RNA interference (RNAi) and the UAS-Gal4 expression system for the identification of *mid*-interacting genes. We then determine whether *mid*-interacting genes guide the differentiation of neurons within the embryonic CNS. We now report that specifically reducing *mid* expression in the larval imaginal eye disc using RNAi results in significantly fewer interommatidial bristles within the adult eye. These results suggest that *mid* functions as a cell-fate determinant during the pupal stage of disc morphogenesis, when specialized accessory cells are recruited to surround an R1-R8 photoreceptor neuron cluster. Results from the genetic modifier screen also show that *mid* collaborates, either directly or indirectly, with the transcription factor *dFOXO* to regulate interommatidial formation, placing *mid* downstream of insulin-stimulated signaling pathways that regulate cell growth, metabolism and survival. We are currently examining whether *dFOXO* also interacts with *mid* to regulate distinct aspects of embryonic CNS development including cell fate specification and axon guidance.

686B

The SUMO pathway promotes bHLH proneural factor activity via a direct effect on the Zn finger protein, Senseless. Lynn M. Powell[1], Yan Chang Huang[2], Angela Chen[2], Andrew P. Jarman[1]. 1) Centre for Integrative Physiology, University of Edinburgh, George Square, Edinburgh, EH8 9XD, United Kingdom; 2) Institute of Biomedical Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan.

During development, proneural transcription factors of the bHLH family are required to commit cells to a neural fate. In *Drosophila* neurogenesis, a key mechanism promoting sense organ precursor (SOP) selection is the synergy between proneural factors and their coactivator the Zn-finger transcription factor, Senseless, in transcriptional activation of target genes. We present evidence that post-translational modification by SUMO enhances this synergy via an effect on Senseless. Our data show that SUMO enhances the ability of Senseless to promote proneural activity in reporter gene assays in S2 cells and to promote neurogenesis *in vivo*. Western blotting of cell lysates reveals that Senseless is a direct target for SUMO modification. Senseless interacts with Ubc9, the SUMO-conjugating enzyme, in a yeast 2 hybrid assay, and Senseless and SUMO interact in a relocalisation assay in HeLa cells. Mutagenesis of a predicted SUMOylation motif in Senseless reduces Senseless/proneural synergy both in cell culture and *in vivo*. We propose that SUMOylation of Senseless promotes its synergy with proneural proteins during transcriptional activation, and hence regulates an important step in neurogenesis leading to the formation and maturation of the SOPs.

687C

Tre1 GPCR signaling orients stem cell divisions in the *Drosophila* central nervous system. Shigeki Yoshiura, Nao Ohta, Fumio Matsuzaki. RIKEN CDB, Kobe, Hyogo, Japan.

During development, directional cell division is a major mechanism for establishing the orientation of tissue growth. *Drosophila* neuroblasts undergo asymmetric divisions perpendicular to the overlying epithelium to produce descendant neurons on the opposite side, thereby orienting initial neural tissue growth. However, the mechanism remains elusive. We provide genetic evidence that extrinsic GPCR signaling determines the orientation of cortical polarity underlying asymmetric divisions of neuroblasts relative to the epithelium. The GPCR Tre1 activates the G protein oα subunit in neuroblasts by interacting with the epithelium to recruit Pins, which regulates spindle orientation. Because Pins associates with the Par-complex via Inscuteable, Tre1 consequently recruits the polarity complex to orthogonally orient the polarity axis to the epithelium. Given the universal role of the Par-complex in cellular polarization, we propose that the GPCR-Pins system is a comprehensive mechanism controlling tissue polarity by orienting polarized stem cells and their divisions.

688A

Drosophila Neto is essential for clustering of glutamate receptors at neuromuscular junction. Young-Jun Kim[1], Hong Bao[2], Liana Bonanno[1], Bing Zhang[2], Mihaela Serpe[1]. 1) NICHD, NIH, Bethesda, MD; 2) Univ. of Oklahoma, Norman, OK.

Neurotransmitter receptor recruitment at postsynaptic specializations is key in synaptogenesis since this step confers functionality to the nascent synapse. The Drosophila neuromuscular junction (NMJ) is a glutamatergic synapse, similar in composition and function to mammalian central synapses. Various mechanisms regulating the extent of postsynaptic ionotropic glutamate receptors (iGluRs) clustering have been described, but none are known to be essential for the initial localization and clustering of iGluRs at postsynaptic densities (PSDs). We identified and characterized the Drosophila neto (neuropilin and

tolloid-like), as an essential gene required for clustering of iGluRs at the NMJ. Neto co-localizes with the iGluRs at the PSDs, in puncta juxtaposing the active zones. neto loss-of-function phenotypes parallel the loss-of-function defects described for iGluR complexes. The defects in neto mutants are effectively rescued by muscle specific expression of neto transgenes. Neto clustering at the Drosophila NMJ coincides with and is dependent on iGluRs. Our studies reveal that Drosophila Neto is a novel, essential component of the iGluR complexes and is required for iGluRs clustering, organization of PSDs and synapse functionality.

689B

***Drosophila* Mitofusin regulates function and development of the neuromuscular junction.** Hector Sandoval[1], Chi-Kuang Yao[2], Kuchuan Chen[1,3], Yong Qi Lin[6], Taraka Donti[1], Manish Jaiswal[1], Vafa Bayat[1,3,4], Ke Zhang[5], Claire Hauter[6], Bo Xiong[1,3], Wu-Lin Charng[1,3], Shinya Yamamoto[1,3], Brett Graham[1], Hugo Bellen[1,3,6]. 1) Human and Molecular Genetics, Baylor College of Medicine, Houston, TX 77030; 2) Institute of Biological Cehmistry, Academia Sinica, Nankang,Taipei 115, Taiwan; 3) Program in Development Biology, Baylor College of Medicine, Houston, TX 77030; 4) Medical Scientist Training Program, Baylor College of Medicine, Houston, TX 77030; 5) Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030; 6) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030.

Mitochondria are dynamic organelles that continually undergo fusion, fission, and trafficking. These cell biological processes control mitochondrial shape, number, size, distribution, and physiology. Neurons utilize mitochondrial dynamics to provide the high demand for energy at synaptic terminals. In a forward genetic screen designed to isolate mutations that cause neurodegenerative phenotypes, we identified 7 mutations in *Marf* (Mitochondrial Associated Regulatory Factor). Marf encodes the *Drosophila* homolog of mammalian mitofusins, proteins that promote mitochondrial fusion. Loss of function mutations in the human Marf homologue cause Charcot-Marie-Tooth type 2A, an axonal peripheral sensorimotor neuropathy primarily affecting lower and extremities. To better understand the neuronal processes that are affected by the loss of Marf, we assessed some neuronal phenotypes. In the eye, mutations in *Marf* lead to a reduced amplitude of the electroretinogram (ERG) recordings and a degeneration of the photoreceptors and their terminals. At the NMJs, loss of Marf causes a severe loss of presynaptic mitochondria, and neurotransmitter release is impaired during prolonged stimulation. *Marf* mutants also exhibit an altered NMJ synapse morphology. We are currently testing several hypothesis related to how the functional and developmental defects arise at the NMJ.

690C

Liquid facets (Lqf) plays novel roles in BMP signaling and retrograde transport. Phillip Vanlandingham, Lerin Luckett-Chastain, Taylor Fore, Hong Bao, Bing Zhang. Dept. of Zoology, University of Oklahoma, Norman, OK.

Maintenance of synaptic growth and function depends on the interaction of cell signaling and intracellular trafficking. Activation of presynaptic BMP receptors leads to phosphorylation of the downstream effector MAD, and translocation to the nucleus where pMAD regulates expression of genes important for synaptic growth and activity. Little is known about the mechanisms through which BMP signaling and pMAD trafficking are linked at the synapse. We have previously shown that Lqf, the Drosophila homolog of epsin, positively controls synaptic growth and function as loss of Lqf decreases synaptic transmission and NMJ size, while neuronal overexpression dramatically increases NMJ growth. Here we test whether Lqf mediates growth and function in a BMP-dependent manner. Using immunofluorescence, we observe higher levels of pMAD at the NMJ in *lqf* mutants relative to control, whereas Lqf overexpression decreases pMAD levels at the NMJ. This result contrasts with mutants such as *nwk* that show higher levels of pMAD at the NMJ correspond to increases in synaptic growth. Therefore, we examined pMAD in the nucleus of motoneurons where we observe no change in *lqf* mutants relative to control, but a dramatic increase in *nwk* mutants. As a result, we predict Lqf-dependent retrograde transport of pMAD to motoneuron nuclei is necessary for BMP growth signaling. Consistent with a defect in retrograde transport of pMAD, lqf genetically interacts with lis1, whose mutations phenocopy the NMJ physiological and pMAD defects observed in *lqf* mutants. We show that pMAD is regulated downstream of Wit as pMAD levels at the NMJ decrease below control levels in *lqf, wit* double mutants. Further, Lqf interacts with Wit as shown by immunoprecipitation, and Wit accumulates at the plasma membrane upon overexpression of Lqf, where Lqf may negatively regulate the receptor. Hence, our results reveal novel dual roles for Lqf in BMP signaling: Lqf negatively regulates BMP receptor activation, and positively promotes pMAD translocation to the nucleus. Supported by NIH/NINDS grant (RO1NS06878).

691A

Phosphorylation of Hts at the MARCKS domain inhibits its ability to regulate Dlg postsynaptic targeting during neuromuscular junction development. Simon Wang[1], Amy Tsai[2], Charles Krieger[2], Nicholas Harden[1]. 1) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada; 2) Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, British Columbia, Canada.

Mammalian adducins are a group of cytoskeletal proteins that regulate actin filaments in several ways such as cross-linking with spectrin and capping of the barbed ends. These roles are inhibited via phosphorylation of the myristoylated alanine-rich C kinase substrate (MARCKS) domain by Protein kinase C (PKC). We have previously shown that phospho-adducin levels are elevated in spinal cord tissue taken from patients who have died from Amyotrophic Lateral Sclerosis, a neurological disease that causes degeneration of motor neurons. To explore further the significance of phospho-adducin in the nervous system, we decided to study *Drosophila* adducin which is encoded by *hu-li tai shao* (*hts*). To do this, we created a phospho-mimetic *hts* transgene where the putative target site of PKC in the MARCKS domain was altered from a serine to an aspartic acid. We provide evidence that expression of a wild-type *hts* transgene in the muscle disrupts the localization of Discs large (Dlg) at the postsynaptic membrane of larval neuromuscular junctions (NMJs). In contrast, disruption of Dlg localization was greatly reduced when the phospho-mimetic *hts* transgene was expressed. Other studies have shown that Dlg postsynaptic targeting is regulated by Partitioning-defective 1 (PAR-1). We show that expression of wild-type *hts*, but not phospho-mimetic *hts*, can elevate PAR-1 protein levels in the muscle causing increased phosphorylation of Dlg. We conclude that Hts is a signalling-responsive component of the cytoskeleton that contributes to synaptic plasticity during NMJ development, at least in part by regulating Dlg postsynaptic localization through PAR-1 dependent phosphorylation.

692B

***Drosophila* Cyfip regulates synaptic growth and endocytosis by suppressing F-actin assembly.** Lu Zhao, Dan Wang, Qifu Wang, Yongqing Zhang.

Institute of Genetics and Developmental Biology CAS, Beijing, China.

Synapses are highly specialized intercellular junctions that transmit information between neurons and their targets. Aberrant synapse morphology and function underline a broad spectrum of neurological diseases. Therefore, the development and function of synapses have been under intensive studies for several decades. Cyfip, also known as Sra-1, is first identified as a specific Rac1-associated protein. It is later shown to interact with the Fragile X mental retardation protein FMRP, hence the name of Cyfip, cytoplasmic FMRP interacting protein. Cyfip is a component of the SCAR/WAVE complex that activates Arp2/3 to initiate F-actin nucleation upon certain signals. Lack of *cyfip* results in dramatically shortened extension of neuromuscular junction (NMJ) synapses with clustered and more numerous boutons. However, how Cyfip affects actin cytoskeleton and functions at synapses remains poorly defined. We report here that Cyfip regulated the organization of F-actin structure in ovary and presynaptic NMJ terminals. Pharmacological and fluorescence recovery after photobleaching (FRAP) assays showed that Cyfip suppressed F-actin assembly. Electron microscopy revealed a significant increase in synaptic vesicle size in *cyfip* mutants. Consistently, the amplitudes of miniature excitatory junctional potentials were increased in *cyfip* mutants. Furthermore, synaptic neurotransmission could not be maintained under high frequency stimulation, indicating a defect in endocytosis or replenishment of synaptic vesicles in *cyfip* mutants. Additionally, reducing the dose of endocytic proteins by half dominantly enhanced *cyfip* mutant NMJ phenotypes. It is known that up-regulation of BMP signaling (a major growth promoting pathway at *Drosophila* NMJ synapses) leads to the formation of satellite boutons in endocytic mutants. As expected, we found an increased level of BMP signaling in *cyfip* mutants. Together, our data demonstrate for the first time that Cyfip regulates synaptic growth and endocytosis by inhibiting F-actin assembly and suppressing BMP signaling.

693C

Reduced TOR Activity Promotes Cap-Independent Translation of Gurken During Drosophila Oogenesis. Malachi A Blundon, Cara L. Doyle, Scott B. Ferguson. Department of Biology, SUNY Fredonia, Fredonia, NY.

Gurken (Grk) expression is required to specify the polarity of the developing oocyte during Drosophila oogenesis. Proper localization and translation of *grk* transcripts is required to achieve proper axis specification. *gkr* translation initiation has been shown to be cap-dependent and require the activity of the DEAD-box RNA helicase, Vasa. Vasa activity can be repressed by the ATK/Chk2-dependent meiotic checkpoint when DNA double strand breaks (DSBs) persist in meiosis. Unrepaired DSBs in oocyte development of *spindle*-class mutants activate this checkpoint and result in inefficient *grk* translation and loss of dorsal fates. This inefficient *grk* translation is thought to be related to reduced Vasa activity.

In a screen for suppressors of the ventralized eggshell phenotype seen in *spindle-B*^{BU} mutants, we identified mutations in the *lnk* and *PyK* genes. We show that *lnk* and *PyK* mutations suppresses the eggshell phenotype independent of the DSB repair delay and Vasa phosphorylation seen in *spn-B* or *spn-A* mutants. This suggests that the eggshell phenotype is corrected by overcoming the translational block of *grk* transcripts seen in *spindle* mutants. Both Lnk and PyK have been recently identified as members of the TOR signaling pathway. Direct inhibition of the TOR kinase with rapamyacin suppresses the ventralized eggshell phenotype in *spn-B* or *vas* mutant females. Lnk and PyK modulate TOR kinase activity through different pathways that converge at the TSC1/2 heterodimer. During dietary starvation, TOR activity inhibits cap-dependent translation by promoting the activity of the translation inhibitor eIF4E binding protein (4EBP). We hypothesize that reduced TOR activity promotes *grk* translation independent of the ATR/Chk2 meiotic checkpoint pathway. Recent data indicates that this may be achieved by way of IRES-dependent translation initiation of *grk* when TOR activity is low. This discovery suggests flies are able to maintain the translation of developmentally important transcripts such as *grk* during periods of nutrient limitation.

694A

The role of the two promoter regions of *hunchback* in its RNA expression pattern in *Drosophila melanogaster* . Maira A. Cardoso[1], Márcio Fontenele[2], Helena Araujo[2], Michelle Diniz[1], Paulo M. Bisch[1], Francisco J. P. Lopes[1]. 1) Institute of Biophysics Carlos Chagas Filho, University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; 2) Institute of Biological Sciences, University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.

Gene expression patterns during cell differentiation are determined by the regulatory activity of morphogenetic proteins on several genes. In *Drosophila melanogaster*, the translation of the Bicoid (Bcd) mRNA, maternally anchored in the anterior pole of the embryo, produces a smooth protein gradient along the embryo. The developmental gene *hunchback* (*hb*) interprets this Bicoid gradient in a position-dependent way, generating an expression pattern with a sharp border in the middle embryo. *hb* regulation is controlled by two distinct promoter regions P1 and P2, and the spatial patterns of hb protein and transcripts show remarkable differences, which have not been well characterized yet. Using specific RNA probes, we characterize the expression pattern of transcripts regulated by both P1 and P2 promoter regions during nuclear cleavage cycle 14. Through fluorescent in situ hybridization and protein staining we simultaneously determined the dynamics of the Hb protein and the transcripts derived from P1 and P2. By expanding a previously proposed model (Lopes, et al., PloS Comput. Biol., 2008), we developed a predictive regulatory network model describing the expression pattern of Hb protein and the *hb* transcripts from both regulatory regions. Our experimental and theoretical approaches allow understanding the role of P1 and P2 regions for generating a central strip in the *hb* expression pattern, called parasegment 4. We show that bistability plays a central role to produce this key structure during the *Drosophila* embryonic development.

695B

Functional Convergence in Embryonic Patterning Determinants. Rhea R. Datta, Jackie Moore, Gozde Yucel, Stephen Small. Department of Biology, New York University, New York, NY.

Homeodomain (HD)-containing proteins control embryonic patterning events in all metazoans. Structural changes in these proteins can result in developmental defects and disease, and can drive speciation events when fixed in a population. The HD containing transcription factor Bicoid (Bcd) is responsible for the patterning events that drive anterior determination in Drosophila melanogaster, and can activate over 40 zygotic targets in the embryo as well as repress translation of posterior proteins. Bcd, while essential for Drosophila development, is not conserved outside higher Diptera. Rather, known anterior embryonic patterning events in other insects are controlled by the HD protein Orthodenticle (Otd). In a fascinating regulatory network twist, otd has evolved to become a strict zygotic target of Bcd in Drosophila. Moreover, the Bcd paralog Zerknullt (Zen) has no role in anterior patterning, suggesting that these genes are under strong selection. We examine the role of HD and amino-acid specificity in activating target genes required for anterior patterning functions. Through key chimeric rescue assays (both with HD swaps and single residue changes), we show that there is some functional convergence between Bcd, Zen and Otd. Maternally supplied Otd is capable of rescuing many anterior structures in bcd mutants, while Zen cannot. We show that Otd can activate Hb, but not Gt expression, and that we can systematically restore Bcd function by adding key resides into Otd-HD. While boundaries of gene expression remain unaffected, we find that swapping BcdHD into Otd and Zen backbones cannot rescue bcd mutants completely. By using this chimeric protein rescue, we hope to link protein structural motifs with specific embryonic patterning functions. In addition to identifying the protein structural requirements for anterior patterning, this work examines the molecular basis for understanding how anterior determinants evolve, both functionally and structurally.

696C

Negative regulation of Epidermal Growth Factor Receptor signalling in the ovary. Scott De Vito[1], Jean-François Biosclair Lachance[1,2], Mariana Fregoso Lomas[1], Laura Nilson[1]. 1) Department of Biology, McGill University, Montreal, Quebec, Canada; 2) Department of Biological Sciences, University of Chicago, Chicago, IL, USA.

During oogenesis the follicular epithelium, which overlies the growing oocyte, is patterned to form the structures present in the eggshell, including two dorsal anterior appendages separated by a dorsal midline domain. The primordia that form these structures are marked by differential expression of the Broad transcription factor complex. Expression of this fate marker is controlled by dorsally localized activation of Epidermal Growth Factor Receptor (EGFR) in the follicle cells by its secreted ligand Gurken (Grk) from the oocyte. We are studying how alterations of EGFR activity lead to changes in the patterning of this tissue. For example, when the negative regulator Sprouty (Sty) is lost, dorsal fates are expanded ventrally as predicted from the expected increase in EGFR activity. Unexpectedly, these domains are also shortened along the anterior posterior (AP) axis. Here we look at two other negative

regulators, D-cbl, which functions as an E3 ubiquitin ligase for EGFR, and Gap1, which promotes the inactivation of Ras. Through clonal analysis we show that loss of D-cbl in follicle cells results in expansion of dorsal fates onto the ventral side of the follicular epithelium. Additionally, these clones display a shortening of dorsal fates along the AP axis reminiscent of the sty phenotype. Through the same analysis we show that loss of Gap1 results in the expansion of dorsal fates ventrally but fails to display any shortening of dorsal fates along the AP axis, possibly suggesting that the function of Gap1 is distinct from that of D-cbl and Sty. We also looked at the regulation of a downstream transcription factor, Capicua, as a more direct readout of EGFR activity. Interestingly, the pattern of EGFR-mediated changes in Capicua localization is expanded in D-cbl and Gap1 mutant clones along both the AP and DV axes. This AP expansion is inconsistent with the observed decrease in dorsal fates in this dimension. This discrepancy is currently being explored.

697A

Pattern formation by graded and uniform signals: gene regulation by Dorsal and Zelda in the *Drosophila* embryo. Bomyi Lim[1], Jitendra S. Kanodia[1], Hsiao-Lan Liang[2], Yoosik Kim[1], Mei Zhan[3], Hang Lu[3], Christine A. Rushlow[2], Stanislav Y. Shvartsman[1]. 1) Department of Chemical and Biological Engineering and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544; 2) Center for Developmental Genetics, Department of Biology, New York University, New York City, NY 10003; 3) School of Chemical and Biomolecular Engineering and Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332.

Graded distributions of maternal transcription factors in the early *Drosophila* embryo provide some of the best understood examples of morphogen gradients. Recent studies revealed that multiple aspects of pattern formation by these gradients depend on uniformly expressed transcriptional activators, such as Zelda. Removal of Zelda influences both the timing and the spatial expression patterns for most of the transcriptional targets of maternal morphogens. We demonstrate that some of these patterning defects, which range from temporal delay to loss of expression, can be rationalized using a mathematical model based on cooperative binding of graded and uniform factors. This model makes testable predictions and suggests an alternative mechanism by which morphogen gradients can establish nested gene expression domains.

698B

Bonus is required maternally for dorsal-ventral pattern formation. Stuart Newfeld, Janine Quijano, Estela Arciniega, Nancy Tran, Ashley Castillo, Michael Stinchfield. Sch Life Sci, Arizona State Univ, Tempe, AZ. 85287-4501.

The discovery that a zygotic extracellular signaling system utilizing the secreted morphogens Dpp and Sog regulates dorsal-ventral axis formation across species was a major advance in our understanding of developmental biology. Recent data from our lab suggests that a conserved maternal intracellular system based on ubiquitination and deubiquitination of the Dpp signal transducer Medea is operating in parallel during this process. Experimental data utilizing mutations in the Fat facets deubiquitinase revealed that these two systems are equally important since the zygotic system cannot function without the maternal system. In an attempt to identify the Medea ubiquitin ligase that functions opposite to Fat Facets in dorsal-ventral patterning we analyzed two candidates: Bonus and Highwire. Bonus is the most closely related fly protein to the family of vertebrate Tif1 proteins, of which Tif1gamma is the Smad4 (Medea homolog) ubiquitin ligase that operates opposite USP9X (Fat facets homolog) in Xenopus dorsal-ventral patterning. Bonus has not been previously connected to Dpp signaling in flies but has been shown to function as a chromatin modifier. Highwire is a known antagonist of Medea activity at the larval neuro-muscular junction but has not been shown to have any vital roles during embryonic development. Here we report that neither Highwire nor Bonus are the sought after ubiquitin ligase for Medea. We found instead that Bonus is required maternally for dorsal-ventral patterning upstream of Dpp via participation in the Dorsal signaling pathway.

699C

***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*). Loss-of-Function (LOF) of *pnr* results in dorsal eye enlargements and antennal duplications in adult flies. We found similar phenotypes in LOF of *defective proventriculus* (*dve*), which encodes a homeobox protein. We investigated if *dve* plays a role in axial patterning during early eye development. We found that Gain-of-Function (GOF) of *dve* results in suppression of eye by downregulating Retinal Determination (RD) genes. We found that *dve* plays an important role in dorsal eye fate selection during early eye development. In the eye imaginal disc, Dve expression is restricted to a small region anterior to the Morphogenetic Furrow (MF) on the dorsal eye margin. This expression domain of Dve also overlaps with Wingless (Wg), which is expressed at the lateral margins of the developing third instar eye discs. Interestingly, we found that *dve* is required to maintain a Wg morphogen gradient in the developing *Drosophila* eye field to promote DV patterning of the *Drosophila* eye. Here we present insights into the novel role of *dve* in dorsal eye fate selection in the *Drosophila* eye.

700A

Specification of Drosophila corpora cardiaca neuroendocrine cells by Daughterless homodimer. Sangbin Park[1], Seung K Kim[1,2]. 1) Developmental Biology, Stanford University, Stanford, CA; 2) HHMI.

Many human diseases result from excessive or inadequate endocrine cell mass or function. Thus, there is intense interest in identifying evolutionarily-conserved transcriptional codes for neuroendocrine cell development and expansion. Drosophila neuroendocrine cells comprising the corpora cardiaca are essential for systemic glucose regulation and represent functional orthologues of vertebrate pancreatic α-cells. Our previous study identified a unique cell signaling context in mesoderm where neuroendocrine precursor cells can be specified by two transcription factors; Tinman and Daughterless. Here, we show that Daughterless homodimer alone can induce ectopic neuroendocrine cells in dorsal trunk mesoderm where cardiac mesoderm is specified, suggesting Daughterless homodimer may convert cardiac precursors to neuroendocrine cells. We speculate the Daughterless homodimers compete with Twist to specify neuroendocrine cell fate in dorsal trunk mesoderm where Tinman expression is high. Using gain and loss of function alleles of EGFR, we also show that the

regulation of EGFR signaling in mesoderm alters the number of neuroendocrine precursor cells. However, unlike Daughterless homodimer, activation EGFR signaling in trunk mesoderm does not induce ectopic neuroendocrine cell fate, suggesting that EGFR signaling is not required for the specification of neuroendocrine cells but rather for regulating the number of neuroendocrine precursor cells.

701B

Conserved MAPK and CK2 sites in E(spl)-M8 regulate repression of Atonal. Mohna Bandyopadhyay, Adam Majot, Bhaskar Kahali, Clifton Bishop, Ashok Bidwai. Biology, West Virginia University, Morgantown, WV.

Inhibitory Notch signaling is required for patterning the founding R8 cells in the developing retina, during which Atonal (Ato) activity is antagonized by the E(spl) repressors. This antagonism requires phosphorylation of E(spl)-M8 by CK2, which appears to be necessary but not sufficient for repression of Ato. The presence of a highly conserved consensus site for MAPK proximal to the CK2 site raised the possibility that multi-site phosphorylation controls M8 activity. MAPK mediates the effects of Epidermal Growth Factor Receptor (EGFR) signaling, and previous studies have shown that excess R8s are specified upon loss of *egfr* or its pathway components. We sought to determine if the MAPK site in M8 is functional, and if it is dependent on modification at the CK2 site. Using site-specific variants of M8, we find that both the CK2 and MAPK sites are necessary for repression of Ato, and that CK2 is epistatic. Accordingly, halved dosage of *egfr* strongly mitigates repression of Ato by a CK2 phosphomimetic variant, indicating that EGFR signaling may impinge on M8, itself. The CK2 site (SDCD) appears to serve as a gatekeeper, as its deletion activates M8 repression of Ato, albeit with a strength that is attenuated when compared to that of the CK2+MAPK mimic. We have also used Gal4 drivers specific for different stages of the morphogenetic furrow (MF) to evaluate when and where repressor activity manifests. Our findings reveal that M8 activation is likely to occur at stage-2/3 of the MF where active MAPK is present and where R8 selection normally occurs. Accordingly, when both CK2 and MAPK sites are replaced with Asp residues inappropriate repression of Ato manifests even at stage-1, where Ato auto-regulation normally occurs. This auto-regulation is important to drive Ato levels to attain a threshold sufficient for the R8 fate. The possibility arises that activation of M8 by multi-site phosphorylation at stage-2/3 acts as a spatial switch that permits Ato repression only after the pre-R8s are formed.

702C

A Novel function of Muscle Myosin II in Drosophila melanogaster Eye Development. Carlos Cano, Landry E. Nfonsam, Jennifer Curtiss. Biology, New Mexico State University, Las Cruces, NM.

Drosophila Mhc encodes the heavy chain of the conventional (muscle) myosin II motor protein, which is important for skeletal muscle function. Nonconventional myosin II family members (e.g. Zipper) have well-known roles in cytokinesis and migration of non-muscle cells. However, recent studies show that Mhc is required for cell migration in two non-muscle contexts in Drosophila melanogaster: in the development of the air sac primordium during tracheal development and in border cell migration during oogenesis. Based on a transcriptomic screen we performed to identify novel eye genes, Mhc is highly expressed in eye tissues. Here, we report a novel role for Mhc in development of the Drosophila eye. We used the FLP/FRT system to generate homozygous clones of the loss-of-function Mhc2L2881 allele. Mhc2L2881 adult mosaic eyes showed strong effects on eye development, including gaps and disruptions in the ommatidial array, loss of photoreceptors and abnormal formation of rhabdomeres. Antibody stainings using anti-DE-Cadherin revealed that the cells that lie between photoreceptor clusters in Mhc2L2881 mosaic eye discs were more irregular in shape and their adherens junctions appeared to be enriched on one side rather than being evenly distributed. We also observed occasional loss of cone cells and photoreceptors in Mhc2L2881 mutant clones, notably the R8 precursors, which are the founding cells of each ommatidium. We found that R8 precursors could still develop in Mhc2L2881 homozygous tissue and in some cases were able to recruit other photoreceptors. However, Mhc2L2881 R8 photoreceptor nuclei were often located more basally within the eye-antennal disc epithelium compared to surrounding wild-type nuclei. These results suggest that Mhc regulates cell morphology and/or cell-cell adhesion during eye development. The stochastic loss of ommatidial cells likely results from failure of signaling between cells that are not well integrated into the epithelium, or from cell death. In conclusion, muscle myosin II has an effect on eye cell morphology, which in turn affects cell fate specification.

703A

Novel function of the kinase Nemo in the negative regulation of Atonal expression in the *Drosophila* eye. Vilaiwan Fernandes, Esther Verheyen. Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia.

The high degree of organization of the *Drosophila* compound eye originates during patterning and morphogenesis of the third larval instar eye imaginal disc. Patterning of the eye is a reiterative process in which a regular lattice of R8 photoreceptors is specified one column at a time posterior to the furrow. Central to this process is the expression and refinement of the bHLH proneural transcription factor Atonal (Ato). Here, we describe a novel role for the kinase Nemo (Nmo) during Ato refinement and R8 spacing. Discs mutant for *nmo* revealed defects in Ato refinement, such that Ato expression fails to refine into intermediate groups (IGs) resulting in frequent R8 doublets. Conversely, overexpression of *nmo* in flipout clones results in loss of IGs and greater spacing between individual R8s, suggestive of a role for Nmo during the stage of R8 lateral inhibition. Notch and EGFR signalling are highly conserved pathways; both of which are known to promote R8 refinement. Using clonal analysis we tested whether expression of targets of either of these pathways are affected by loss of *nmo*. Intriguingly, we find that loss or reduction of *nmo* results in reduced expression of the Notch targets, Enhancer of split and Daughterless, as well as the EGFR targets, Rough and *pointed P1*. Additionally, we find that levels of the EGFR effector pMAPK are reduced in the absence of *nmo*. Collectively, our data suggest positive roles for Nmo in both Notch and EGFR signalling during eye development. We aim to determine whether Nmo's effect on Ato refinement stems from its regulation of one or both of these signalling pathways. Our study will provide further insight into the complex regulation of Ato expression in the developing retina, a process that has many parallels throughout neural fate specification.

704B

Homeodomain-interacting protein kinase interacts with the retinal determination gene network and is required for development of the *Drosophila* compound eye. Jessica A Gardner[1], Wendy Lee[2], Esther Verheyen[1]. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby B.C., B.C, Canada; 2) Dept. Cell Biology, Harvard, Boston, MA, USA.

The *Drosophila* eye-antennal imaginal disc is an excellent system for studying tissue growth, cell-specification, patterning and signal transduction

cascades. Found within this tissue is the Retinal Determination Gene Network (RDGN); a dynamic signalling network which includes *twin of eyeless (toy), eyeless (ey), sine-oculus (so), eyes absent (eya), dachshund (dac)*, among other factors. Additionally, signalling factors such as Dpp, Hh, N, and Wg, feed into multiple points in the RD network having both protagonistic and antagonistic effects in a context dependent manner. Homedomain interacting protein kinase (Hipk) is a conserved serine threonine kinase and has been reported to act in multiple signalling pathways. Previous studies have shown that *hipk* plays a role in Notch mediated growth of the *Drosophila* eye imaginal disc by antagonizing the global co-repressor Groucho. Here we provide evidence that *hipk* holds additional roles in eye development and eye specification. Loss of *hipk* leads to partial and or complete loss of both the compound eye and the ocellar complex similar to phenotypes observed with loss of RDGN components. Conversely, overexpression of *hipk* induces ectopic eye fields on the head, abdomen and in rare cases the leg disc. Preliminary ectopic eye assays with overexpression of *toy* reveal that modulating levels of Hipk in this background influence both size and number of ectopic eyes induced. Additionally, clonal analysis with loss of *hipk* has revealed altered transcriptional and protein levels for the RD components toy, ey, so, and eya suggesting Hipk has the ability to modulate the levels and likely the activity of the RDGN. With further studies, we aim to mechanistically describe *hipk's* role in eye specification and its involvement with the RDGN.

705C

Study the functions of eye selector genes in Drosophila eye-antenna disc primordium. Hui-Yu Ku[1,2], Henry Sun[1,2]. 1) Institute of Molecular Biology,Academia Sinica, Taipei, Taiwan; 2) Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan.

The origin of Drosophila compound eyes can be traced back at embryonic stage as eye-antenna disc primordium (EADP), a pair of epithelium-derived sacs posterior to the dorsal pouch. Clonal and histological analysis suggest that these EADP cells stop motisis since 12 hr embryo, and resumed cell division at late first instar larval (L1) stage to generate the eye-antennal imaginal disc. The eye-antennal discs then contribute to the compound eyes, antennae, ocelli, maxillary palps and head cuticle through morphogenesis. Mitotic clonal analysis suggested that the eye and antenna fates do not segregate till second instar (L2). Interestingly, a number of important transcription factors are expressed in EADP. These include the four Pax genes *eyeless (ey)*, *twin of eyeless (toy)*, *eye gone (eyg)* and *twin of eyg (toe)*. *ey* and *toy* are selector genes to determine the eye fate, while *eyg* and *toe* control cell proliferation in eye disc. Although these genes play important roles in eye development after L2, their mutations do not affect EADP development, indicating that they might function redundantly in EADP. Transient knockdown/inactivation (using an early eyg enhancer) of all of them from embryonic to L1 caused headless (21%) and no eye (26%) phenotypes. We will dissect whether these genes play roles in eye field specification and/or promote proliferation at early eye development stage.

706A

Alleles of *CK2* and *EGFR* modulate the neural defects of *Nspl* and *E(spl)D*. Adam Majot, Mohna Bandyopadhyay, Christa Bryan, Bhaskar Kahali, Clifton Bishop, Ashok Bidwai. Biology, West Virginia University, Morgantown, WV.

Dynamic expression of Atonal (Ato) marks the onset of retinal neurogenesis. During this process single R8s are selected from Ato-positive pre-R8 clusters. Specifically, the bHLH repressors encoded by the *Enhancer of split complex* (*E(spl)C*) inhibit Ato activity in all but the future R8 cell. However, ectopic E(spl)-M8 fails to repress Ato and R8 patterning is unaffected. In contrast, the dominant *m8* allele *E(spl)D* strongly represses Ato and R8 fate. This mutation encodes truncated M8 that lacks co-repressor binding and consensus sites for phosphorylation by CK2 and MAPK. *E(spl)D* in combination with the sensitized *Nspl* allele potently inhibits Ato and the R8 fate to block eye development, suggesting that full-length M8 is likely to be subject to post-translational modification. However, direct genetic evidence for these kinases has not been forthcoming, as they are haplosufficient and when fully removed are cell lethal. We reasoned that the synergy between *Nspl* and *E(spl)D* should yield a phenotype sensitive to modulation. Indeed, the CK2-DN allele *Timekeeper* (*Tik*) strongly rescues the reduced eye and R8 defects, providing direct genetic evidence for a role for this kinase. The eye and R8 defects of a CK2-hypomorphic allele also appear consistent with such a role. We have also tested *egfrf24*, a null allele, in the *Nspl/+; E(spl)D/+* background through analysis of adult phenotypes and staining of eye discs. Consistent with a role in M8 activation, decreased EGFR dosage rescues the reduced eye and R8 defects. As *egfrf24* does not modulate *Nspl*, rescue may reflect hypo-phosphorylation of wild type M8 encoded by the *E(spl)+* allele. A similar case would apply to *Tik*. Additionally, we have used clonal analysis of EGFR and *ato* enhancer-LacZ reporters to further understand repression by M8. Our findings are consistent with the proposal that some levels of EGFR signaling are necessary for lateral inhibition to occur.

707B

Functional analysis of Abd-B protein. Jesús R. Curt[1], Nagraj Sambrani[2], Samir Merabet[2], Ernesto Sánchez-Herrero[1], Yacine Graba[2]. 1) Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Universidad Autónoma de Madrid, Nicolás Cabrera 1, Cantoblanco, Madrid, Spain; 2) Institut de Biologie du Développement de Marseille Luminy, CNRS, Université de la Mèditerranée, Marseille, France.

The Hox genes are a group of genes that determine distinct structures along the anteroposterior axis of bilaterians. They encode proteins containing a DNA-binding domain, the homeodomain, and act as transcription factors, regulating downstream genes. In Drosophila, the Abdominal-B (Abd-B) Hox gene is required for the formation of the posterior abdominal segments and the genitalia. When paralog Hox proteins sequences are compared, some conserved residues and domains are observed. To analyze the function of these conserved aminoacids we have modified the Drosophila Abd-B Hox protein and study the effect of these changes. The modifications concern residues with unknown function, presumptive interaction domains with the cofactor Extradenticle, residues that contact the DNA, and homeodomain swaps with other Hox proteins. The functionality of these modifications were assessed both in the embryo and in the adult. In the embryo, we tested the induction of posterior spiracles by expressing ectopically the modified proteins with the Gal4/ UAS method. In adults, we tested the repression of the seventh abdominal segment in males and the formation of the female genitalia by expressing the modified proteins also with the Gal4/UAS method, but in an Abd-B mutant background. Our results allow drawing a map of Abd-B domain requirements for embryonic and adult functions and identify the context-dependent use of protein domains as a salient feature of AbdB activity. The work also highlights functional differences in the mode of action of AbdB when compared to anterior Hox proteins.

708C

A missense allele in the evolutionarily-conserved octapeptide motif of Sex combs reduced, a Drosophila Homeotic selector gene, represents the first of a novel class of mutant alleles. Lovesha Sivanantharajah, Anthony Percival-Smith. Dept. of Biology, The University of Western Ontario, London,

Ontario, Canada.

The Homeotic selector (Hox) genes are required for body patterning in Bilaterans. In Drosophila melanogaster, Hox expression along the anterior-posterior axis of the embryo establishes segmental identity. The Hox gene, Sex combs reduced (Scr), is essential for the identity of the labial and prothoracic segments. A characterization of 15 Scr mutant alleles identified Scr14, a missense allele in the evolutionarily-conserved octapeptide motif. Scr14 was initially grouped as an antimorphic allele, but distinct properties were noted resulting in its reclassification. In the classic mechanism for an antimorphic allele, dominant negativity, inactive proteins expressed from the mutant allele form a complex with wildtype proteins rendering the complex inactive. Scr14 is distinct from the traditional dominant negative because SCR14 alone has wildtype activity; inactivation is only observed in the presence of other SCR polypeptides. We observed a significant reduction in sex comb bristle number on the prothoracic legs of Scr+/Scr14 males, indicating formation of inactive complexes between SCR+ and SCR14 proteins. A further decrease in Scr activity occurred when truncated SCR null peptides (with wildtype octapeptides) were expressed with SCR14, due to only SCR14 homodimers being active. Surprisingly, truncated SCR14 peptides expressed in vivo did not inhibit endogenous SCR+. In contrast, expression of truncated SCR+ peptides suppressed endogenous SCR14 activity, showing that the effect of the Scr14 missense mutation is non-reciprocal. This may explain why in vitro experiments failed to show complex formation with SCR+. Currently, we are trying to demonstrate stable complex formation between SCR14 and truncated SCR+ peptides. The observation of non-reciprocality suggests that in Scr+/Scr14 flies it is the wildtype allele that acts antagonistically to the mutant allele, not the reverse. Scr14 represents a novel class of alleles we have termed: anti-dominant negative.

709A

The role of disco in specification of the Drosophila leg. Juan Bautista Rosario. Genetics Dept, North Carolina State Univ, Raleigh, NC.

In Drosophila, the origin of the appendages is established during embryogenesis as a group of cells are set aside to from the imaginal discs. The imaginal discs have been studied as a paradigm for understanding the genetic control of organ development, and a model describing this process to understand how the leg develops has been proposed but some questions remain unanswered. The finding that ectopic expression of the Drosophila disco gene in the dpp domain (Patel et al. 2007) suggest that this model is incomplete, as well as raises questions about imitation of leg primordial and transdetermination. I will present further data on the transformation caused by ectopic disco, addressing the molecular basis of the transformation and how this relates to normal leg development. Further, I will present new information about the normal role of the Disco protein during leg development as well as a new role in the regulation of a Distal-less enhancer previously identified as an early cis-regulatory element expressed in the thoracic imaginal disc primordial during embryogenesis.

710B

New allele of *engrailed* associated with three spermathecae in *Drosophila melanogaster* female. Masanobu Itoh[1,2], Akiko Sawada[3]. 1) Center for Bioresource Field Science, Kyoto Inst Tech, Kyoto, Japan; 2) Insect Biomedical Research Center, Kyoto Inst Tech, Kyoto, Japan; 3) Dept. Applied Biology, Kyoto Inst Tech, Kyoto, Japan.

We isolated a recessive mutation (*NK14*) in number of the spermathecae in *Drosophila melanogaster* from the wild population. Females of *NK14* have three, but not two, functional spermathecae with the penetrance of 95% or more. We investigated the gene(s) responsible for this female specific morphological abnormality. Chromosome mapping using the balancer chromosomes demonstrated that the responsible gene(s) for the *NK14* phenotype are mainly located to the second chromosome and some additional genes on the third chromosomes show an epistatic enhancement the *NK14* phenotype. Moreover, *NK14* showed a partial complementation with en^{spt}, of which females are also known to have a variety in number of the spermathecae depending on the developmental temperature, although *NK14* females show no temperature-sensitivity. By determining the structure of *engrailed* gene, a deficiency of 15 nucleotides was found in the first exon of *en* gene in *NK14*. The deficiency results in an amino acid substitution (S/T) and a deletion of five consecutive amino acid residues in EN, which may causes a structural impediment of normal EN function. Our results suggest that *NK14* is a novel allele of *engrailed* gene.

711C

Identification of the gene responsible for the *wings apart* phenotype in *Drosophila melanogaster*. Ginny Morriss, Carmelita Jaramillo, Bianca Garcia, Richard Cripps. Biol, Univ New Mexico, Albuquerque, NM.

The Drosophila *wings apart* (*wap*) locus contains a semi-lethal gene that when mutated leads to the absence of the Tergal Depressor of Trochanter (TDT or jump) muscle. *wap* has been mapped to the proximal X chromosome but it is unclear what gene is mutated to produce the *wap* phenotype. The aspect of muscle development disrupted in *wap* mutants leading to TDT loss is also unknown. To identify the *wap* gene, we performed complementation mapping of *wap* mutants crossed with known X chromosome deletions. We sectioned thoraces of progeny from these crosses to observe if these flies exhibit the TDT phenotype associated with *wap*. Results of mapping analysis and phenotypic characterization suggest the most likely candidate for the *wap* gene is *DIP1*. PCR of *DIP1* is underway in wild-type and wap mutant flies to detect the mutation leading to the observed phenotype. We detected an alanine to threonine amino acid substitution in the *DIP1* coding region in wap^{9} mutants. Excision of *DIP1* was carried out to determine if the *wap* mutant phenotype could be reproduced. Excisants exhibit the same semi-lethal phenotype observed in *wap* mutants. These flies are being sectioned to determine if they also exhibit the TDT phenotype characteristic of *wap* mutants. The results of these excision experiments will be presented. Gain-of-function assays are in progress to determine if over-expression of *DIP1* rescues the *wap* mutant phenotype. The impact of the *wap* mutation will be analyzed by determining at which step in development jump muscle formation is disrupted. Results indicate that TDT founder cells are specified early but are lost later in development. The broad goal of this research is to identify mechanisms of muscle formation in the *Drosophila* adult. Since similar developmental mechanisms are used in vertebrate and invertebrate muscle formation, this study can aid in understanding processes which may impact vertebrate muscle formation and whose mis-regulation may lead to muscular diseases.

712A

An interdisciplinary approach to studying the evolution of BMP signaling. Matthew G. Niepielko[1,2], Kuhn Ip[2,3], Jitendra S. Kanodia[4], Desmond S. Lun[2,3], Nir Yakoby[1,2]. 1) Biology Department, Rutgers University, Camden, NJ 08102, USA; 2) Center for Computational and Integrative Biology, Rutgers

University, Camden, NJ, 08102, USA; 3) Department of Computer Science, Rutgers University, Camden, NJ 08102, USA; 4) Department of Chemical and Biological Engineering, Lewis Sigler Institute for Integrative Genomics, Carl Icahn Laboratory, Princeton University, Princeton, NJ 08544, USA.

The bone morphogenetic protein (BMP) signaling pathway is a conserved regulator of cellular and developmental processes in animals. The mechanisms underlying BMP signaling activation differ among tissues and mostly reflect changes in pathway components. BMP signaling is one of the major pathways responsible for patterning the *Drosophila* eggshell, a complex structure derived from a layer of follicle cells (FCs) surrounding the developing oocyte. Activation of BMP signaling in the FCs is dynamic; initially, signaling is along the anterior-posterior (A/P) axis. Later, signaling acquires dorsal-vernal (D/V) polarity. These dynamics are regulated by changes in the expression pattern of the type I BMP receptor *thickveins* (*tkv*). We found that signaling dynamics and *tkv* patterning are highly correlated in the FCs of multiple *Drosophila* species. In addition, we showed that signaling patterns are spatially different among species. Using a mathematical model to simulate the dynamics and differences of BMP signaling in numerous species, we predicted that qualitative and quantitative changes in a receptor can lead to differences in the spatial pattern of BMP signaling. We tested these predications experimentally in three different *Drosophila* species and through genetic perturbations. Based on the results, we concluded that changes in *tkv* patterning are responsible for the differences in the patterns of BMP signaling activation found in the FCs of *Drosophila* species.

713B

Patterning potential of the terminal system in segmentation of the *Drosophila* embryo. Yoosik Kim, Kate M. Fitzgerald, Stanislav Y. Shvartsman. Department of Chemical and Biological Engineering and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544, USA.

Segmentation of the *Drosophila* embryo is initiated by four maternal signals. Anteriorly localized Bicoid controls gap genes along the anteroposterior (AP) axis by directly activating them in the anterior half of the embryo and also by repressing translation of Caudal, generating the posterior-to-anterior gradient of the protein. Nanos is localized at the posterior pole and regulates gap genes by repressing translation of maternal *hunchback*. The non-segmented termini are patterned by localized activation of the Mitogen-Activated Protein Kinase (MAPK) pathway. Together, these four systems control gap genes at appropriate positions along the AP axis, which then establish multidomain expression patterns of pair-rule genes. Removal of the terminal signal from the wild-type embryo affects only the terminal regions, suggesting that it is responsible for patterning of only a small fraction of the AP axis. Here we demonstrate that, in absence of anterior and posterior signals, the terminal system can segment the AP axis on its own. Using quantitative imaging and modeling approaches, we analyzed the expression of six gap genes (*hunchback*, *giant*, *knirps*, *Kruppel*, *tailless*, and *huckebein*) and one pair-rule gene (*eve*) in this mutant background. We found that this embryo develops a symmetric cuticle pattern along the AP axis, with two segments in each half. We propose a mathematical model that can explain both this cuticle pattern and the underlying patterns of gene expression.

714C

Description and visualization of motor neuron morphology in three dimensions. Prateep Mukherjee[1], Jennifer Brazill[2], Michael D. Kim[2], Gavriil Tsechpenakis[1]. 1) Computer and Information Science, Indiana University-Purdue University Indianapolis, IN; 2) Department of Molecular and Cellular Pharmacology, University of Miami School of Medicine, FL.

Dendrites are fundamental determinants of neuronal function and the morphological properties of dendrites have a direct influence on the types and numbers of synaptic inputs a neuron receives, and consequently, the way in which a neuron integrates and processes these impinging signals. In vertebrates, motor neurons that target different muscles establish distinct dendrite arborization patterns within the spinal cord and respond to sensory stimulation with different latencies. Consequently, the selectivity of synaptic input is largely determined by the differential orientation and positioning of motor neuron dendrites in the spinal cord. Although understanding how motor neurons achieve their final dendritic morphologies remains an important and understudied problem in motor circuit formation, the overwhelming number and complexity of motor neurons and connections in the vertebrate spinal cord remains a critical barrier in addressing this problem. In this work, we use a hybrid model- and appearance-based Computer Vision approach to automatically segment neuron volumes in three dimensions and simultaneously partition them into three distinct compartments, namely axon, soma and dendrites, from z-series image stacks of single-neuron MARCM clones. This morphology estimation yields a compact numerical description of each individual motor neuron, which allows for the unbiased comprehensive analysis of mutations that specifically alter the dendritic arborization patterns of distinct motor neuron subtypes. Our study provides important insight into how different motor neuron subtypes organize and pattern their dendrites in the CNS and will reveal novel molecular mechanisms that control synaptic connectivity in the Drosophila motor circuit.

715A

Using a variable expressivity mutant to study pair-rule gene regulation via evolution *in silico*. Alexander V. Spirov[1,2], Francisco J.P. Lopes[3], David M. Holloway[4]. 1) The I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, St-Petersburg, Russia; 2) Computer Science and CEWIT, State Univ New York, Stony Brook, NY; 3) Instituto de Biofisica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; 4) Mathematics, British Columbia Institute of Technology, Burnaby, BC, Canada.

bcd^{K57R} flies are defective in the cooperative binding of the Bicoid (Bcd) ligand (Lebrecht et al., 2005), and are a way to study the stochastic aspects of gene penetrance and expressivity at the maternal level (Lopes et al., 2008; Holloway et al., 2011). bcd^{K57R} flies display a wide range of anterior shifts in expression of the downstream target *hunchback* (*hb*), from quite weak to very strong (nearly equal to those seen in *bcd*-/- or *stau* mutants). Even-skipped (Eve) pair-rule patterning in the bcd^{K57R} background also shows a range of discrete outcomes, from nearly WT, to mild, to severe cases reminiscent of bcd^{E3} embryos. We are modeling these downstream effects at the cis-regulatory module (CRM) level (Spirov & Holloway, 2010; 2012), the simplest biologically reasonable level at which to treat pair-rule patterning, since each of the (usual) seven pair-rule stripes (or pair of stripes) is under the control of autonomous stripe-specific regulatory elements. The known mapping of regulator binding sites is entered into the model, and we find the strengths of these interactions through a selective Evolutionary Computation process. These computational experiments allow us to understand how CRM structure and binding strengths combine to both read out pair-rule patterns and buffer against upstream variability in maternal factors and *hb*. Our modeling has found complex patterns that recapitulate the variable anterior shifting seen in bcd^{K57R}. In these solutions, disturbed pair-rule patterns are in part due to *hb* shifts (also seen experimentally). Our results demonstrate how maternal variability (from a mutant condition) can be amplified, through the gap level, down to pair-rule expression.

716B

Odd-skipped genes organize growth and patterning along the notum anterior-posterior axis. Steven J. DelSignore, Victor Hatini. Cell, Molecular & Developmental Biol, Tufts Univ Sackler School Biomedical Sciences, Boston, MA.

The Drosophila wing imaginal disc undergoes extensive growth and patterning to give rise both to the wing blade and the dorsal thoracic body wall, or notum. The coordination of growth and patterning has been well studied in the wing, but remains poorly characterized in the notum. Because the body wall lacks the proximo-distal organization of the wing appendage, it is of interest to characterize whether these tissues share similar growth and patterning mechanisms. We previously found that the odd-skipped proteins Drm, Odd, Sob, and Bowl accumulate along the anterior margin of the notum, and are required for notum formation or expansion (Nusinow, 2008). Here we utilized loss- and gain-of-function clonal analyses to test the hypothesis that odd-skipped gene activity at the margin promotes the identity and expansion of the notum anterior-posterior (AP) axis. We found that ectopic expression of Drm promoted Bowl accumulation both within and adjacent to clones. Additionally, it promoted the anterior notum patterning gene *eyegone* (*eyg*), both within and many cell diameters from clones. These patterns of gene activation recapitulate the endogenous relative accumulation of Drm, Bowl and Eyg. Though the cell-autonomous induction of Bowl and Eyg required Bowl function, the non-autonomous effects were Bowl-independent. These data suggest that Drm promotes a long range signal to pattern and expand the anterior part of the notum. Loss of function studies indicate that one or more proteins act redundantly with Drm to promote Bowl and Eyg, while ectopic expression reveals that Odd and Sob are each sufficient to promote Bowl accumulation. By contrast, at L2 we find that Bowl is uniquely necessary to promote Bar-h expression to pattern the anterior notum. Our data suggest that Odd-skipped genes exhibit both unique and complementary functions to elaborate pattern across a field of cells, and suggest an important role for the anterior notum margin in coordinating growth and patterning across the notum AP axis.

717C

A calibrated examination of the influence of dosage variance in *brinker* on multivariate wingshape. Anne Sonnenschein, David Arnosti, Ian Dworkin. Michigan State University, East Lansing, MI.

Cis-regulatory regions control the gene-dosage output of protein-coding genes. Variation in these regulatory regions is prevalent, and has been proposed to contribute to within-species phenotypic differences as well as the heritability of complex diseases. Mutations in cis-regulatory regions can have little to no effect, or induce dramatic mutant phenotypes depending on the location and severity of the molecular change. There is no clear gradient of gene dosage between natural phenotypic variation and clear mutational effects, therefore, a quantitative exploration of this phenomenon is required to understand how genetic systems respond to this variation. . The transcriptional repressor Brinker plays a pivotal role in *Drosophila melanogaster* wing development. Its regulatory region is complex, including multiple regulatory modules with overlapping function. This built in redundancy might indicate a system that is highly sensitive to subtle variation in gene dosage. We are conducting a series of experiments to establish a ‘gene dosage series’, to demonstrate the range of variation that can be expected from varying degrees of up-regulation and down-regulation of Brinker. We are using the power of multivariate analysis of wing-shape is sufficiently sensitive to capture small differences in effect-size. Our study will provide a framework for later classification of *brinker* regulatory mutant alleles and mapping of contributions by within-species variation to phenotype.

718A

Identification of BMP target genes in the Drosophila larval wing precursor. Alexander Springhorn[1], Milica Jevtic[1], Marco Blanchette[2], Britta Hartmann[3], Giorgos Pyrowolakis[1]. 1) Department of Developmental Biology, Institute of Biology 1, Freiburg, Germany; 2) Stowers Institute for Medical Research, Kansas City, USA; 3) Center for Biological Systems Analysis (ZBSA), Freiburg, Germany.

BMPs are secreted signaling proteins which are able to spread from their site of secretion into non-expressing tissue. In the larval precursor of the fly’s wing the Drosophila BMP ligand Dpp forms a long-range morphogen gradient. BMP signaling along this gradient is needed for the growth and patterning of the organ as well as for cell survival, maintenance of epithelial integrity and - by means of feed-back regulation - proper formation of the BMP signaling activity gradient itself. We aimed at identifying new BMP target genes which mediate such functions. To this end, we hyper-activated or blocked the BMP pathway in a spatially and temporarily controlled manner and assessed transcriptome changes by RNA-sequencing. Expression-profiling allowed us to reduce the high number of significantly responding targets to a handful of candidates which display a consistent response at all analyzed conditions. We will present results from the ongoing functional analysis of shortlisted genes and report on cis-regulatory modules that mediate their BMP-signaling responsiveness.

Poster Full Abstracts - Physiology and Aging

Poster board number is above title. The first author is the presenter

719B

Establishing a caloric restriction paradigm in *Drosophila*. Sany Hoxha, Sarah Rollins, William Ja. Metabolism & Aging, The Scripps Research Institute, Jupiter, FL.

Caloric restriction (CR), the reduction of nutrient intake short of malnutrition, extends the lifespan of various organisms and can improve measures of human health. Whether mechanisms of lifespan extension are conserved between humans and model organisms is unknown. In mammals, implementing CR is easily achieved by providing a restricted group with a fraction of the food consumed by an "ad libitum" fed group, which has unlimited food access. Due to the difficulty in directly controlling *Drosophila* food intake, caloric restriction, performed similarly to the mammalian paradigm, has never been tested in flies. Here, we demonstrate a system that allows measurement of food intake throughout life. Flies are housed individually in chambers that permit another cohort of flies to be provided with a limited and measurable amount of food. This system will be used to measure fly lifespan under caloric restriction analogous to current mammalian studies. Our work will help tease apart the differences between the various caloric and dietary restriction paradigms in *Drosophila*, strengthening our understanding of how fly models relate to mammalian systems.

720C

The effect of resveratrol and diet on lifespan and nutrient storage in Drosophila. Michael J Polen[1], Nikolai J Kolba[2], Andrew Montgomery[3], Neha Sirohi[3], Timothy Rudolph[2], Hemlata Mistry[2,3], Justin R DiAngelo[4], Alexis Nagengast[1,2]. 1) Dept Chemistry; 2) Dept Biochemistry; 3) Dept Biology, Widener University, Chester, PA; 4) Dept Biology, Hofstra University, Hempstead, NY.

Caloric restriction is a conserved mechanism that extends lifespan in a variety of organisms from mice to flies. Humans with a calorie restricted diet demonstrate decreased abdominal fat storage and experience health benefits such as protection against cardiovascular disease and cancer. However, the mechanism underlying lifespan extension in caloric restricted individuals remains unclear. The polyphenol resveratrol is naturally found in high levels in grape skins and studies have shown that dietary exposure mimics caloric restriction and extends lifespan. To better understand the link between lifespan and nutrient storage, we exposed a genetically homogenous strain of Drosophila to a high carbohydrate diet or 100 or 350μM resveratrol in a standard cornmeal-molasses-yeast food source and compared their lifespan to flies raised on 0μM resveratrol in the standard food. Male flies raised on 350μM resveratrol and the high carbohydrate diet showed an extension in lifespan. To determine if nutrient storage correlates with lifespan extension, we aged these flies for 7, 21 and 42 days and examined triglyceride levels. Flies exposed to 100μM and 350μM resveratrol exhibit decreased triglyceride levels similar to those raised on the high carbohydrate food in the longest lived male flies. This may suggest some mechanism other than caloric restriction plays a role in lifespan extension. One potential explanation for the lifespan phenotype is resistance to age-related oxidative damage. We are testing this by exposing the aging flies to paraquat to induce reactive oxygen species. Future directions also include identifying the site of resveratrol accumulation in the fly to better understand the affected target metabolic tissue.

721A

Steroid control of the mid-oogenesis checkpoint in Drosophila. Martina Galikova, Peter Klepsatel, Thomas Flatt, Chantal Dauphin-Villemant. Institute of Population Genetics, Vienna, Austria.

The mid-oogenesis checkpoint at stage 8/9 is tightly regulated by nutrition via ecdysteroid (20E/EcR) signaling. Under normal conditions baseline 20E levels allow egg chambers to pass the checkpoint and to develop to stage 10, whereas under starvation programmed cell death (PCD) of mid-oogenic chambers is triggered. To gain further insights into the steroid regulation of mid-oogenesis we genetically up- and downregulated 20E/EcR signaling in adult females. Overexpression of the biosynthetic enzyme shadow was sufficient to increase both ovarian 20E levels and PCD at stage 8/9, thus mimicking the effects of starvation. By contrast, when we decreased 20E by ubiquitous overexpression of the catabolic enzyme Cyp18a1 and RNAi against the biosynthetic enzyme phantom, we observed accumulation of early vitellogenic chambers that failed to develop beyond stage 9. These defective chambers were not cleared away via PCD typical of mid-oogenesis, since follicle cells failed to increase in size and to phagocytose nurse cell remnants. Remarkably, when either up- or downregulating 20E/EcR signaling, nurse cells of the most distal chambers exhibited apoptotic changes. This suggests that 20E/EcR signaling is dispensable for apoptosis of nurse cells but is required for initiating phagocytosis of nurse cells remnants in follicle cells. We next examined which cells are responsible for the arrest at stage 9 by decreasing 20E/EcR signaling in somatic ovarian cells via EcR-RNAi, thus leaving signaling in nurse cells unaffected. In contrast to ubiquitous impairment of 20E/EcR, EcR silencing in follicle cells did not affect 20E titers, with egg chambers proceeding to stage 10. Thus, 20E/EcR signaling appears to be required in nurse cells, but not in follicle cells, for egg chambers to pass the checkpoint. Together, our data suggest that under normal conditions nurse cells require physiological 20E levels to pass to stage 10, whereas under starvation conditions 20E/EcR signaling is required in follicle cells for clearing nurse cell remnants and completion of the PCD program.

722B

Localized Tissue Damage Disrupts Ecdysteroid Biosynthesis and Developmental Progression in Drosophila. Jennifer Hackney, Omid Zolali-Meybodi, Peter Cherbas. Department of Biology, Indiana University, Bloomington, IN.

In insects injury to specific tissues can result in a global developmental delay (e.g. prolonged larval/pupal stages) often associated with decreased levels of ecdysone - a steroid hormone required for developmental transitions in insects. I use Drosophila as a model to examine the pathway by which tissue injury disrupts developmental progression. Here I present data that demonstrate that imaginal disc damage inflicted early in larval development triggers developmental delays while the effects are reduced or eliminated in older larvae. I examine the relationship between the switch in injury response (e.g. delay/no delay) and the mid-3rd instar transition - a developmental time-point that is associated with a small pulse of ecdysone and marks the initial steps of metamorphosis. Finally, I show that developmental delays triggered by tissue damage are associated with decreased expression of Halloween genes which encode enzymes required for ecdysteroid biosynthesis.

723C

The Nuclear Receptor dHNF4 Regulates Carbohydrate Metabolism During Metamorphosis and Adulthood in *Drosophila*. William E. Barry, Jason M. Tennessen, Carl S. Thummel. Department of Human Genetics, University of Utah, Salt Lake City, UT.

Little is known about how energy homeostasis is maintained during Drosophila development, despite the significant changes in nutrient availability and

energy demands as the animal progresses through each stage in the life cycle. At the onset of metamorphosis, for example, the growing larva ceases feeding and enters a period of developmentally-programmed starvation until adult eclosion. The nuclear receptor dHNF4 is a critical regulator of energy homeostasis during starvation in larvae, where it is required to activate a transcriptional program for the catabolism of stored fat (triacylglycerol). In addition, under standard growth conditions, dHNF4 mutants display lethality just prior to, or immediately following, eclosion from the pupal case. This suggests that dHNF4 may also play a vital role in energy homeostasis during metamorphosis and possibly adulthood. Consistent with this, dHNF4 mutants display significantly reduced amounts of glycogen (stored sugar) and trehalose (circulating sugar) during late pupal stages. Surprisingly however, they maintain relatively normal levels of triacylglycerol, in contrast to what is seen in starved dHNF4 mutant larvae. Interestingly, decreasing the amount of dietary sugar, either during development (larval diet) or adulthood, dramatically suppresses the lethality of dHNF4 mutants. Metabolite measurements are currently being performed using these different dietary conditions. Our findings suggest a critical role for dHNF4 in the regulation of carbohydrate metabolism during both metamorphosis and adulthood.

724A

The Let-7 microRNA Complex Extends Longevity and Alters Fat Metabolism in *Drosophila Melanogaster*. Christi Gendron, Scott Pletcher. Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI.

Objective: Given the wide range of physiological processes that microRNAs (miRNAs) are involved in, we hypothesized that specific miRNAs will influence organismal longevity and fat metabolism. An initial screen for miRNAs that influence *Drosophila* longevity identified the let-7-complex (containing let-7, miR-100, and miR-125 miRNAs) as candidates. Therefore, our goal was two-fold: (1) to confirm if the let-7-complex influences both organismal longevity and fat metabolism and (2) to identify which component within the let-7-complex was responsible for the observed changes. Methods: Mated female flies were maintained on standard sugar/yeast food with 12:12 light/dark cycle and constant humidity for longevity experiments. Fly triglyceride (TAG) and protein levels were measured using the Infinity TAG assay or the Pierce BCA Protein assay, respectively, according to the manufacturer's instructions. Starvation assays were performed with young female flies kept on agar-only media. Results: Let-7-complex over-expression resulted in a significant increase in lifespan over control flies. This effect is likely due to miR-100, because over-expression of let-7 or miR-125 individually did not extend lifespan. Whole body let-7-complex over-expression led to increased TAG stores in female flies, with little effect in males. Increased female TAG stores were also observed with let-7 over-expression alone. Conversely, let-7 deletion leads to thinner flies. Let-7-complex over-expression does not provide additional starvation resistance, suggesting that there are alterations in the ability of these flies to mobilize fat stores. Conclusions: We identified the let-7-complex, and likely miR-100, as having the ability to extend fly longevity. Surprisingly, this was not due to miR-125 overexpression, which has been shown to increase longevity in *C. elegans*. Furthermore, over-expression of the let-7-complex, and let-7 itself, alters female fat levels and mobilization. Work is ongoing to identify the mechanisms behind these observations.

725B

Biosynthesis and regulation of *Drosophila* molybdoenzymes. Marina L Georgiou[1], Zvonimir Marelja[2], Silke Leimkühler[2], Fanis Missirlis[1]. 1) School of Biological and Chemical Sciences, Queen Mary, University of London, London, UK; 2) Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany.

The *rosy*, *maroon*, *maroon-like* and *cinnamon* fly mutants were named after a characteristic brownish eye color phenotype. The studies of Art Chovnick, Victoria Finnerty and their co-workers showed that rosy represents the structural gene of xanthine dehydrogenase (XDH) and that the gene products of *cinnamon* and *maroon-like* are involved in the maturation of XDH. XDH is an enzyme composed of two identical and independent subunits, binding one molybdenum cofactor (Moco), two non-identical iron sulfur (FeS) clusters and a flavin adenine dinucleotide. As a continuation of the studies of rosy maturation, we screened for genes that play a role in Moco and FeS cluster biosynthesis by analyzing the activities of the *Drosophila* molybdoenzymes XDH, aldehyde oxidase and sulfite oxidase following RNA interference of various candidate genes that are suggested by sequence homologies to play a role in Moco and FeS cluster biosnythesis. Furthermore, we are interested in the *maroon* locus that was isolated by Calvin Bridges in 1912. Our preliminary experiments suggest that *maroon* differentially regulates molybdoenzyme activities and we are using the genome sequence in an effort to map the original mutation. Finally, although a single aldehyde oxidase activity is revealed following native protein gel separations, four aldehyde oxidase homologues are present in the *Drosophila* genome and show distinct developmental expression patterns. We wish to uncover their respective functions.

726C

Identification of metabolic phenotypes and mechanisms of metabolic regulation by TGF-β signaling in *Drosophila melanogaster*. Arpan Ghosh, Michael O'Connor. GCD, University of Minnesota, Minneapolis, MN.

The TGF-β superfamily is a highly conserved and frequently employed signaling pathway in the animal kingdom that regulates many developmental events. Recent evidence indicates that TGF-β signaling is involved in regulating metabolism and homeostatic processes in vertebrates. However, the mechanism(s) by which TGF-β signaling regulates metabolism remain unknown. We have found that loss of TGF-β-signaling components manifests a number of metabolic defects in *Drosophila*. Loss of the TGF-β ligand *dawdle* (*daw*) causes larval lethality on standard cornmeal food. This phenotype can be rescued by rearing the larvae on yeast food and, as substitution experiments show, is partly caused by sensitivity to propionic acid present in the cornmeal food recipe. Loss of *daw* also leads to a unique sugar-dependent pupal lethality phenotype, causes a small drop in total TAG content, and affects total glycogen content in the larvae. Notably, loss of *daw* causes a significant increase in circulating sugar (CS) concentration in both feeding and starving third instar larvae. The phenotype is aggravated by simultaneous loss of *dAct* indicating that these related ligands function redundantly. While *daw* mutants do not show significant change in *dIlp* (*2,3&5*) expression, IHC experiments show that *daw* larvae are defective in secretion of the dIlp2 peptide from the IPCs. The diabetic phenotype is also observed in feeding *smox* and *babo* larvae. However, unlike *daw*, starvation suppresses the phenotype in *smox* and *babo* mutants indicating an alternate role of the pathway in positively regulating CS levels during starvation. Consistently we observe that loss of *smox* (RNAi clones) in fatbody cells leads to loss of dFOXO nuclear localization upon starvation. Since FOXO is known to be involved in nutrient mobilization during starvation we believe that loss of dFOXO translocation in *smox* mutants impairs release of stored sugar and counteracts the diabetic phenotype caused by loss of *daw*. Current work is focused on defining the mechanisms by which TGF-β signaling regulates CS levels in both feeding and starving conditions.

727A

The *Drosophila PGC1*-α Homolog *spargel* Modulates the Physiological Effects of Endurance Exercise. Lindsey Healy, Martin Tinkerhess, Matthew Morgan, Erin Matthys, Li Zheng, Robert Wessells. Univ Michigan, Ann Arbor, MI.

Endurance exercise is a promising, inexpensive intervention that is thought to provide substantial protection against several age-related pathologies, as well as inducing acute changes to endurance capacity and metabolism. Recently, it has been established that endurance exercise induces conserved alterations in physiological capacity in the invertebrate *Drosophila* model. If the genetic factors underlying these exercise-induced physiological alterations are widely conserved, then invertebrate genetic model systems will become a valuable tool for testing of genetic and pharmacological mimetics for endurance training. Here, we assess whether the *Drosophila* homolog of the vertebrate exercise response gene *PGC1*-α, *spargel (srl)*, is necessary or sufficient to induce exercise-dependent phenotypes. We find that reduction of *srl* expression levels acutely compromises mobility and fatigue resistance, as well as exercise-induced improvement in both assays. Conversely, muscle/heart specific *srl* overexpression improves mobility and cardiac performance in unexercised flies. In addition, we find that *srl* overexpression acts additively to facilitate the impact of endurance exercise on mobility, fatigue resistance and cardiac performance, indicating that other factors also act in parallel to *srl* to regulate exercise-induced physiological changes in muscle and heart.

728B

Effects of dietary fatty acids and temperature on mitochondrial function. Marissa A. Holmbeck[1], David M. Rand[2]. 1) Molecular Biology, Cell Biology, and Biochemistry Dept., Brown University, Providence, RI; 2) Ecology and Evolutionary Biology Dept., Brown University, Providence, RI.

Energy metabolism is modulated by both temperature and diet. These environmental variables can alter cellular functions as well as the fatty acid composition of membranes. According to the fluid mosaic model of membrane structure, phospholipids are arranged in a fluid bilayer with integral proteins free to move within this structure. The degree of membrane saturation and temperature may interact to modulate the fluidity of the membrane and activity of membrane-associated enzymes. This is particularly important in mitochondria where the integrity of the double membrane structure of the organelle is critical to the production of cellular energy. To dissect the interaction between temperature and fatty acid diets on mitochondrial function, flies were raised on media containing specific saturated, monounsaturated, or polyunsaturated fatty acids supplements at low concentrations. Flies were maintained on control and fatty acid diets for ten days at two different temperatures. To assay mitochondrial function under these conditions mitochondrial respiration, reactive oxygen species production, and membrane potential were measured. A large thermal effect on respiration was observed, while only subtle effects of diet were seen on activity of electron transport chain complexes. Additionally, longevity was measured to dissect the interactions of fatty acid and temperature manipulations on lifespan, and the results are not consistent with the simple mitochondrial free radical theory of aging. We hypothesize that the varied effects of temperature and fatty acid supplementation are modulated by acclimation of membrane composition in order to maintain membrane fluidity, regardless of diet.

729C

Catalytically Inactive Triosephosphate Isomerase Rescues TPI Deficiency. Bartholomew P Roland[1,2,3], Kimberly Stuchul[1], Michael J Palladino[1,3]. 1) Pittsburgh Institute for Neurodegenerative Diseases, Pittsburgh, PA; 2) University of Pittsburgh Graduate Program in Molecular Pharmacology, Pittsburgh, PA; 3) University of Pittsburgh Department of Pharmacology & Chemical Biology, Pittsburgh, PA.

Triosephosphate isomerase (TPI) is a glycolytic enzyme that converts dihydroxyacetone phosphate (DHAP) into glyceraldehyde-3-phosphate (G3P). Dysfunction in TPI will lead to a number of diseases collectively known as TPI deficiency glycolytic enzymopathies. We have isolated a point mutation in the Drosophila TPI gene called sugarkill that causes symptoms similar to those in human patients, including seizures, paralysis, premature death, and neurodegeneration. We have established that this mutation increases the degradation of TPI, and animal phenotypes can be attenuated by inhibiting the proteasome or overexpressing the mutant TPI. Here we show that TPI sugarkill behavioral phenotypes can be rescued through the addition of a catalytically inactive TPI enzyme.

730A

The regulation of fat storage by *Mio* in *Drosophila*. Eric D. Sassu, Jacqueline E. McDermott, Brendan J. Keys, Justin R. DiAngelo. Department of Biology, Hofstra University, Hempstead, NY.

During nutrient excess, triglycerides are synthesized and stored to provide energy during times of famine. One of the major pathways controlling fat synthesis during nutrient excess leads to the activation of carbohydrate response element binding protein (ChREBP), a transcription factor that induces the expression of a number of glycolytic and lipogenic enzymes. However, the molecular mechanisms regulating ChREBP activation and function are not fully understood. In this study, we characterized the role of the *Drosophila* homolog of ChREBP, *Mlx interactor (Mio)*, in controlling fat accumulation in larvae and adult flies. Lowering *Mio* levels using RNAi specifically in the larval or adult fat body leads to a lean phenotype. This phenotype results from decreasing the amount of fat stored per cell while the total number of fat body cells produced remains unchanged. A lean phenotype is also observed when the gene *bigmax*, the fly homolog of the ChREBP binding partner Mlx, is decreased in the fat body suggesting that Mio and bigmax may be acting together to promote triglyceride storage. Interestingly, depleting *Mio* in the fat body results in decreased feeding providing a potential cause of the lower triglycerides observed in these animals. Together these data indicate a role for *Mio* in controlling fat accumulation in *Drosophila* and suggests that it may act as a nutrient sensor in the fat body to coordinate feeding behavior with nutrient availability.

731B

Transgenerational Inheritance of Metabolic State in *Drosophila*. Rebecca A Somer, Matt Sieber, Carl Thummel. University of Utah, 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 parental diet can also have a dramatic impact on the metabolic state of our children. 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, as it can be inherited from either parent before conception. All of these studies, however, are correlative,

and the mechanism of the inheritance of metabolic state is still unknown. To determine if parental diet influences progeny metabolism in *Drosophila*, we fed mature wild type adults either a complete diet or a nutrient depleted diet. We have found that the adult progeny of parents fed the nutrient-depleted diet display higher levels of triacylglyceride and lower levels of glycogen than the progeny of parents fed the complete diet. These effects are independent of progeny diet, suggesting that they are the result of an inherited metabolic program. In addition, we see changes in the expression levels of several genes controlled by the nuclear receptor DHR96 between progeny of parents fed either the complete or nutrient depleted diets. This preliminary data provides the foundation to study the genetic and molecular mechanisms underlying the transgenerational inheritance of metabolic state in an easily manipulated genetic system, as well as to understand the physiological effects of parental diet on offspring.

732C

***dFatp* Regulates Nutrient Distribution and Long-term Physiology in *Drosophila*.** Alyson Louise Sujkowski, Samantha Morley, Joanna Jennens, Nicole Piazza, Lindsey Healy, Martin Tinkerhess, Li Zheng, Robert Wessells. Univ Michigan, Ann Arbor, MI.

Nutrient allocation and usage plays an important part in regulating the onset and progression of age-related functional declines. Dietary restriction, for example, extends lifespan and protects against multiple stresses in various organisms. Endurance exercise, by contrast, extends functionality during aging without extending maximal lifespan. The discovery of mimetics that replicate positive and additive effects of diet and exercise programs is thus an important research goal. Here, we describe a dominant mutation in *Drosophila (dFatp)* that alters nutrient distribution by limiting usage of fatty acids. *dFatp* mutants have increased lifespan and stress resistance, altered feeding behavior and fat storage, increased respiration and increased mobility. Concurrently, mutants experience impairment of cardiac function. We show that cardiac impairment of mutants is rescued by exercise training without reversing lifespan extension and present a model to explain these results. These findings establish a novel conserved genetic target for regulating the functional effects of diet and exercise on the physiology of aging animals.

733A

Impact of Glutamate Dehydrogenase (GDH) and Isocitrate Dehydrogenase (IDH) on Lifespan and Starvation Resistance in Varying Nutrient Conditions. Brittany Barnett, Matthew Talbert, Walter Eanes. Ecology and Evolution, SUNY Stony Brook, Stony Brook, NY.

Nutrient state is partly governed by ATP/ADP and metabolic cofactors, which are established by central metabolic enzymes. A fed or fasting state in secretory neural and endocrine tissues results in molecular profiles that impact lifespan and life history traits. We altered activity of two metabolic enzymes and investigated impact on lifespan and starvation resistance of *D. melanogaster* in the context of low and high nutrient (LN and HN). Gdh regulates protein entrance into metabolism, critical in states of starvation or low nutrient intake and regulates NAD+ levels. Idh functions in the TCA cycle and supplies NADPH. Null alleles, derived from P-element excision, were background replaced and crossed with w; 6326; 1, yielding flies with 70% Gdh activity and 50% Idh activity compared to full alleles also crossed with 6326. To assess interaction of nutrient availability with any lifespan effects, flies were subjected to HN (16g yeast, 16g sucrose/100 mL H_20) or LN (4g yeast, 4g sucrose) cornmeal-based food medium at 25°C (n=400). Starvation studies were run at room temperature using sealed plastic vials with a damp cotton base (n=200). There was no effect on lifespan regardless of Idh activity on either diet; however, a calorie restriction effect was observed with LN flies living longer than HN flies. Full activity Idh flies survived starvation ~10 hrs longer than the low activity flies (p=.031). Again a calorie restriction effect was observed in Gdh flies; however, low activity Gdh flies lived 5 days longer than the full activity Gdh flies within the LN cohort (p=.020). In addition, low activity Gdh flies survived starvation ~8 hrs longer than the full activity Gdh flies (p=.037). The data suggest that Gdh is an important mediator of lifespan under LN conditions. On HN, the intake of nutrients may be too great for differences in Gdh activity to matter. The data also suggest that Idh is not an important mediator of lifespan, but it is of starvation resistance.

734B

Role of Conventional Odorant Receptors in *D.melanogaster* Lifespan and Aging Physiology. Ceyda Bilgir[1], Xiaowen Chu[2], Yuzhong Liu[1], Brian Y. Chung[1], Scott D. Pletcher[1]. 1) Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 2) Huffington Center on Aging, Baylor College of Medicine, Houston, TX.

Olfaction is an ancient sensory system present in many species from bacteria to humans. Exposure to nutrient-derived odorants can regulate aging and longevity. In the absence of the broadly expressed atypical Odorant Receptor 83b (Or83b), conventional odorant receptors (ORs) are rendered in the cell body and olfaction is compromised. Anosmic Or83b homozygous null flies are long-lived and they present enhanced stress resistance as well as increased fat storage. Because Or83b is required for proper localization of conventional ORs to dendritic membranes, which is imperative for their function, we hypothesize that the longevity phenotype and related physiological phenotypes seen in Or83b null flies are due to the loss of function of one or a subset of conventional ORs. To dissect the specific effects of individual ORs, we assayed lifespan, metabolism, and stress resistance in a range of mutant lines in which one to three ORs were deleted. Our results indicate that absence of one or a small number of conventional ORs can affect longevity and that they often act in different manners. Flies that do not have a functional OR implicated in 11-*cis*-vaccenyl acetate-induced mating behavior, for example, are long-lived. However, they have lower resistance to starvation compared to controls. On the contrary, certain receptor deletions result in a reduced lifespan while several other ORs have little to no effect on longevity. This establishes that some individual ORs promote longevity while others limit it. Together our data suggest that sensory systems rival the insulin-signaling, TOR, and translation-related pathways in the sheer number of effective manipulations that share a similar function and induce potent and reproducible effects on organism lifespan.

735C

***Spargel*, a mammalian PGC-1 homologue is involved in nutrient sensing pathway acting downstream to TOR and S6k.** Subhas Mukherjee, Claudette Davis, Atanu Duttaroy. Biology Department, Howard University, Washington, DC.

Peroxysome proliferator-activated receptor gamma coactivator-1 (PGC-1) is a transcriptional coactivator in mammals. Among the two PGC-1 isotype, PGC-1α is a master regulator of mitochondrial activities including gluconeogenesis, fatty acid oxidation, regulation of thermal tolerance, skeletal muscle fibre determination and initiation of mitochondrial biogenesis. PGC-1β on the other hand is well known for fat synthesis. The single *PGC-1* gene of Drosophila called spargel carries significant homology to both α and β forms at the RNA Recognition Motif. Spargel is a nutrient sensor since it gets upregulated following the addition of nutrients after starvation. A recent study and our results confirmed that spargel is part of the insulin- signaling

pathway. More over, the smaller body size, reduced ATP, protein and fat content of spargel hypomorph, *srl*^{l} suggests that spargel is required for nutrients utilization. Therefore, our objective is to place spargel in the insulin-signaling pathway appropriately in relation to other partners in the pathway. Since over expression of spargel in the fat body increases the production of fat and ATP this data indicates that spargel facilitates better utilization of nutrients which is also a hallmark function of TOR (Target of Rapamycin) and S6 kinase. Both TOR and S6K are integral parts of a highly conserved metabolic pathway starting from yeast to Human. Therefore, down regulation of TOR and S6K reduces the viability in flies. Absence of S6k reduces the cell size severely. We found that *spargel* over expression successfully rescues the S6k dominant negative mutant. Absence of TOR causes premature death in Drosophila around 2nd instar larval stage, but Spargel over expression effectively rescued this TOR dominant negative phenotype by helping the mutants to live up to the pupal stage and some adult eclosion was also evident. This data strongly suggests that spargel controls cell growth and metabolism by acting downstream to Tor/S6k in the nutrient utilization pathway.

736A

Impact of Supplementing Taurine in the diet of *Drosophila*. Lee A. Smith[1], Feras Alhourani[1], Ishtiaq Habib[1], Brian Talon[1], Stephanie Shirkey[1], Jeremy Nadolski[2]. 1) Dept Biological Sci, Benedictine Univ, Lisle, IL; 2) Dept Mathematical and Computational Sci, Benedictine Univ, Lisle, IL.

As the amino acid taurine is being used more frequently in human diets, the exact physiological role and benefit have not been fully elucidated. To determine if taurine can impact long term physiology, we investigated the effects of a chronic taurine supplemented diet on adult life span, egg-laying, and larval development. A comparison of survival curves found a significant difference between male and female fruit flies supplemented with a taurine diet as well as a difference between taurine and normal fed fruit flies with respect to gender. Current experiments are investigating if the benefit of taurine is due to its anti-oxidant ability. The effect of taurine does not provide a benefit during early development of the fly, rather it reduces the number of hatched larvae in a concentration-dependent manner. Given the sensitivity of eggs to taurine, we are testing whether females avoid laying eggs given a choice between normal and taurine supplemented food.

737B

Genome wide association study for visual decline in a population of aging *Drosophila melanogaster*. Mary A. Carbone[1,2], Tess A. Brune[1], Akihiko Yamamoto[2,3], Michael M. Magwire[1,2], Trudy F.C. Mackay[1,2], Robert R.H. Anholt[2,3]. 1) Department of Genetics, North Carolina State University, Raleigh, NC; 2) W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC; 3) Department of Biology, North Carolina State University, Raleigh, NC.

Age-related eye diseases affect more than 3 million Americans over the age of 40. Such disorders include cataracts, glaucoma and macular degeneration. Genes that maintain phenotypic variation in vision disorders in natural populations are unknown. Here, we used a population of wild-derived *Drosophila melanogaster*, known as the *Drosophila* Genome Reference Panel (DGRP), to characterize variation in visual acuity in aging flies, by assaying their phototaxis behavior using a countercurrent apparatus. We performed a genome wide association study (GWAS) and identified polymorphisms (SNPs) significantly associated with phototaxis in the genes *cacophony* and *rugose*. The gene *cacophony* is involved in the detection of light stimulus for visual perception, while *rugose* functions in the differentiation of compound eye cone-cells. We demonstrate the use of *Drosophila melanogaster* as a model system to decipher the genetic networks that modulate the natural variation in age-related decline in visual acuity.

738C

Cellular and Physiological Basis of Thermal Plasticity of Body and Organ Size in *Drosophila melanogaster* . Shampa Ghosh Modak, Alexander W. Shingleton. Zoology, Michigan State University, East Lansing, MI.

Body size and temperature show an inverse relationship in most organisms including *Drosophila*, a phenomenon commonly known as the ‘temperature size rule’. In addition, different organs of the fly show different levels of thermal sensitivity during pre-adult growth. However, the developmental genetic underpinning of thermal regulation of body and organ size in flies, or any organism, is almost completely unknown. Size in *Drosophila* is regulated by three aspects of pre-adult development: (i) critical size; (ii) growth rate during the terminal growth period (TGP) *i. e.* the time between critical size and the cessation of growth; and (iii) the duration of the TGP. Temperature presumably generates plasticity in body and organ size by affecting one or all of these three parameters. Here we describe the extent to which changes in critical size, growth rate and TGP contribute to thermal plasticity in *D. melanogaster*. Such data are an essential prelude for understanding how temperature influences the developmental genetic mechanisms that regulate each parameter and hence body size as a whole. Further, we use clonal analysis to show that the thermal sensitivity of cell proliferation varies among organs. These data explain, in part, why there is variation among organs in their size response to temperature.

739A

Life history Variation and Reproductive Senescence in Three Recently Caught Wild-type Populations of Drosophila melanogaster. Peter Klepsatel, Martina Galikova, Nicola de Maio, Christian Schlötterer, Thomas Flatt. Institute of Population Genetics, Vienna, Austria.

The life history of D. melanogaster is relatively well studied, but life history data are typically obtained by averaging over many individuals rather than following single individuals over time. Moreover, most studies have examined life history traits in lab strains kept for a long time rather than working with individuals freshly sampled from natural populations and may thus be confounded by effects of lab adaptation and inbreeding. Here we focus on variation in life history traits (fecundity, ovariole number, hatchability, lifespan) measured on freshly collected individuals from three different wild-type populations of D. melanogaster. We found significant variation among populations for all traits, in particular for different fecundity traits, and used these data to create a mathematical model of individual fecundity and reproductive senescence. Fecundity data were best described by a model with four distinct phases during a female’s life: onset of maturity, reproductive senescence, fecundity "collapse", and a postovipository period. Interestingly, the onset of the postovipository period was a good predictor of death, with females dying 6-9 days after laying their last egg, irrespective how long they lived. We also examined phenotypic correlations between traits. We failed to find negative correlations (trade-offs) between early and late fecundity or between different fecundity measures and lifespan. Ovariole number was positively correlated with early daily fecundity in all populations, but uncorrelated with late or total fecundity. In general, most fitness components were positively correlated. This preponderance of positive correlations is unlikely due to inbreeding or positive mutational covariance but is probably best explained by optimal lab conditions. Fitness traits measured in the lab might thus reflect unconstrained physiological state

(health) under optimal conditions.

740B

Quantifying main ecdysteroides throughout the *Drosophila* developmental cycle. Oksana Lavrynenko, Maria Carvalho, Jonathan Rodenfels, Julio Sampaio, Suzanne Eaton, Andrej Shevchenko. MPI CBG, Dresden, Germany.

Ecdysteroides are major regulators of developmental timing in *Drosophila*. Although it has been generally assumed that developmental transitions are accompanied by puls releases of ecdysteroides [1], little is known on how concentrations of individual hormones are changing throughout the larvae development. Current biological enzyme immunoassay (EIA) and radioimmunoassay (RIA) methods lack specificity towards individual hormones, are laborious and relatively inaccurate. We developed LC-MS/MS method for simultaneous quantification of major ecdysteroides in Drosophila larvae: ecdysone (E), 20-hydroxyecdysone (20H) and makisterone A (Maki A) with the detection limit of 3 pg and better than 7% RSD. We then acquired the full time course of these hormones from the beginning of embryonic stage to adult's emergence with the time intervals of four hours. We observed synchronized pulses of 20H and Maki A in the middle embryonic period, the end of first and second instars directly followed by cuticle formation and molting. Maximal content of 20H was 180 pg during wings and legs formation metamorphosis. The maximal release of ecdysone was observed 8 hours prior to 20H and Maki A pulses. Overall, the highest E concentration was 800 pg/animal which is 5 to 10 times higher compared with 20H and Maki A. The total of all ecdysteroides was reaching 1 ng per animal. We were able to quantify ecdysteroides in a single larvae and keep track on individual variability of the hormone concentration throughout larvae population. Within white pupa hormone concentration could deviate for as much as 30%. While it has become possible to quantify individual ecdysteroides in their active form, further efforts are directed towards comprehensive quantitative profiling of the full ecdysteroidome, including multiple storage and metabolized forms. [1] Molecular Mechanisms of Developmental Timing in C. elegans and Drosophila, Carl S. Thummel, developmental Cell, Vol. 1, 453-465, 2001.

741C

The Involvement of the Electron Transport Chain in the Isoflurane Response in Drosophila melanogaster. Christopher R. Pope[1], Gerald B. Call[2]. 1) Department of Biomedical Sciences, College of Health Sciences, Midwestern University, Glendale, AZ; 2) Department of Pharmacology, Arizona College of Osteopathic of Medicine, Midwestern University, Glendale, AZ.

Over 160 years has passed since the ground breaking public demonstration of the use of general anesthetics (GA) in surgery. However, the mechanism for GA action is still unknown. Drosophila exhibits all four anesthetic endpoints: analgesia, amnesia, muscle relaxation and loss of consciousness. Because of this and the numerous genetic tools available, Drosophila has been recognized as an excellent model organism for identifying the mechanism of GAs through genetic approaches. The *bellwether* gene, which codes for the beta subunit of ATP Synthase, was one of a few genes identified in an initial screen of Drosophila mutants for sensitivity or resistance to the commonly used GA, isoflurane. These results, along with other scientific evidence linking mitochondrial function and GAs, led to this study on determining the involvement of the mitochondrial electron transport chain (ETC) in GA action. Available mutants from the Bloomington stock center, along with RNAi stocks from different collections (Harvard TRiP, NIG and VDRC), for different nuclear encoded ETC genes were used. RNAi lines were crossed with the ELAV-GAL4, UAS-Dcr2 stock to produce pan-neuronal silencing of the ETC genes. Adult flies (100-150, with a minimum of four repetitions) are tested in an inebriometer with 1% isoflurane. The inebriometer is a device that can quantitatively measure the response of a population of flies to GAs. The data from each mutant or RNAi line is then compared to appropriate controls to characterize whether a gene confers sensitivity or resistance to isoflurane. Preliminary results from mutants or RNAi lines includes: complex I (*Pdsw*=67% of wt,*CG6020*=46%), complex II (*SdhA*=196%, *SdhB*=166%), complex III (*RFeSP*=321%, *CG3560* 49%), complex IV (CG11015=300%, CG11043=288%), ATP Synthase (*CG5389*=302%, *Oscp*=285%). More comprehensive data will be presented at the meeting. Currently, this is the most comprehensive study to determine the role of ETC genes in the isoflurane response.

742A

The Involvement of Ion Channels in the Response to Isoflurane. Ryan Stopher-Mitchell[1], Krista Pearman[2], Erik Nelson[1], Michael J. Murray[3], Gerald B. Call[2]. 1) Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ; 2) Department of Pharmacology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ; 3) Department of Anesthesiology, Mayo Clinic, Scottsdale, AZ.

For more than 160 years volatile anesthetics (VAs) have been used, yet the workings of VAs upon the central nervous system is unknown. Different and separate mechanisms have been proposed for the effects of amnesia, analgesia, and immobility in addition to different target locations in the brain and spinal cord. Recent evidence suggests that these effects occur through multiple targets as opposed to a single common mechanism. Drosophila melanogaster is a useful species to study the effects of VAs: it passes through each stage of anesthesia at equivalent human dosages, its central nervous system is more complex than other invertebrates, its genome has been completely mapped, it has a short life cycle, and it has an easily altered genome. An inebriometer was used to quantitatively study the effects of the common VA, isoflurane, on Drosophila. Publically available Drosophila with UAS-RNAi constructs provides an ideal resource for gene-specific silencing. Previous studies have indicated that ion channels, particularly sodium and nicotinic channels, may be potential candidates for the VA mechanism. We identified 380 potential ion channels within the D. mel. genome. So far, 300 of these have been used in an RNAi screen to determine potential candidates for future studies of the mechanism of VAs. RNAi silencing of genes such as *Nckx30C*, a K^+ dependent Na^+/Ca^+ exchanger, showed sensitivity, while others like *CG7333*, a secondary active organic cation transporter, and *nAcRβ-64B*, a nicotinic acetylcholine receptor showed resistance. Analysis of the data reveals many potential pathways for VA action and confirms the efficacy of using this method to further study anesthetics in vivo. The full results of our screen will be presented at the meeting. This study is the first comprehensive analysis of ion channels in the response to isoflurane.

743B

Arginine kinase function in adult tissues. Glen E. Collier. Dept Biological Sci, Univ Tulsa, Tulsa, OK.

Arginine kinase is encoded by a single locus (*Argk*) in *Drosophila melanogaster* that produces six putative alternative transcripts. These all share a common C-terminal catalytic domain, but differ in amino acid sequences at the N-terminus of the protein products. One product (PD) is extremely abundant in muscle tissue, but is found in other tissues as well. The other protein products of this locus are less abundant and are more restricted in tissue distribution.

The single available null mutation affects the common catalytic domain and is an embryonic lethal, thus hampering investigation of the function of these products in adult tissues. The diversity of these functions have been investigated using tissue-specific GAL4 drivers and RNAi constructs. Ubiquitously expressed GAL4 drivers (i.e. *tubP*GAL4) in combination with *Argk* RNAi results in lethality with the same lethal phase as the EMS-induced null. In contrast, muscle-specific expression of GAL4 (i.e. *Mef2*GAL4) in combination with Argk RNAi bypasses the embryonic lethality and results in a flightless phenotype and premature mortality supporting the importance of PD and PA products in flight muscle metabolism. GAL4 drivers restricted to germline expression (i.e. *da*GAL4) in combination with *Argk* RNAi also bypasses the embryonic lethality, yet results in a unique female sterile phenotype in which eggs fail to develop. These eggs are deficient in the PB product that is produced in nurse cells and transported to the egg suggesting this form is necessary for early embryonic development. A testis-specific paralog of *Argk*, CG4546, results in a unique male sterile phenotype when eliminated by RNAi. In these males the individualization process is blocked and functional, mature sperm are not produced.

744C

Genetic and imaging analyses of Drosophila sperm storage. Xiangyi Lu, Benjamin Burger. Wayne State Univ, Detroit, MI.

The human polycystic kidney disease gene 2 (PKD2) encode a conserved calcium channel of the TRPP family. In variety of organisms studied, PKD2 channels localize and function on cilia and flagella, which are microtubule axoneme-containing, sensory and/or motile organelles of a common evolutionary origin. PKD2 on renal epithelial cilia is mechano-sensitive and it generates calcium influx in response to fluid flows in the nephron. PKD2 mutations cause PKD due to, presumably, a disruption of cilium-mediated fluid sensation. However, signaling events downstream of calcium entry are mostly unknown. Drosophila Pkd2 localizes on the sperm flagella. Although Pkd2 null Drosophila are viable, the mutant males are largely sterile due to inability of the sperm to move into sperm storage organs. Sperm storage or accumulation of sperm at a defined location of the female reproductive tract is a reproductive process of many internally fertilizing species, including insects, birds and mammals. Movement of sperm into the storage site appears to be guided by external signals, but not much is known. Using GFP imaging, we showed that Drosophila sperm are bidirectional swimmers. The sperm show complex motility regulation in the reproductive tract, involving changes in both flagellar waveforms and wave directions. Pkd2 mutation affects the sperm's tail-leading movement for entering the sperm storage organs without affecting the head-leading movements for exiting the storage organ and for fertilizing the egg, suggesting that Pkd2 regulates sperm movement via possibly sensory responses to sperm storage signals. Moreover, mutagenesis screens have led to five new mutant loci that cause sperm storage phenotypes similar to that of Pkd2. One of these encodes Lobo, which is localized on the sperm flagellum and appears to be an integral protein of the outer doublet microtubules. Genetic analyses indicate that Pkd2 and Lobo function in the same pathway. Other Pkd2-like genes encode protein kinase, ATPase, and proteins of unknown functions - all are conserved from flies to humans. Our studies have shed a new light on the Pkd2 pathway and sperm interaction with the female reproductive tract which drives speciation of insects.

745A

Female reproductive glands play essential roles in reproduction that may have been conserved during evolution. Jianjun Sun, Allan Spradling. Howard Hughes Medical Institute, Department of Embyology, Carnegie Institution for Science, Baltimore, MD. 21218.

Glands are associated with the female reproductive tract in diverse organisms. Yet the cellular and molecular pathways controlling their formation, and the roles played by glandular secretions in sperm storage, capacitation, sperm-ova interactions, and early embryo implantation remain poorly known. We have better characterized *Drosophila* spermathecal and parovarial development and used this knowledge to probe reproductive gland function. Three-cell secretory units comprising a gland cell, a canal cell and an accessory cell generate the mature tissues, a process likely to be characteristic of diverse insect glands. The transcription factor Lozenge specifies precursor cells within the genital disc while the nuclear hormone receptor Hr39 times and controls subsequent gland formation. Notch signaling and its downstream target *hindsight* are essential for gland cell differentiation. Using genetic tools to specifically alter gland cell formation, we demonstrate that secretions not only mediate sperm storage in both seminal receptacle and spermathecae, but also control ovulation. Our work shows that *Drosophila* is a powerful model for analyzing conserved genes and mechanisms underlying sperm storage, sperm capacitation, ovulation, and fertilization.

746B

dJun and Vri/dNFIL3 regulate age related cardiac senescence in Drosophila. Herve TRICOIRE[1], Veronique MONNIER[1], Magali ICHE-TORRES[2], Michael RERA[1], Vincent CONTREMOULINS[3], Nathalie LALEVEE[2], Laurent PERRIN[2]. 1) Unité de Biologie Fonctionnelle et Adaptative (BFA, Univ Paris Diderot, Sorbonne Paris Cité, PARIS, France; 2) IBDML, UMR 6216 Campus de Luminy, 13288 Marseille Cedex 9 France; 3) Institut Jacques Monod, CNRS-University Paris Diderot 75205 Paris cedex 13, France.

Cardiac aging is characterized by a progressive senescence of organ's physiology, including a decrease of cardiac reserve, modifications of heart rate and increased arrhythmias. To identify the molecular pathways involved in heart senescence, we identified biomarkers of aging by tissue specific transcriptome comparison of young (10 days) versus aged (40 days) fly hearts. Data mining suggested specific pathways and regulatory inputs involved in heart aging. In particular, the JNK/AP1 pathway but also the vri/dNFIL3 transcription factor were pointed, as well a potential role of oxidative stress (OS) in the process. Tissue specific genetic manipulations were performed in the aging heart, and parameters of cardiac senescence were analyzed in vivo. Reducing or increasing OS specifically in the cardiac tissue by manipulating Catalase activity respectively delayed or enhanced cardiac senescence. In addition, targeted dJun and Vri knockdown both delay cardiac senescence, pointing to a central role of these transcription factors in heart aging.

747C

Identification and Characterization of Upstream Regulators of Nrf2 Signaling in *Drosophila melanogaster*. Nirmalya Chatterjee[1], Kerstin Spirohn[2], Michael Boutros[2], Dirk Bohmann[1]. 1) Dept. of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY; 2) Division Signaling and Functional Genomics, German Cancer Research Center, Heidelberg, Germany.

Oxidative stress causes widespread damage to macromolecules, cells and organisms. Accumulation of such damage over time is thought to drive the aging process. Multiple signaling pathways respond to oxidative stress by regulating processes which limit or repair cell damage. The transcription factor Nrf2 is a principal regulator of such antioxidant and stress defense mechanisms. Previous work by this and other labs indicates that Nrf2 also delays age associated functional decline and influences longevity. Consistent with a role in aging, metabolic and longevity signals known to modulate longevity can regulate Nrf2

signaling pathways. A comprehensive molecular description of Nrf2 regulation is necessary to understand the function of Nrf2 as an effector of different environmental and physiological signals and as a mediator of oxidative stress tolerance and longevity. Nrf2 binds to antioxidant response elements (AREs) in DNA of different antioxidant and detoxification genes. The key components of the Nrf2 signaling are conserved between *Drosophila* and mammals and CncC is the homolog of Nrf2 in flies. In order to study CncC activity in Drosophila and in cell culture, complementary *in vivo* and cell-based reporters that carry reporter transgenes in which GFP, RFP or luciferase are regulated by synthetic ARE promoter elements, were generated. These reporters are specific to CncC signaling and are responsive to genetic and chemical activators of Nrf2. A kinome-wide dsRNA library screen in S2 cells with the cell-based reporter identified several novel regulators of CncC signaling. The putative regulators were validated in Drosophila using the *in vivo* reporters and were further characterized. This study advances our understanding of the mechanism and consequences of environmental and physiological regulation of Nrf2.

748A

Transcriptional down regulation of two nuclear genes with *Frag1* and *Protein Kinase* motifs confers oxidative stress resistance and extends lifespan. Atanu Duttaroy, Dondra Bailley, Sanjay Nag. Dept Biol, Howard Univ, Washington, DC.

Reactive Oxygen Species (ROS) poses many threats to an organism. In addition to the threats triggered by oxidative stress, ROS action is biphasic because at low levels mitochondrial ROS intermediates many cellular signaling pathways essential to variety of biological processes. Thus ROS is already tied up with the regulation of growth factor receptors, src kinase, ras signaling, mitogen-activated protein kinases, etc. In Drosophila, the Jun N-terminal kinase (JNK) signal transduction pathway was identified as one of the main systems with which the fruit fly protects itself against ROS induced oxidative insults. More recently, mitochondria and choloroplast have been identified and shown to exert their influence on nuclear gene transcription activity through a process called retrograde signaling. The loss of function mutants of mitochondrial *Sod2* gene in Drosophila *Sod2n283*, demonstrably produces high flux of mitochondrial superoxides. We asked if this high mitochondrial superoxide environment in *Sod2n283* is capable of activating some novel cell-signaling pathway? Microarray analysis of *Sod2n283* followed by RNAi mediated suppression of the induced transcriptomes helped us to identify two genes, *CG4945*, is a Serine/Threonine Protein Kinase and *CG7990* is homologous to mammalian Frag1 (FGF receptor activator) superfamily gene. While both *CG4945* and *CG7990* are upregulated more than two folds in *Sod2n283*, their RNAi mediated suppression renders the flies as highly resistant to paraquat induced oxidative stress. Such high paraquat resistance led us to measure the lifespans of *CG4945IR* and *CG7990IR* following ubiquitous activation of the RNAi with *Tub-GAL4* driver. Reduced *CG4945* and *CG7990* expression helps the animals to live longer, so they have an extended life span compared to the controls. Our results demonstrate that expression of CG7990 and CG4945 are negatively required against oxidative stress protection as well for achieving normal life span very much like the JNK, although unlike JNK these two genes are not essential for the survival of the organism.

749B

Nitric oxide signals developmental delay during regeneration. Jacob Jaszczak, Adrian Halme. Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA.

In many organisms the capacity to regenerate is closely linked to development. *Drosophila* larvae are capable of robust regenerative growth within damaged imaginal discs during larval stages. However, regeneration of damaged imaginal discs is restricted near the end of the last larval instar. We have previously demonstrated that damage to the imaginal tissues activates a developmental checkpoint, producing a delay in development and extending the period of regenerative competence. We have shown that the TNF-JNK pathway is necessary for producing this delay during regenerative growth, however the systemic signals that coordinate local tissue repair with developmental progression are still unknown. Nitric oxide has been shown to be an important signal in producing systemic responses to hypoxia or infection. Here we demonstrate that nitric oxide synthase (NOS) transcription is activated in third instar larvae by damage to the imaginal tissues. We observe that expression of NOS protein is increased in circulating hemocytes after irradiation or targeted imaginal disc damage, and this correlates with an increase in nitric oxide levels across the whole larva. Finally, we demonstrate that the induction of NOS expression is sufficient to produce developmental delay, but only during a specific responsive period in the third larval instar. Based on these observations, we propose that nitric oxide is an important regulator of developmental delay following imaginal tissue damage.

750C

Potential role of V-ATPases in autophagy regulation. Caroline C Mauvezin, Thomas Neufeld. Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

Macroautophagy (here after named autophagy) is a degradative process essential for cell survival. Indeed, autophagy is involved, for example, in the degradation of large unhealthy organelles such as damaged mitochondria. The recent increase in awareness and interest in this field stems from the discovery of the genes that control autophagy - the ATG genes - and the subsequent finding that autophagy can play either harmful or beneficial roles in a wide range of areas important to human health, including cancer, neurodegeneration and aging. The core autophagic machinery is conserved from yeast to human. Autophagy is activated by scarcity of nutrients through TOR signaling. In autophagy, double-membrane autophagosomes envelop and sequester intracellular components and then fuse with lysosomes to form autolysosomes, which degrade their contents to regenerate nutrients. In the laboratory, we are taking advantages of the powerful genetics of Drosophila melanogaster as a model of study to characterize the mechanisms underlying autophagic regulation. Recently, it has been described that reactivation of TOR in mammals is autophagy-dependent and requires the degradation of autolysosomal products. Lysosomal acidification is necessary for the proper degradation of autophagic cargo, fusion with autophagosomes and this process is directly linked with the assemblage and the well-functioning of V-ATPase proton pump. V-ATPases are formed by two subunits V0 and V1 that are required to assemble at the membrane of the acidic vacuoles. Each of the subunit is composed by several proteins that together form a functional complex. Interestingly, the role of V-ATPase in membrane dynamics required for the uptake of autophagic cargo is far from fully understood and it is unknown whether V-ATPases assembly or function is regulated by nutrients-TOR signaling. Here, we propose to target V-ATPase subunits as new regulators of autophagy. By using RNAi lines, we propose to investigate potential variations of autophagosome induction and blockade of the autophagic flux in fat bodies of fruit flies larvae.

751A

***Drosophila*, an *in vivo* model to evaluate reprotoxic damage.** PATRICIA RAMOS[1,2], BLANCA HERNANDEZ[2], OLGA RAMIREZ[1]. 1) Lab Genética y Toxicología Ambiental, Depto. Biología, Facultad de Ciencias, CU, Universidad Nacional Autónoma de México, D.F., Coyoacan; 2) Drosophila Stock

Center México, Facultad de Ciencias, UNAM, México.

Sexual differentiation is a key activity during the development of organisms. In a particular species, the proportion of individuals of either gender is balanced. Some environmental pollutants act as endocrine disruptors by altering the hormonal signaling cascade involved in sexual differentiation (1). In mammals, the action of endocrine disruptors as beta-estradiol lead to the feminization of males (2). Herbicides like Atrazine are endocrine disruptors on birds, amphibians and mammals (3). Goal: In this work we propose an *in vivo* experimental strategy to evaluate the ability of pollutants to cause reprotoxic damage. Methods. We used two species of flies: *D. melanogaster* (DME) and *D. mojavensis* (DMO). The gene cascade involved in DME sexual differentiation has been reported. DMO, is a cactophlic fly widely used in studies of speciation (4). Upon emerging, the testes of BMD males are colorless but become red when ripe, so no dissection is needed to count the number of gonads per male (2, 1 or 0 testis). The last third of larval development was chose for the exposure to genotoxins (Exterpro^{TM}, glyphosate). 15 successive dilutions were prepared which were administered in the food for a semi-chronic exposure. Adult flies recovered were counted, sexed and mated as follows (t, treated flies; nt untreated flies): ntFxtM, tFxntM and tFxtM. For each concentration were evaluated 15 families individually. Results and Discussion. Treatment toxicity was similar for DME and DMO flies. The amount of progeny decreased in the lower and higher concentrations assayed being the effect stronger when the females or both parents were exposed ($p<0.05$). This methodology allows the in vivo assessment of reprotoxic damage. It needs to broaden the type of disruptors assayed. Acknowledgments. To the UC San Diego *Drosophila* Stock Center for biological material; to Estefania Arroyo, Fernanda Ramírez, Estefanía García for technical support.

752B

Drosophila melanogaster as a model system to study macrophage migration inhibitory factor (MIF). Blanka Rogina[1], Tahereh Ziafazeli[1], Maria Renna[2], Danny Soares[2], Cynthia Staber[3], Richard Bucala[4], George Kuchel[2], Robert Reenan[3]. 1) Dept Gen & Dev Biol, Univ Connecticut Hlth Ctr, Farmington, CT; 2) UConn Ctr Aging, Division of Geriatric Medicine, Univ Connecticut Hlth Ctr, Farmington, CT; 3) Dept Mol Biol, Cell Biol and Biochemistry, Brown University, Providence, RI; 4) Dept Medicine, Yale Univ School of Medicine, New Haven, CT.

Increased inflammation is often associated with age-associated disorders such as Alzheimer's disease, sarcopenia, and frailty. MIF is a proinflammatory cytokine involved in variety of processes such as inflammation, glucose metabolism, and tumorigenesis. MIF counterbalances anti-inflamatory effect of glucocorticoids by increasing the expression of cytokines such as TNF-a, IL-2, IL-6 and others. High levels of MIF were found in long-lived calorie restricted, long-lived Snell dwarf and growth hormone receptor knockout mice (GHR-KO) suggesting that increased MIF levels may be beneficial. However, MIF-knockout mice are long lived illustrating complex relationship between MIF levels and longevity. Here describe how different levels of MIF mRNA affect stress resistance, longevity and metabolism by using *Drosophila melanogaster* as a model system. Fruit flies do not have MIF but have the ABC MIF transporter, and other components of MIF pathways. We established 20 stable transgenic lines that contain *Tribolium mif* gene MIF1A or MIF1B. These transgenic lines allow us to express MIF pan-neuronally, in fat bodies or in muscles by using tissue specific Gene-Switch drivers: ELAV, S^{1}106 drivers, or MHC respectively. Overexpression of MIF1B in head resulted in a small beneficial effect on fly longevity and stress resistance. However, overexpressing MIF in fat bodies has negative effect on longevity and stress resistance of flies, illustrating complex relationship between the levels and tissue of MIF mRNA expression. The preliminary data suggest that flies can be used to determine effects of ectopic expression of the MIF gene on fly intermediary metabolism in aging and under stress conditions.

753C

Sexual dimorphism for water balance mechanisms in montane populations of Drosophila kikkawai. Vineeta Sharma[1,2], Ravi Parkash[1], Bhawna Kalra[1]. 1) Genetics, Maharshi Dyanand,University, Rohtak, India; 2) Centre for Cellular and Molecular Biology,Uppal Road, Hyderabad,India.

Conservation of water is critical to the ecological success of Drosophila species living in the drier montane localities of the Western Himalayas.We observed clinal variation in desiccation resistance for both sexes of Drosophila kikkawai from an altitudinal transect (512-2226 (m).a.s.l.).Since more than 90 per cent of body water is lost through cuticular transpiration, the target of selection may be cuticular lipids or cuticular melanization. We tested whether melanic females and non-melanic males of D. kikkawai have similar mechanism of desiccation resistance.There is clinal variation in the amount of cuticular lipids per fly in males, but not in females.In contrast, for females, elevational increase in melanization is positively correlated with desiccation resistance and negatively with cuticular water loss, but there is no variation in the amount of cuticular lipids. Thus, sexual dimorphism for the mechanism of desiccation resistance in D. kikkawai matches the water proofing role of body melanization as well as cuticular lipids.

754A

A Novel p38 MAPK/Mef2/MnSOD Regulatory Mechanism in Aging and Oxidative Stress. Alysia D. Vrailas-Mortimer[1,2], Subhabrata Sanyal[1,2]. 1) Cell Biology, Emory University, Atlanta, GA; 2) Center for Behavioral Neuroscience, Atlanta, GA.

Oxidative stress has been associated with a variety of diseases including neurodegenerative disorders, inflammation, and cancer. To best understand the pathophysiology underlying oxidative stress-dependent diseases, it is essential to delineate the pathways that regulate oxidative stress. One such pathway is the p38 MAPK (p38K), which is activated in response to oxidative stress in mammals and has been linked to aging and neurodegenerative disorders. Although p38K is a bona fide stress activated protein kinase, specific stress-related signaling pathways regulated by p38K remain poorly understood. Similarly, while p38K has been linked to aging and neurodegenerative disorders, the presence of four p38K genes in the mammalian genome has complicated mechanistic investigation of p38K in these processes. Therefore, we have utilized Drosophila, which has two p38K genes (p38Ka and p38Kb) in order to explore the function of p38K, with particular emphasis on the regulation of stress and aging. Loss of p38K leads to decreased viability, increased levels of endogenous oxidative stress, shortened lifespan, and age-related locomotor behavior deficits, which can be rescued by add back of wild type p38Kb in muscle, but not neurons. Conversely, over-expression of p38K in the muscle extends lifespan and is protective against environment oxidizing agents such as Paraquat. Furthermore, we have found that p38K regulates expression of the mitochondrially localized antioxidant enzyme, MnSOD (SOD2), with loss of p38K leading to a 50% decrease in MnSOD protein levels. Furthermore, over-expression of MnSOD in the p38K mutant background rescues lifespan, while inhibition of MnSOD abolishes p38K-mediated lifespan extension. Finally, we find that p38K regulation of MnSOD is mediated by the muscle specific transcription factor Mef2 as inhibition of Mef2 leads to decreased MnSOD expression. These results suggest that p38K might regulate lifespan through the transcription factor Mef2 by modulating a SOD2-dependent oxidative stress response pathway in flies.

755B

Dose dependent stress response to high levels of Sir2 over-expression in flies. Rachel E Whitaker, Shakeela Faulkner, Reika Miyokawa, Lucas Burhenn, Will Donovan, Stephen Helfand. Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI.

It has been reported that a 10-25 fold increase in expression of dSir2 leads to cell lethality[1]. Based upon these studies it was concluded that dSir2 is highly lethal to cells and the previous beneficial effects of increased dSir2 expression were due to the simultaneous expression of the neighboring DNAJ-H gene in the particular overexpression system used[2]. We examined several new UAS-dSir2 transgenic lines with dSir2 expression of up to 360 fold over normal without developmental lethality. Despite these transgenes being in locations distant from the DNAJ-H gene, we found DNAJ-H was up-regulated as much as 5-fold when dSir2 was expressed at very high levels, but not when only moderately over-expressed. Heat shock proteins Hsp27 and Hsp70 levels were found to increase in the same pattern as DNAJ-H. This suggests that the DNAJ-H up-regulation noted by Griswold et al[1] is not related to its genetic location near dSir2, but rather is a stress response to very high levels of dSir2. Recalculation of the levels of dSir2 in the "lethal" line from Griswold et al[1] using a gene-switch inducible system which preserves the cells in which dSir2 is being expressed showed extraordinarily high levels of dSir2, at least double our highest expressing line. Additionally, utilizing the gene switch system to express the "lethal" dSir2 transgenic lines at "lower" levels we found this line also up-regulated both DNAJ-H and heat shock proteins, demonstrating that very high levels of dSir2 lead to the induction of stress responses. Our studies show that it is unlikely that dSir2 normally plays a role in regulating cell death directly in over-expression studies. Instead, extremely high levels of dSir2 over-expression are likely causing lethality due to protein stress pathways. 1. Griswold et al PNAS 2008 Jun 24;105(25):8673-8 2. Rogina et al PNAS 2004 Nov 9;101(45):15998-6003.

756C

Drosophila GAGA Factor is required for full activation of dE2F1 and Yki/Sd common targets in the wing. Battuya Bayarmagnai[1], Brandon Nicolay[1], Abul Islam[2], Nuria Lopez-Bigas[2], Maxim Frolov[1]. 1) Biochem & Mol Gen, Univ Illinois-Chicago, Chicago, IL; 2) Research Unit on Biomedical Bioinformatics, Dept of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona 08003, Spain.

The Hippo signaling pathway regulates organ size: inactivation of the Hippo pathway or hyper-activation of its most downstream effector, transcriptional co-activator Yorkie (Yki), leads to tissue overgrowth. Yki relies on its binding partners, including Scalloped (Sd), to recruit it to its target gene promoters. However, this interaction only partially explains the mechanism of function of Yki. We recently showed that Yki/Sd cooperates with dE2F1 on a common set of target genes to bypass cell cycle exit. Bioinformatics analysis suggested that GAGA Factor (GAF) may also play a role in regulating these genes. Consistently, genome-wide location analysis of promoters bound by GAF (modENCODE) revealed significant enrichment for cell cycle targets common to dE2f1 and Yki/Sd. Here we demonstrate that GAF, Yki/Sd and dE2f1, directly activate a common set of cell cycle genes. Reduction of GAF levels by RNAi lead to a smaller wing, and lower expression of the identified subset of dE2f1, Yki/Sd cell cycle target genes. Furthermore, depletion of GAF rescued a lethal phenotype induced by the overexpression of activated Yki in the wing. Consistently, Sd expression is essential for wing development. The interaction of GAF with Yki/Sd was also observed in the eye, where expression of Yki induced ectopic proliferation, and co-depletion of GAF reduced the number of interommatidial cells. GAF and RBF1, the negative regulator of dE2f1, co-localized on polytene chromosomes and bound common target gene promoters. Furthermore, a direct physical interaction was revealed in co-immunoprecipitation experiments. Therefore, we propose that GAF is required for the full activation of cell cycle targets common to dE2f1 and Yki/Sd, and suggest that GAF does so by physically interacting with RBF1, thereby limiting RBF1-mediated repression of these genes.

757A

X-Signal amplification by Runt mediated antagonism of Groucho. Sharvani Mahadevaraju, James W Erickson. Dept of Biol, Texas A&M Univ, College Station, TX.

Runt is one of the X-linked signal elements (XSEs) that signals X chromosome dose to the establishment promoter (*Pe*) of the sex determination switch gene *Sex-lethal* (*Sxl*). The mechanism of how a two-fold difference in X dose, and XSE protein concentrations, leads to the all-or-none response of *SxlPe* remains unknown. We are testing the idea that X-dose-sensitivity is achieved via a signal amplification mechanism that depends primarily on the maternal co-repressor Groucho (Gro). In the absence of Gro, *Sxlpe* is expressed nearly in direct proportion to the X chromosome dose in both sexes. Several lines of evidence led us to the hypothesis the Runt has a direct role in X signal amplification by interfering with Gro-dependent repression. Runt and other Runx family members can act as both repressors and activators. Runt has conserved DNA binding domain and a C-terminal WRPY motif that interacts with Gro co-repressor proteins. We tested the notion that Runt acts as an activator at *SxlPe* by antagonizing Gro through its WRPY motif by generating *runt* transgenes carrying runt with modified C-terminal Gro-interacting sequences. A *runt* transgene lacking the WRPY motif failed to activate *SxlPe* expression. In contrast when the WRPY domain was replaced with the more potent Gro interaction domain, WRPW, Runt's *Sxl* activation function was retained. Because Runt's XSE function at *SxlPe* may not require sequence-specific DNA binding we are testing the idea Runt can act independent of both of its normal heterodimerization partners, the Bro, or Bgb, subunits. Neither maternal, nor zygotic, *Big-brother* (*Bgb*) or *brother* (*bro*) mutants have any effect at *Sxl* activation suggesting either that the genes are redundant or unnecessary for Runt function at *SxlPe*. We are attempting to create *Bgb bro* double mutants using male recombination to determine whether either is required for *Sxl* activation.

758B

Nemo phosphorylates Eyes absent and enhances output from the Eya-Sine oculis transcriptional complex during Drosophila retinal determination. Santiago A. Morillo[1], Lorena Braid[2], Esther M. Verheyen[2], Ilaria Rebay[1,3]. 1) Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL; 2) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 3) Ben May Dept for Cancer Research, University of Chicago, Chicago, IL.

The retinal determination gene network comprises a collection of transcription factors that respond to multiple signaling inputs to direct Drosophila eye development. Previous genetic studies have shown that *nemo*, a gene encoding a proline-directed serine/threonine kinase, can promote retinal specification through interactions with the retinal determination gene network, although the molecular point of cross-talk was not defined. We report that the Nemo kinase positively and directly regulates Eyes absent (Eya). Genetic assays show that Nemo catalytic activity enhances Eya-mediated ectopic eye formation and potentiates induction of the Eya-Sine oculis (So) transcriptional targets *dachshund* and *lozenge*. Biochemical analyses demonstrate that Nemo forms a complex with and phosphorylates Eya at two consensus mitogen-activated protein kinase (MAPK) phosphorylation sites. These same sites appear crucial for Nemo-mediated activation of Eya function in vivo. We propose that Nemo phosphorylation of Eya potentiates its transactivation function to enhance transcription of Eya-So target genes during eye specification and development.

759C

Involvement of Polycomb/Trithorax group proteins in the regulation of the sex determination master switch, *Sex-lethal*. Janel Rodriguez, Jamila Horabin. Biomedical Sciences, Florida State University, Tallahassee, FL.

In *Drosophila*, one of the earliest developmental decisions made is that of determining sex. Key to the process of sex determination is the regulation of the X chromosome sensing promoter of the master switch *Sex-lethal* (*Sxl*), at the establishment promoter Sxl_{Pe}. Sxl_{Pe} is only activated in female embryos resulting in SXL protein. Splicing of the late *Sxl* transcripts from the maintenance promoter, Sxl_{Pm}, is then switched by SXL from the default male mode into the female mode, to bypass inclusion of the male specific exon which prematurely truncates the translation open reading frame. Our lab has previously shown that heterochromatin proteins are required for proper Sxl_{Pe} regulation. In analyzing the role of these proteins on the sex determination decision, we have found that the Polycomb/Trithorax (Pc/Trx) group proteins also have an influence. Pc/Trx group proteins are chromatin-modifiers, known regulators of gene expression. We have investigated the role of enhancer of zeste (E(Z)), absent, small or homeotic discs 1 (ASH1) and the heterogeneous nuclear ribonucleoprotein *Drosophila* dodeca satellite protein 1 (DDP1) in regulating Sxl_{Pe}. DDP1 is known to regulate gene expression by interacting with heterochromatin protein 1 (HP1) in an RNA dependent manner. We observe that E(Z), ASH1 and DDP1 are necessary for proper Sxl_{Pe} expression in female embryos. Using chromatin immunoprecipitation (ChIP) assays we also observe that these proteins are necessary for proper histone 3 lysine 4 (H3K4) and

histone 3 lysine 9 (H3K9) methylation at the promoter. Using ChIP analysis we find that ASH1 protein associates with Sxl_{Pe} sequences, placing it at the promoter during Sxl_{Pe} expression. Our data suggest that these proteins modify the epigenetic environment at Sxl_{Pe} to ensure proper regulation of the sex determination process in males and females.

760A

Retinal determination factor Eyeless and type I bHLH protein Daughterless directly induce onset of *atonal* expression and synergistically induce retinal development. Miho Tanaka-Matakatsu, Wei Du. Ben May Dept Cancer Res, Univ Chicago, Chicago, IL.

Induction of the proneural gene *atonal* (*ato*) expression is a key step for the retinal progenitor cells to initiate photoreceptor neuron differentiation. Retinal determination factor Eyeless (Ey) has been shown to directly activates the *ato* early eye enhancer, *ato-3'*, through a conserved Ey binding site. The initiation of photoreceptor differentiation and *ato-3'* expression are negatively regulated by EMC, which functions by binding to the type I bHLH transcription factor Daughterless (Da). We show that Ey and high level of Da overlap near the Morphogenetic Furrow (MF) region and that *da* mutant clones spanning the MF exhibit delayed onset of the *ato-3'* enhancer activation while Da over-expressing clones near the MF show precocious activation. Furthermore, expression of Da and Ey in wing tissues synergistically activates the *ato-3'* enhancer and increases the ectopic eye sizes, suggesting that Ey and Da synergistically activates the *ato-3'* enhancer and controls proper timing of photoreceptor cell differentiation during eye development. To dissect the mechanisms involved in the synergistic activation, we found that the Ey site in *ato-3'* enhancer overlaps with an E box binding site. Mutations that disrupt either the Ey binding site or the E box binding site impairs the activity of *ato-3'* enhancer, indicating both Ey and Da-Da are required for the full *ato-3'* enhancer activity. Furthermore, the observed interactions between Ey and Da are conserved in mammalian systems and that observed effect of Da is likely mediated by Da-Da homodimer since linked Da homodimer but not linked Da heterodimer is functional. We will present a model by which the retinal determination factors and the bHLH family of proteins coordinate to control the onset of photoreceptor differentiation.

761B

Transcriptional and Metabolic Adaptation to Hypoxia is Driven by HIF-Independent Actions. Keith D. Baker[1], Yan Li[1], Divya Padmanabha[1], Luciana B. Gentile[1], Catherine I. Dumur[2]. 1) Biochemistry and Molecular Biology, VCU School of Medicine, Richmond, VA; 2) Pathology, VCU School of Medicine, Richmond, VA.

Hypoxia-inducible factors (HIFs) are the vanguard of the transcriptional response to hypoxic conditions, where they direct oxygen-sensitive changes that allow for homeostatic adaptation. While it is assumed that HIF-mediated actions shift metabolic strategy to allow for adaptation, the contribution that HIFs provide has not been well examined. Here, we examine hypoxic responses in wild-type and *dHIF* mutant animals using transcriptional and metabolic profiling and provide integrated views of the transcripts and metabolites that change in response to hypoxia in *Drosophila*. Unexpectedly, we find dHIF has a greater impact on metabolism in normoxia than in hypoxia, and that a majority of hypoxia-induced metabolic changes still occurs in *dHIF* mutants. Consistent with the dissociative nature of HIF from hypoxia-induced shifts, we show that dHIF-independent actions are responsible for inducing glycolytic transcripts. Our analysis also reveals distinct roles of dHIF-dependent and -independent activity on various aspects of adaptive metabolism. These data suggest that low oxygen conditions trigger different response pathways, with the driver of metabolic adaptation being dHIF-independent responses.

762C

The *Drosophila melanogaster* gene *tfiia-s-2* encodes a male germline-expressed homolog of the small subunit of the TFIIA general transcription factor. Amory Brandt, Cameron Jernigan, Margaret Wood, Cynthia Cain, Mark Hiller. Biological Sciences, Goucher College, Baltimore, MD.

Eukaryotic general transcription factors are protein complexes that help position RNA polymerase at promoters and initiate transcription. The general transcription factors TFIIA and TFIID assemble on promoters early in the process of transcription initiation. TFIIA consists of three protein subunits. In *D. melanogaster*, a single gene, *tfiia-l*, encodes a 48 kD polypeptide that is proteolytically cleaved to form two proteins of 30 kD and 20 kD. *tfiia-s* encodes the small subunit of 14kD. We have shown that a homolog of the small subunit, *tfiia-s-2* (CG11639), is expressed only in the male germline. We have also shown that two transcripts are encoded by *tfiia-s-2*, and that both proteins are able to interact with the generally expressed large subunit when expressed in *E. coli*. Together, this suggests that three different forms of TFIIA might be present in the male germline. TFIID is comprised of TATA-binding protein (TBP) and up to fourteen TBP-associated factors (TAFs). Several testis-specific TAFs have been identified, and mutations in the testis-TAFs cause defects in transcription and block spermatid differentiation. It is possible that complexes containing testis-TAFs and testis-specific TFIIA-S-2 function together to regulate gene expression in the testis. We are examining this possibility by making a mutation in the *tfiia-s-2* gene by homologous recombination in order to examine the phenotype of a *tfiia-s-2* mutation. We are also characterizing the ability of complexes containing TFIIA-S-2 to physically associate with TFIID subunits and testis-expressed homologs of TFIID subunits.

763A

The investigation of a novel Y chromosome specific gene in *Anopheles stephensi*. Frank Criscione, Yumin Qi, Zhijian Tu. Department of Biochemistry, Virginia Tech, Blacksburg, VA.

Anopheles mosquitoes are the main malaria vectors and their sexual determination is controlled by a dominant male-determining factor on the Y-chromosome. However, the properties of this Y-chromosome factor responsible for the initiation of the sexual determination cascade have yet to be elucidated, but would provide strong support to genetic strategies of vector population control. We use *Anopheles stephensi*, one of the main mosquito vectors of the malaria parasite in India and the Middle East, as a model to study Y-specific genes that may be involved in sex determination. Using next-gen sequencing and a comparative bioinformatic approach, we identified a small number of Y-chromosome gene candidates and confirm localization to the Y chromosome. This study focuses on male-specific gene 1(MSG1) that is transcribed at the very onset of the maternal-zygotic transition and expressed solely in the early embryo. Using RT-PCR, we confirmed the expression pattern of this gene. We hypothesize that MSG1 is the male-determining factor. Biological function of MSG1 is being tested through the use of both transient and transgenic siRNA and ectopic expression of MSG1.

764B

The transcription factor network patterning Drosophila photoreceptors. Hui-Yi Hsiao, Robert Johnston, Dave Jukam, Claude Desplan. Dept Biol, New

York Univ, New York, NY.

In the Drosophila eye, each ommatidium contains eight photoreceptors (PRs) arranged in a trapezoid shape. PR cell fates are specified at late larval stages. After their recruitment, they are defined as outer PRs (R1-R6) vs. inner PRs (R7 and R8), which are located at the center of the trapezoid formed by the outer PRs. Then, during late pupal stages, all PRs undergo terminal differentiation and express specific Rhodopsins (Rh). Even though outer PRs all express Rh1, each of them can be distinguished by a unique transcriptional profile. Although some of the genetic programs controlling outer/inner fate determination are well characterized, many regulatory steps remain unclear. To address the underlying mechanisms, we performed an RNAi screen knocking down all known transcription factors (~950 TFs) and using rh1-GFP to visualize patterning phenotypes of PRs in the adult eye. The expression of UAS-RNAi is controlled spatially and temporally by a combination of two eye-specific GAL4 drivers, ey-Gal4 and lGMR-Gal4.By using rh1>GFP as a readout, couple of genes has been found with a mutant cell fate phenotype showing extra rh1-expressing PRs. Inner rhodopsins are normally expressed in the ectopic rh1-GFP expression PR suggests those candidates function in repression of rh1 in inner PRs rather than in the determination of inner/outer fate. Further study shows that only de-repression of rh1-GFP has been detected in mutant candidates rather than Rh1 protein. The result leads to the explanation that rh1 might be post-transcriptionally regulated.

765C

Characterizing the Transcriptional and Metabolic Response to Hypoxia in *Drosophila Melanogaster*. Yan Li[1], Catherine Dumur[2], Keith Baker[1]. 1) Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA, 23298; 2) Department of Pathology, Virginia Commonwealth University, Richmond, VA, 23298.

Low oxygen tension plays important roles in normal and pathological conditions, including tumorigenesis. The cellular response to hypoxia shifts metabolic homeostasis at the transcriptional and post-transcriptional level. Hypoxic adaptation is thought primarily to proceed through the conserved hypoxia-inducible factor 1(HIF-1), which is a member of the PAS domain family. Although HIF-1 biology has been examined extensively, HIF-mediated metabolic transitions have never been characterized. In this study, we examine the response to low oxygen conditions in *Drosophila*. We measure the global temporal-dependent changes in transcripts and metabolites in 4% hypoxia in wild type or *hif-1* mutants using microarray analysis and GC-MS and/or LC/MS/MS. Unexpectedly, we find that in hypoxia the dHIF-independent pathway is responsible for the transcriptional induction of glycolytic enzymes. Furthermore, the majority of hypoxia induced changes of intermediary metabolites still occurred in the absence of dHIF. We find that dHIF has a greater impact on metabolism in normoxia than in hypoxia. Our data suggest that low oxygen induces both dHIF dependent and dHIF independent actions, which elicit compensatory adaptation responses that impact transcription and metabolism. We conclude that dHIF-independent actions drive hypoxic transitions.

766A

Examining the role of EcR binding sites on ecdysone inducible polytene chromsome puffs. Alexander D. Ostapenko, Rebecca F Spokony, Dmitri Novikov, Kevin P. White. Institute for Genomics & Systems Biology, University of Chicago, Chicago, IL.

The steroid hormone ecdysone plays a crucial role in the development of insects. In *Drosophila melanogaster*, ecdysone pulses regulate the developmental transition of both molting and metamorphosis by inducing genome-wide changes in gene expression. The ecdysone response can be visualized by puff formation on polytene chromosomes from late third instar salivary glands. The *Ecdysone Receptor* (*EcR*) has been shown to be required for this response, but it is not yet known which EcR binding sites are necessary and sufficient for puff formation and gene regulation. We hypothesized that EcR binding sites contained within ecdysone responsive puff regions are necessary and sufficient for the puff response. We studied the ecdysone response by visual examination of the puffing on polytene chromosomes. Our results indicate that EcR and its binding sites are required for puff formation. Using immunohistochemistry on polytene squashes we confirmed that EcR binds to previously described ecdysone inducible puff regions. By inactivating EcR with a heat-shock inducible RNAi, we produced alterations within the polytene structure as and confirmed a reduction of puff sizes at the primary ecdysone response loci *74EF*, *75B*, and *Broad-Complex*. Using BAC recombineering, we generated transgenic fly lines with insertions of eGFP-tagged ecdysone-inducible genes containing several EcR binding sites in large BACS at attP sites on chromosomes complementary to their endogenous locations. We found that *74EF* and *EcR* genomic regions are sufficient to induce ectopic puffs. We confirmed EcR binding to both ectopic and endogenous puffs using immunohistochemistry. We used Chromatin Immunopreciptiation and Sequencing to finely map EcR binding in these regions. We are currently testing whether these binding sites are necessary and sufficient for puffing by using BAC recombineering techniques to delete EcR binding sites from the ectopic puff-inducing loci, as well as inserting EcR binding sites (without the coding regions) into the attP sites and looking for a reduction or increase in puffing, respectively.

767B

Molecular analysis of 5' regulatory region of *Lim3* locus associated with *D. melanogaster* lifespan control. Olga Y. Rybina, Elena G. Pasyukova. Inst Molec Gen RAS, Moscow, Russian Federation.

Lim3 encodes an RNA polymerase II transcription factor with a key role in neuron development and specification. It was also identified as a candidate gene that affects lifespan. These pleiotropic effects indicate the fundamental significance of the potential interplay between neural development/functioning and lifespan control. The goal of this study was to analyze the causal relationships between *Lim3* structural variations, and gene expression and lifespan changes. *Lim3A*, a transcript of *Lim3* locus, was shown to be functional during *Drosophila* neuron development. We sequenced a 2092 bp DNA fragment including 5' regulatory region of *Lim3A* in fifty *Drosophila* lines containing second chromosomes from Raleigh natural population. Five polymorphic markers located within 380 to 680 bp upstream of the *Lim3A* transcription start sites (TSS) were significantly associated with the amount of *Lim3A* transcript, as evaluated by real time RT-PCR. Two of these five markers formed a haplotype which was also significantly associated with lifespan. Haplotype variations could cause a six-fold change in gene transcription and a 25% change in lifespan. Several significant markers were located in binding motifs of Polycomb/Trithorax group proteins (Rybina, Pasyukova, 2010). The DNA region located within 380 to 680 bp of the *Lim3A* TSS appeared to be very conservative throughout *D. melanogaster* group, in accordance with low sequence variation of this region observed in Raleigh population. To evaluate the role of different parts of *Lim3A* regulatory region in *Lim3A* transcription we used reporter constructions containing the firefly luciferase gene under the control of fragments of *Lim3A* regulatory region of different length. The deletion of the whole region located within 380 to 680 bp of the *Lim3A* TSS provided two fold decrease of expression of the reporter construct in S2 *Drosophila* cell culture. Partial deletions of this region affected the reporter gene

expression in various degrees. These data directly prove that this part of *Lim3A* regulatory region is significant for *Lim3A* transcription control.

768C

Sequential activation of Pointed isoforms during eye development amplifies EGFR signaling. Arkadi Shwartz, Eyal D Schejter, Ben-Zion Shilo. Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

EGFR signaling in flies and in vertebrates involves activation of MAP kinase (MAPK), and culminates in triggering of ETS-domain transcriptional activators, which in *Drosophila* is executed by Pointed (Pnt). The *pnt* gene harbors two promoters, and generates two alternative transcripts, *pntP1* and *pntP2*, which are under distinct modes of regulation. Transcription of *pntP1* is induced by an unknown MAPK-dependent activator, and PntP1 protein activates transcription constitutively. PntP2 protein, on the other hand, is directly activated by MAPK phosphorylation, but its transcription is EGFR independent. We have undertaken a detailed dissection of this process during *Drosophila* imaginal eye disc development. Full disruption of *pnt* function revealed a photoreceptor recruitment phenotype, in which only R8 cells are present in the ommatidia of newly developed eye discs. Generation of flip-out mutant clones for each isoform alone showed the same phenotype as complete *pnt* disruption, thus demonstrating that each isoform is essential for photoreceptor recruitment. Further analysis showed that the Pnt isoforms are activated in a sequential manner: MAPK activates PntP2, which is only capable of inducing *pntP1* transcription. Once expressed, PntP1 is sufficient to induce target genes essential for photoreceptor development. The induction of EGFR-target genes may thus be sustained by PntP1 activity, beyond the time window where local MAPK activity is triggered by EGFR.

769A

Identification of a *wingless* pair rule response element. Kimberly Bell[1,2], Kevin Chen[1], J. Peter Gergen[1]. 1) Department of Biochemistry and Cell Biology and the Center for Developmental Genetics, Stony Brook University, Stony Brook, NY; 2) Graduate Program in Genetics, Stony Brook University, Stony Brook, NY.

Prior studies on early transcriptional regulation in the Drosophila embryo identified two distinct pair rule response elements for the segment polarity gene *sloppy-paired-1* that interact in a non-additive manner to establish the initial striped expression pattern. These two elements are separated by more than 4 kb of intervening DNA, suggesting that long distance non-additive enhancer interactions may be a common phenomenon in the pair rule to segment polarity transition, and transcription regulation in general.

It was previously demonstrated that a 4.5kb region upstream of transcription unit of the segment polarity gene *wingless (wg)* drives expression in odd numbered stripes with very weak expression of the even number stripes. The ChIP-on-Chip data from the University of California Berkeley Drosophila Transcription Network Project identifies two regions within the *wg* locus that show association with four different pair rule transcription factors. One of these regions spans from 3.9 kb to 1.1 kb upstream of the transcription start site and the other is located within an intron. Here we demonstrate that reporter gene constructs containing the upstream 2.8kb region, in either orientation show robust expression that mimics the early striped pattern of *wg*. Both loss and gain of function experiments demonstrate that this region is a bona fide pair-rule response element. Reporter expression is observed to come on earlier than endogenous *wg*, and in some mutant backgrounds there are clear differences in the expression of the *lacZ* and endogenous *wg* mRNAs. We propose that non-additive interactions between this upstream enhancer and other cis-regulatory sequences from the *wg* locus, potentially the putative pair-rule response element within the intron, are responsible for generating the full *wg* pattern.

770B

***EvoPrinter* and *cis*-Decoder Facilitate Analysis of Enhancer Structure.** Thomas Brody, Alexander Kuzin, Mukta Kundu, Jermaine Ross, Ward F. Odenwald. Neural Cell-Fate Determinants, NINDS/NIH, Bethesda, MD.

We have developed two computer algorithms to discover and analyze cis-regulatory sequences. The phylogenetic footprinting program *EvoPrinter* identifies conserved sequence clusters (CSCs); functional analysis reveals that many of these serve as cis-regulatory modules. *cis*-Decoder identifies both unique and repeat sequence elements that are shared among CSCs and are essential for enhancer function. We have identified several enhancers that consist of multiple sub-modules that function semi-autonomously to drive expression in a sub-pattern of the total expression profile of the CSC. Analysis of a *castor* NB enhancer, *cas-6*, reveals that it consists of two sub-clusters separated by 250 bp of less conserved DNA. *cis*-Decoder reveals several conserved octamer sequences in one of the sub-regions. One sub-region drives expression in a single pair of NBs per segment and a second, containing the octamer repeats and a Single-minded site, drives expression in a single ventral midline NB. The entire enhancer expresses both in the midline and in a larger set of NBs, indicating that the two halves can function semi-autonomously, but that both are necessary for the full biological function of the enhancer. Similarly, we have found that another *cas* temporal network NB enhancer consists of three semi-autonomous modules that drive expression in a sub-pattern of the total expression profile of the intact enhancer. Finally, one of the *D. melanogaster* NB enhancers for late temporal network determinant *grainyhead* appears in several other *Drosophila* species as two separate CSCs. We tested these two regions, and found that one, containing three well-conserved POU transcription factor binding sites, expresses in embryonic and larval brain precursors, while the second, containing two identical conserved novel 12mer sequences, expresses in ventral cord NBs but does not express in larval precursors. We conclude that combined *EvoPrinter* and *cis*-Decoder analysis can reveal sub-modules within CSCs and therefore prove of use in investigating the integrity of CSCs and the evolution of cis-regulatory DNA.

771C

A machine learning approach for identifying novel cell type-specific transcriptional regulators of myogenesis. Brian Busser[1], Leila Taher[2], Yongsok Kim[1], Terese Tansey[1], Ivan Ovcharenko[2], Alan Michelson[1]. 1) National Heart Lung and Blood Institute, Bethesda, MD; 2) National Library of Medicine, Bethesda, MD.

A complete understanding of the structure and function of transcriptional enhancers that are active in related cell types requires identification of the sequence features to which co-regulatory transcription factors (TFs) bind. To address this problem, we developed a computational approach that profiles the TF binding sites (TFBSs) governing the transcription of a set of co-expressed genes and applied it to discover novel components of the transcriptional regulatory network controlling myoblast differentiation. Our approach involved assembling a small number of enhancers with activity in somatic muscle founder cells (FCs). We then used evolutionary profiling to increase the size of this enhancer set by incorporating orthologous but diverged sequences from other *Drosophila* species. Putative enhancer orthologs were found to be active in largely similar patterns as their *D. melanogaster* counterparts, even though

there was extensive evolutionary shuffling of known TFBSs. We next built and trained a classifier to identify other functionally related enhancers based on the presence or absence of known and putative TFBSs. Predicted FC enhancers were over-represented in proximity to known FC genes, and the presence of a candidate enhancer was predictive of expression of the associated gene in FCs. In addition, we found that many of the TFBSs learned by the classifier are critical for activity of FC enhancers, including those for POU homeodomain, Myb, Ets, Forkhead and T-box TFs. In particular, we used gene co-expression, cis-trans tests of enhancer function and embryo RNAi to show that the T-box protein encoded by *optomotor-blind-related-gene-1* is a previously uncharacterized regulator of muscle cell identity. In summary, machine learning combined with evolutionary sequence analysis can be used to recognize novel TFBSs and to facilitate the identification of cognate *trans*-acting factors that coordinate cell type-specific developmental gene expression patterns.

772A

Regulatory DNA of the engrailed and invected genes. Yuzhong Cheng, Judith Kassis. Program in Genomics of Differentiation, NICHD, Bethesda, MD.

engrailed (en) and invected (inv) form a gene complex that extends about 100kb. These two genes encode highly related homeodomain proteins that are co-expressed in a complex manner throughout development and are co-regulated by enhancers that stretch over a 70 kb region. en/inv are co-regulated by the Polycomb group genes (PcG), and the entire en/inv domain is covered with tri-methylated histone H3 (H3K27me3), the distinctive mark of PcG-regulated genes. In addition, en and inv both have Polycomb-response elements (PREs), DNA elements that bind PcG-proteins. We would like to understand how PREs act with en/inv enhancers to regulate en/inv expression. In order to do this, we need to identify the en/inv enhancers. We used two types of constructs to identify en/inv enhancers: P-element based reporter constructs with small pieces of en/inv DNA fused to the en promoter driving the expression of lacZ and large constructs using the phiC31 system and HA-tagged en and inv. We report two important findings. First, the sum of the parts is not equal to the whole; that is, some of the enhancer activities we see in the small constructs are not recapitulated in the large constructs. Second, imaginal disk enhancer activity was not found in any small reporter construct, but is present in a 45kb-en-HA transgene. We suggest that the activity of the imaginal disk enhancer depends on two or more DNA regulatory elements and that chromatin structure may also play a role in its activity.

773B

A Common Sequence Motif Regulates *broad* and *pipe* Expression in Response to EGFR Signaling. Lily S. Cheung[1,2], Alisa Fuchs[3], Enrica Charbonnier[3,4], Stanislav Y. Shvartsman[1,2], George Pyrowolakis[3,4]. 1) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544; 2) Department of Chemical and Biological Engineering, Princeton University, Princeton NJ 08544; 3) Institute for Biology I, Faculty of Biology, Albert-Ludwigs-University of Freiburg, Hauptstrasse 1, 79104 Freiburg, Germany; 4) BIOSS Centre for Biological Signalling Studies, Albert-Ludwigs-University of Freiburg, 79104 Freiburg, Germany.

Activation of the *Drosophila* Epidermal Growth Factor Receptor (EGFR) homolog by Gurken, a ligand secreted from the oocyte, determines the dorso-ventral (DV) axis of the follicular epithelium and the future embryo. Although multiple transcription factors involved in EGFR gene regulation in the follicle cells have been characterized, the regulatory DNA sequences that interpret these factors remained unknown. In order to define *cis*-elements responsive to EGFR signaling, we undertook an unbiased reporter analysis to identify regulatory sequences responsible for the regulation of *broad*, an EGFR target crucial for the formation of dorsal respiratory structures in the eggshell. We found that the dynamic pattern of *broad* is regulated by two non-overlapping enhancers, which are differentially regulated by the Iroquois transcription factor Mirror. We show that Mirror directly binds one of the enhancers through a non-canonical binding site, and that this sequence is conserved in *pipe*, the first component in the cascade that will determine the DV axis of the embryo. We further show that this sequence is sufficient to account for the cell autonomous repression of *pipe* by Mirror *in vivo*. Our work establishes Mirror as a key effector of the EGFR pathway, and provides one of the first regulatory sequences controlling patterning during oogenesis.

774C

An In Vivo Titration of Transcription Factors in the Drosophila Embryo. Matthew D. Davis[1], Michael B. Eisen[2]. 1) Department of Molecular and Cell Biology UC Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute Department of Molecular and Cell Biology UC Berkeley, Berkeley, CA.

The DNA binding domains of transcription factors bind their ligands with varying affinity depending on the sequence of the ligand. The thermodynamic relationship between these molecules demonstrates classic cooperative Hill kinetics in vitro. In vivo, transcription factors bind their targets with quantitatively varying specificity that is not well-explained by sequence affinities measured in vitro. Thus, the thermodynamic relationship established in vitro is poorly understood in vivo, where other proteins and higher order interactions are presumably prevalent. We have titrated the in vivo concentration of the transcription factors bicoid and Kruppel and measured their relative DNA binding with chromatin immunoprecipitation followed by short-read sequencing. These experiments allow us to assess which sites in the genome are saturated by these transcription factors when they act in the early embryo, and which sites are susceptible to differential binding as concentration varies. Similarly, it is unknown if increased activator or repressor bound to an enhancer region will affect the output of the target promoter. To address this question, we have collected commensurate RNA sequencing and conofcal microscopy data in these lines.

775A

The *Drosophila Niemann-Pick Type C-2 (NPC2c)* gene is a direct target of VP16-DHR96 protein. Niloofar Farboodi. Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

Excess cholesterol is associated with pathogenesis of cardiac and vascular diseases, diabetes and cancer. However, cholesterol is also essential for proper functioning and structure of animal cells and therefore its concentrations have to be highly regulated. In *Drosophila*, this regulation occurs in part by a nuclear receptor called *Drosophila hormone receptor 96* (*DHR96*). *DHR96* binds cholesterol *in vivo* and acts as a cellular cholesterol sensor. We recently showed that *DHR96*^{1} mutants fail to survive on a low cholesterol diet, while control flies develop normally. Thus, it is presumed that DHR96 functions by protecting cellular cholesterol levels from dropping below a critical threshold. Consistently, genes with roles in cholesterol metabolism and trafficking are not properly regulated in *DHR96*^{1} mutants. It is unclear; however, which of these genes are direct targets of DHR96 and what DNA sequences are recognition sites of this nuclear receptor. To approach this question, transgenic lines expressing *DHR96* fused to the activation domain of *VP16* were generated. We predicted that direct target genes of DHR96 would be significantly induced as a result of VP16-mediated activation. *ACAT* and *NPC2c* were the two genes with strong upregulation in their transcript levels. *NPC2c*, whose mammalian ortholog has known roles in intracellular cholesterol trafficking,

was further examined to identify functional elements required for transcriptional activation. In a transgenic approach, a 1.7 kbp fragment upstream of *Npc2c* resulted in a 10-fold induction in a *lacZ* reporter gene, suggesting a regulatory role for this region. Chromatin immunoprecipitation was performed to identify VP16-DHR96 DNA binding sites upstream of *NPC2c*. The highest enrichment level was found at about 2.6 kbp upstream of *Npc2c* transcription start site; however, regions closer to this transcription start site were also enriched in the chromatin immunoprecipitate. These data demonstrate that VP16-DHR96 fusion protein binds *NPC2c* upstream sequences and consequently nominate this gene as a direct target of DHR96 *in vivo*.

776B

***REDfly:* The Regulatory Element Database for *Drosophila*.** Marc S. Halfon[1,2,3,4], Steven M. Gallo[2,5], Michael Simich[1,2], Benjamin Des Soye[1,2], Casey M. Bergman[6]. 1) Department of Biochenistry, 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, Cellular, & Developmental Biology Department, Roswell Park Cancer Institute, Buffalo, NY; 5) Center for Computational Research, SUNY at Buffalo, Buffalo, NY; 6) Faculty of Life Sciences, University of Manchester, Manchester, UK.

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 any sequence reported as functionally tested in a transgenic reporter gene assay regardless of whether it showed regulatory activity or has activity redundant with other, shorter regulatory sequences. Graphical views show the position of each CRM within its genomic locus, the location of each CRM with respect to its associated gene is provided, and conservation of local synteny between CRMs and their target genes across nine species of *Drosophila* is assessed. Curation of TFBSs includes sites identified by electrophoretic mobility shift assay (EMSA, “gel shift”) and DNAase I footprinting. Extensive abilities exist for database searching and results filtering. We have undertaken a major increase in curation activity over the past year: over 40% of records have been added within the past six months, including over 1400 new reporter construct entries and over 400 new TFBS entries. In all, REDfly contains more than 4250 records of reporter constructs and TFBSs drawn from over 560 publications. 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.

777C

Cis-regulatory contributions to the regulation of sloppy-paired-1 transcription initiation and elongation. Saiyu Hang, J. Peter Gergen. Biochemistry and Cell Biology and the Center for Developmental Genetics, Stony Brook University, Stony Brook, NY.

The Drosophila segmentation pathway provides a valuable system to study the in vivo mechanisms of transcription regulation. The expression of sloppy-paired-1 (slp1) in the gastrula stage embryo is controlled by the interaction of 4 transcription factors Runt, Eve, Ftz, and Opa with 2 cis-regulatory elements DESE and PESE. Both of the enhancers can be repressed by Runt, whereas only DESE mediates Runt-dependent activation. Runt and Opa activate DESE in cells in the posterior half of odd-numbered parasegments, while Runt and Ftz repress DESE in cells in the anterior half of the even-numbered parasegments. Using site-specific transgenesis and a number of reporter constructs, I found DESE facilitates pre-initiation complex formation at the promoter and that DESE-dependent activation is influenced by the extent of promoter proximal DNA upstream of the transcription start site. Chromatin IP experiments that compare activated versus repressed states of slp1 as well as a DESE-lacZ reporter gene indicate that Runt and Ftz repress expression by blocking the elongation step of the transcription cycle which involves the regulated association of the elongation factor P-TEFb and phosphorylation of Ser2 in the C-terminal domain of RNA polymerase II.

778A

Overlapping but distinct roles for Odd-paired and Unpaired in transcription activation in the Drosophila blastoderm embryo. Michael L. Higgins[1,2], Liujing Xing[1], J. Peter Gergen[1]. 1) Department of Biochemistry and Cell Biology and the Center for Developmental Genetics, Stony Brook University, Stony Brook, NY; 2) Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, NY.

The Drosophila sloppy-paired-1 (slp1) gene provides an attractive model for investigating the mechanisms of regulation by the primary pair-rule transcription factor Runt. The slp1 expression pattern consists of 14 two-cell wide stripes in the posterior half of each parasegment in the early Drosophila embryo. Runt works with the Zn-finger transcription factor Odd-paired (Opa), to activate the odd-numbered stripes, but the factor responsible for activation of the even-numbered stripes is not yet established and has been referred to as Factor X. We present genetic experiments indicating that both Opa and D-Stat, a transcription activator in the Drosophila JAK-STAT pathway, contribute to Factor X activity. The changes in expression of slp1 and different slp1-lacZ reporter genes in embryos that are mutant for opa alone, unpaired (unpaired encodes a ligand that activates the JAK-STAT pathway) alone, and embryos doubly mutant for these two factors reveal that Opa plays a major role in activating both even-numbered and odd-numbered stripes whereas JAK-STAT signaling results in activation of the even-numbered stripes when opa is not present. We have identified DESE and PESE as two distinct pair-rule response elements of slp1, both of which are capable of generating the even-numbered stripes. Interestingly, only DESE mediates activation in response to JAK-STAT signaling. We will present results suggesting that JAK-STAT dependent activation of DESE is Runt-independent, and that the Runt-dependent activation of DESE occurs via a distinct and mutually exclusive pathway. We hope that defining the specific roles of Runt, Opa and D-Stat in slp1 activation will provide a foundation for future studies that are relevant to understanding the roles of homologs of these transcription factors in human development and disease.

779B

A systems-level analysis of *giant* regulation in *Drosophila melanogaster*. Astrid Hoermann, Damjan Cicin-Sain, Hilde Janssens, Johannes Jaeger. EMBL/CRG Research Unit in Systems Biology, Centre de Regulació Genòmica, 08003 Barcelona, Spain.

Eukaryotic transcription is very complex, and we are far from a satisfactory biochemical understanding of it. We aim to use data-driven mathematical modeling to investigate how different binding sites form a regulatory element, and how these elements then together establish the expression pattern of the endogenous gene. These questions are addressed by quantitative analysis and mathematical modeling of the expression pattern of the gap gene *giant* (*gt*) in the *Drosophila melanogaster* blastoderm. It is expressed in a posterior and an anterior domain, which refines into two stripes over time, and finally also

expression at the anterior tip is visible. The regulatory region of *gt* contains binding sites for the maternal activators Bicoid and Cadual, and repressor sites for other gap genes, such as Krueppel and Hunchback. Several partially overlapping *cis*-regulatory elements (CRE) for *gt* were previously predicted through detection of clusters of binding sites. I quantified their expression patterns with a high-throughput method for extracting concentrations from confocal microscope images. These data will be fed into a mathematical model of transcriptional control in order to predict spatio-temporal expression patterns of CREs from sequence. The model gives the combinations of binding sites required for correct expression as output. It can predict expression patterns for all kinds of modifications and combinations within the regulatory regions, and the most interesting ones will then be verified *in vivo* with constructs harbouring mutations in binding sites, or by testing a CRE in a mutant background lacking a certain transcription factor.

780C

Decoding transcriptional control at the IAB7b *cis*-regulatory module in the bithorax complex. Jessica S. Kurata, Michael J. Nevarez, Robert A. Drewell. Harvey Mudd College, Claremont, CA.

The body plan for *Drosophila* is determined in early development by the spatially-regulated expression of homeotic (Hox) genes. This spatial patterning is itself dictated by the distribution of transcription factors (TFs) in the embryo and controlled primarily through enhancer *cis*-regulatory modules (CRMs). The 330 kb bithorax complex (BX-C) contains three Hox genes: ultrabithorax (*ubx*), abdominal-A (*abd-A*), and Abdominal-B (*Abd-B*). Expression of each gene in segments along the anteroposterior axis is controlled by CRMs in the neighboring infraabdominal (*iab*) regions.

The IAB7b enhancer is responsible for the expression of *Abd-B* in the seventh abdominal segment (A7) of the *Drosophila* embryo. Investigation of the IAB7b enhancer has revealed an evolutionarily conserved signature motif, consisting of a cluster of two FUSHI-TARAZU (FTZ) and two KRUPPEL (KR) TF binding sites. The signature motif, when isolated from the rest of the IAB7b enhancer, drives reporter gene expression in A5, A7, and A9. We examined the role of the transcriptional repressor KNIRPS in restricting the IAB7b-directed expression pattern and investigated the functional importance of spacing between the two FTZ sites.

781A

Spatial regulation of *achaete* via global activation and repression by Hairy and Delta. Ji Inn Lee, Meghana Joshi, Teresa Orenic. Dept Biological Sci, Univ Illinois, Chicago, Chicago, IL.

During vertebrate and invertebrate development, organs and tissues must be precisely patterned and periodic proneural gene expression is an early and essential event in neuronal pattern formation. The *Drosophila melanogaster* sensory bristles are a good model system to study the molecular mechanisms involved in the precise proneural gene expression. There are two classes of sensory bristles: early specified (mechanosensory macrochaetae and chemosensory microchaetae) and late specified (mechanosensory microchaetae) bristles. Previous studies suggest that patterning of early specified bristles requires induction of the proneural gene expression at specific epidermal locations: proneural gene expression in primordia of early specified bristles is controlled by discrete modular *cis*-regulatory elements (CRE) (Campuzano and Modolell 1992; Gomez-Skarmeta et al., 2003). Our studies, however, suggest that a different mechanism is used to pattern late specified bristles: expression of proneural gene *achaete* (*ac*) in primordia of late specified bristles is controlled by a single CRE. On the surface of the *Drosophila* leg, small mechanosensory microchaetae (mC) are organized in a series of longitudinal rows along the leg circumference. In the prepupal leg, *ac* is expressed in longitudinal stripes which comprise the leg microchaete primordia. We have found that Hairy (H) and Delta (Dl) function concertedly and non-redundantly to define periodic *ac* expression. This process involves broad and late activation of *ac* expression and refinement in response to a prepattern of repression, which is established by Hairy and Delta. These findings have allowed us to formulate a general model for generation of periodic bristle patterns in the adult leg and this model is supported by the analysis of a CRE that specifically directs *ac* expression in the leg proneural fields. This CRE contains an activation element, which directs broad expression of *ac* along the circumference of prepupal legs, and repression elements, which are Dl/N and Hairy responsive.

782B

A post-blastoderm role of Zelda as regulator of CNS midline and tracheal gene expression. Joseph C. Pearson, Joseph D. Watson, Stephen T. Crews. Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Changes in *cis*-regulatory sequences such as enhancers have been proposed as an important force of evolutionary change. One mechanism by which *cis*-regulatory evolution can occur involves developing redundant mechanisms, using different regulators, to control a gene's expression. This redundancy allows flexibility in subsequent gain or loss of individual binding sites. We have identified a 285 b.p. enhancer controlling all embryonic expression of *CG13333*, including in midline glia and tracheal primordia. The pattern controlled by the *CG13333* enhancer resembles patterns controlled by enhancers regulated by the bHLH-PAS transcription factors Single-minded (Sim) and Trachealess (Trh). However, mutating the Sim/Trh consensus site did not significantly alter midline or tracheal expression. Instead, a set of four sequences (T1-4, consensus AGGTA/GG) were required for this and other *CG13333* expression. These sequences resemble binding sites for the transcription factor Zelda (Zld), a master regulator of transcription at the maternal-to-zygotic transition. *CG13333* expression was lost in *zld* mutants, and ChIP-Seq has demonstrated Zld binding to this enhancer at blastoderm stage (Harrison et al., 2011, PLoS Genetics). While some aspects of *CG13333* expression were eliminated by mutation of high-affinity Zld binding sites (sites T1 and T3), midline and tracheal expression was maintained. In contrast, mutating sites T1, T3, as well as a third site that diverges from the optimal Zld binding site (T4), eliminated midline and drastically reduced tracheal expression. Similarly, mutating high-affinity Zld sites T1 and T3 as well as the Sim/Trh site essentially eliminated midline and tracheal expression. Thus, both Zld and Sim/Trh directly regulate *CG13333* expression in CNS midline and tracheal cells. Surprisingly, prominent midline expression of *CG13333* is unique to *D. melanogaster*, although both *zld* and Sim are expressed in midline cells in all *Drosophila* species; we are testing whether the *D. melanogaster CG13333* midline expression is due to changes in *CG13333* sequences or an upstream regulator.

783C

Distinct transcription factor binding strategies at the intersection of growth and patterning in the Hippo signaling pathway. Matthew Slattery[1,2], 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 in both invertebrates and mammals. 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 proliferation and other "housekeeping" functions, 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, and have led to characterization of adjacent, but separate, Sd- and Hth-dependent wing and eye enhancers for the *bantam* microRNA. 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.

784A

Sculpting the insect abdomen: molecular characterization of sexually dimorphic regulation of Wingless by Abd-B and Doublesex. Wei Wang, John Yoder. Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL.

Diverse abdominal morphologies found throughout the order Diptera present an attractive system for investigating developmental and evolutionary mechanisms underlying morphological novelty. One such trait, shared by all Cyclorrhaphan diptera, is adult abdominal segment number; males possess fewer segments than females. The Hox protein Abdominal-B (Abd-B) and products of the sex-determination gene Doublesex (Dsx) coordinately regulate reduction of the posterior-most adult male abdominal segments. We have shown this reduction is principally controlled through male-specific transcriptional repression of the morphogen Wingless (Wg) in the posterior-most abdominal segment A7. 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. We have identified two non-overlapping genomic fragments each capable of driving expression of a reporter gene in patterns reflecting endogenous abdominal Wg protein. Expression driven by both fragments responds to Abd-B and Dsx expression levels suggesting that a minimum of two distinct CREs regulate Wg expression in the Drosophila pupal abdomen. We are currently characterizing smaller sub-clones of these fragments to identify minimal sequences necessary for correct spatial control of Wg abdominal expression. In order to investigate direct regulation of Wg abdominal expression by Abd-B and Dsx these minimal CREs will be subjected to electrophoretic mobility shift experiments and mutational analyses. Characterization of Abd-B and Dsx as direct regulators of Wg expression will provide the necessary genetic context in which to explore the hypothesis that the evolution of abdominal segment number within the higher diptera (Cyclorrhapha) occurred through cis-regulatory changes at the Wg locus.

785B

Disentangling the Sources of Species-Specific Gene Expression Patterns in Drosophila Embryos. Zeba Wunderlich[1], Meghan Bragdon[1], Kelly Eckenrode[1], Charless Fowlkes[2], Angela DePace[1]. 1) Systems Biology, Harvard Medical School, Boston, MA; 2) Computer Science, University of California, Irvine, CA.

Phenotypic divergence in populations and between species has been traced to changes in gene expression, but we cannot yet predict how sequence changes affect regulatory sequence function. To investigate the quantitative connection between regulatory sequence and function, we experimentally and computationally characterized the expression driven by several sets of orthologous *cis*-regulatory elements (CREs) in blastoderm-stage *Drosophila* embryos. Using quantitative 3D cellular resolution imaging techniques, we created atlases of gene expression for key transcription factors (TFs) in the anterior-posterior patterning network in several *Drosophila* species. To disentangle contributions of different sources to expression divergence, we created a cell-by-cell model to relate the levels of the input TFs to the endogenous expression pattern directed by each CRE. To assess the degree of regulatory function conservation of orthologous CREs, we fit the model's parameters in one species and apply them to the other species. We find that regulatory function conservation drops off with phylogenetic distance, but the majority of expression divergence between species is explained by the differences in expression patterns of input TFs. To investigate the contribution of sequence changes in CREs to expression divergence, we made transgenic lines with orthologous CREs driving a reporter in *D. melanogaster*. We use a simple measure of sequence function to fit the quantitative differences between these lines. In closely related species, we can explain virtually all the observed endogenous expression differences as a combination of changes in input TF expression patterns and CRE sequence change, while in more distantly related species, there is evidence for compensatory mutations outside annotated CREs. We discuss how to generalize this approach to measure how sequence divergence effects regulatory function and implications for the evolution of gene regulation.

786C

Regulation of the Sex-determination Transcription Factor Doublesex by the Hox protein Abdominal-B. Shun Yan[1], Wei Wang[1], Michelle Arbeitman[2], John H.Yoder[1]. 1) University of Alabama, Tuscaloosa, AL; 2) College of Medicine, The Florida State University, Tallahassee, FL 32306.

Posterior abdomen of adult Drosophila melanogaster displays several sexually dimorphic traits including male-specific pigmentation as well as sex-specific segment morphology and segment number. Two principle transcription factors that control these abdominal morphologies are the Hox protein Abdominal-B (Abd-B) and products of the sex-determination gene Doublesex (Dsx). These proteins function cooperatively to confer sex-specific repression or activation upon downstream target genes including bric-a-brac (bab) and Wingless (Wg). However, little is known about the interaction between these key transcriptional regulators. Expression studies have shown that Dsx transcription is temporally and spatially dynamic throughout development. We therefore investigated Dsx expression during early pupation when Dsx and Abd-B function cooperatively to reduce segment number in male flies. We found that Dsx is transcriptionally regulated downstream of Abd-B. To investigate whether this regulation is direct we have screened genomic fragments from the Dsx locus for the ability to drive reporter gene expression in the developing pupal abdomen. We have identified two non-overlapping fragments each containing Abd-B responsive Dsx abdominal enhancers. Together these fragments recapitulate Dsx abdominal expression. Analyses of the regulation of these elements have revealed complex genetic regulation beyond the Hox input.

787A

Identification of regulatory elements mediating trans-interactions at metabolic loci in Drosophila melanogaster. Xinyang Bing, Thomas Merritt. Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario, Canada.

Trans-interactions mediate gene expression in somatic cells through interactions between homologous chromosomes. Trans-interactions have been recently documented in Drosophila melanogaster at the Malic enzyme (Men) locus, and we have evidence that similar interactions modify gene expression at the Triose phosphate isomerise (Tpi) locus. To identify regulatory elements responsible for trans-interactions, we continue to annotate the regulatory regions of genes that do, and do not, show these trans-interactions. We use P-element excision mediated mutagenesis to create deletion mutant alleles varying in size and location, and quantify the effect of these deletions on gene expression, protein level and activity when the mutant alleles are in trans to wild-type alleles. Putative regulatory elements are predicted in silico, and mapped to deletions in mutant alleles. Significant deviations from expected gene expression (i.e. 50% wildtype in heterzygotes) of each mutant allele can then be attributed to these deleted elements. Identification of regulatory elements mediating trans-interactions will be systematically completed for both Men and Tpi loci. We are concurrently annotating the regulatory regions of two genes that do not appear to exhibit trans-interactions (isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase). Comparison of regulatory elements across these four loci will identify elements that are, and are not, involved in trans-interactions, allowing us to draw conclusions about the regulatory elements that are critical to trans-interactions, and improving our understanding of the specific mechanisms by which these trans-interactions are mediated or precluded in D. melanogaster.

788B

REMSA and mutational analysis reveal a novel role for full-length dADAR in *Drosophila rnp-4f* 5'-UTR alternative splicing regulation during embryogenesis. Sushmita Ghosh, Girija Lakshmi, John Cook, Gabriel Jones, Roshni Parikh, Bridgette Rawlins, Jack Vaughn. Zoology, Miami University, Oxford, OH.

The *Drosophila rnp-4f* gene encodes a splicing assembly factor that dimerizes U4- and U6-snRNPs during spliceosome formation. 5'-UTR pre-mRNA intron processing results in two major isoforms, unspliced and alternatively spliced. The unspliced isoform has a secondary structure where an intron pairs with adjacent highly evolutionarily-conserved exon 2 to form a stem-loop. The coding potential for the two isoforms is identical, raising interesting questions as to the control mechanism and functional significance of this 5'-UTR intron splicing decision. It is known that the unspliced isoform localizes largely in the developing fly central nervous system, as do *dADAR* mRNAs, and that dADAR uses dsRNAs for substrate during A-to-I RNA editing. These observations suggested an hypothesis in which dADAR protein may bind to the *rnp-4f* pre-mRNA stem-loop and inhibit splicing. To test this hypothesis, RNA Electrophoretic Mobility Shift Assay (REMSA) was carried out using *in vitro* transcribed stem-loop RNA incubated with embryo protein extract. Two RNA-protein complexes are detected by shifted RNA bands. Protein extract from a *dADAR* null mutant fly line results in only one shifted band, and recombinant dADAR results in a band shift. A mutated stem-loop in which the conserved exon 2 sequence is changed but secondary structure maintained results in diminished band shifts. To determine if unspliced mRNA levels are correlated with presence of dADAR protein during embryo development, qRT-PCR was carried out using embryo protein extracts from wild-type and the *dADAR* mutant. A dramatic decrease in unspliced mRNA levels occurs in the *dADAR* mutant, a finding consistent with the REMSA results. These observations demonstrate a novel non-catalytic role for dADAR protein in *rnp-4f* 5'-UTR alternative intron splicing regulation. A model is proposed to explain the results. We are now attempting to identify the regulatory protein(s) which bind to the stem-loop using MALDI-TOF technology.

789C

The role of *Drosophila* ATF4(crc) in the Unfolded Protein Response. Min-Ji Kang, Josepher Li, Dowhan Kim, Hyung Don Ryoo. Dept Cell Biol, New York Univ Sch Med, New York, NY.

Stress in the endoplasmic reticulum (ER) activates transcriptional response pathways, widely referred to as the Unfolded Protein Response (UPR). These pathways require transmembrane proteins that can detect stress in the ER, and transmit that information to the cytoplasm. In mammals, three pathways are particularly well-established, mediated by ER-stress sensors, ATF6, IRE1 and PERK. Upon detection of ER-stress, PERK phosphorylates eIF2alpha, which leads to a selective activation of ATF4 translation to induce the Unfolded Protein Response. Here, we report the function of *Drosophila* ATF4. This protein is encoded in the cryptocephal (crc) locus, and is essential for development. *Drosophila* ATF4 has two upstream open reading frames (uORFs) that are inhibitory and normally block downstream ATF4 expression. Upon ER-stress, or other conditions that lead to eIF2alpha phosphorylation, ribosomes can bypass such uORFs to synthesize ATF4. Using this feature, we developed the ATF4 reporter, which is activated by misexpression of misfolded Rhodopsin-1 that is a model for Autosomal dominant retinitis pigmentosa (ADRP). Once activated, *Drosophila* ATF4 stimulates the transcriptional activation of XBP1, a mediator of the IRE1 branch of the UPR. In addition, ATF4 induces Thor that suppresses cap-dependent protein translation. By enhancing UPR and reducing cap-dependent translation, ATF4 is thought to relieve unfolded protein overload in the ER, not only in response to excessive stress, but also during normal development.

790A

Sub-type specific regulation of *Drosophila* glutamate receptor production by the novel receptor mRNA associated genes *optimus-prime* (*opr*) and *bumblebee* (*bbe*). Julie E. Karr[1], Subhashree Ganesan[2], Magdalena M. Paces[3], David E. Featherstone[4]. 1) Science and Mathematics , Columbia College Chicago, Chicago, IL; 2) Neurosciences Institute, Stanford School of Medicine, Stanford, CA; 3) Loyola University Chicago, Chicago, IL; 4) Biological Sciences, University of Illinois at Chicago, Chicago, IL.

Postsynaptic receptor abundance is a critical determinant of synapse strength. We are identifying and studying mechanisms that control glutamate receptor (GluR) abundance in *Drosophila* embryonic/larval neuromuscular junctions (NMJ). One process that appears particularly important is regulation of the production, trafficking, stability, and translation ofGluR mRNA. GluR subunit mRNA in embryonic/larval NMJs is associated with messenger ribonucleoprotein (mRNP) complexes distributed throughout the cytoplasm of postsynaptic muscle cells. Here, we show two novel proteins that appear to associate specifically with *GluRIIA* mRNA and regulate GluRIIA protein abundance. The first of these genes is CG12149, which we named '*optimus-prime* (*opr*)'. Mutants and muscle-specific RNAi knockdown of *opr* leads to loss of GluRIIA protein but no change in *GluRIIA* mRNA quantity or loss of other GluR subunits. A polyclonal antibody raised against Opr shows immunoreactivity distributed throughoutmuscle cells, but Opr is not seen at motor neuron

terminals. Mutants of the second gene CG17816, named *bumblebee* (*bbe*), also leads to loss of GluRIIA but no disruption in production of other GluR protein subunits. Both opr and bbe are novel highly conserved proteins. Optimus prime is the founding member of a novel protein family. SNPs in the human opr homolog are associated with autism spectrum disorders.

791B

Investigating Duplicated Ribosomal Proteins Reveals Differential Post-Translational Modification of the RpL22e Family in the Male Germline: Evidence for SUMOylation of RpL22. Michael Kearse, Jill Ireland, Vassie Ware. Department of Biological Sciences, Lehigh University, Bethlehem, PA.

Duplicated ribosomal protein (Rp) genes are found throughout eukaryotic genomes, at times encoding highly similar or identical proteins. Whether these paralogues are functionally redundant or provide a unique cellular role, not limited to translation, remains largely unknown. We have focused on the the eukaryotic-specific RpL22e family members in *Drosophila*, RpL22 and RpL22-like. Using paralogue-specific C-terminal peptide-derived polyclonal antibodies, Western analysis shows RpL22-like is primarily testis-specific, found at its predicted molecular weight (MW), and is a component of ribosomes (Kearse *et al*., 2011). Furthermore, RpL22 is detected not only at its expected MW of 33kD, but more predominantly at a higher MW of ~50kD, suggestive of post-translational modification. Computational probing predicts a SUMOylation consensus motif, localized at different sites within the fly-specific N-terminal extension of both family members. In this report, we investigate the possible SUMOylation of RpL22. Results from S2 cell-based experiments replicate the RpL222 higher MW pattern when a FLAG-tagged RpL22, but not a K39R mutation within the proposed SUMOylation site, is co-expressed with HA-SUMO. These results support the *in silico* prediction and demonstrate that RpL22 can be SUMOylated, likely with two SUMO moieties. Interestingly, Western analysis shows that SUMOylated RpL22 is more predominant in adult testis than in S2 cells. Western analysis of RpL23a-FLAG-affinity purified complexes from S2 cells shows that SUMOylated RpL22 is only a minor component of complexes containing RpL23a. Whole-mount testis immunohistochemistry reveals distinct nucleoplasmic, but not nucleolar localization for RpL22 and cytoplasmic localization for RpL22-like within the germ cells. Taken together, these data suggest that RpL22 and RpL22-like have distinct roles within the germline.

792C

The evolutionarily canalized expression of *eve* stripe 2 in *Drosophila* and the Sepsidae. Ah-Ram Kim[1,2], Carlos Martinez[1], Bin He[1], Michael Ludwig[1], Martin Kreitman[1], John Reinitz[1,3]. 1) Department of Ecology and Evolution, Chicago Center for Systems Biology, University of Chicago, IL, U.S.A; 2) Department of Biochemistry and Cell Biology, Stony Brook University, NY, U.S.A; 3) Departments of Statistics and Molecular Genetics & Cell Biology, University of Chicago, IL, U.S.A.

We employ a theoretical model that is intermediate between, on one hand, a content-based picture in which only the number of binding sites for each factor in an enhancer is significant, and, on the other hand, a grammar-based approach in which a precise arrangement of binding sites is required for regulatory function. The model quantitatively treats 1) proteins binding to DNA; 2) Steric competition for binding sites; 3) Cooperative binding; 4) Short range repression by quenching; 5) Coactivation; 6) Direct repression; and 7) Transcriptional initiation as a diffusion limited enzymatic process driven by an Arrhenius rate law. In our model, the physical arrangement of binding sites is quite important, but it is specified by rules that are sufficiently flexible to permit many solutions, reflecting the observed variability in binding site arrangement. Here we report new progress in understanding the naturally evolved robustness of transcriptional control at single functional binding site resolution. It is a remarkable fact that *eve* enhancers from 12 *Drosophila* species and from the Sepsidae, which bear almost no homology to those from melanogaster, nevertheless express in that organism in patterns only slightly different from the native ones. We make use of the fact that our model can be used to untangle the relative roles of many simultaneously acting regulatory mechanisms to show how the evolutionarily canalized expression of eve stripe 2 in *Drosophila* and the Sepsidae is achieved.

793A

Turning off Bruno-dependent Translational Repression. Goheun Kim[1], Keiji Sato[2], Akira Nakamura[2], Paul Macdonald[1]. 1) Molecular Cell & Developmental Biology Dept, 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 the viability of the embryo. Bruno (Bru) directly binds to the *osk* mRNA and represses translation during mRNA localization to the posterior pole. In one model for repression, the interaction between Bru, Cup and eIF4E is thought to inhibit translational initiation. In another model, Bru promotes oligomerization of multiple *osk* mRNAs into large particles that are inaccessible to the translational machinery. Evidence from our lab suggests that translational activation of *osk* involves at least two spatially distinct mechanisms: one acts throughout the oocyte and requires *cis*-acting elements; another appears to act only at the posterior pole of the oocyte and may be independent of *cis* elements. Insights into the second mechanism come from analysis of Bru protein interactions and phosphorylation. We showed that Bru dimerizes, which can explain how Bru can oligomerize *osk* mRNA. We mapped domains involved in Bru-Bru and Bru-Cup interactions, and found that aa1-146 and aa334-416 contribute to both interactions, with a stronger requirement for aa1-146. We found that a small fraction of Bru is phosphorylated. Several predicted sites of phosphorylation in Bru lie within the regions important for Bru-Bru and -Cup interactions, and could be targets for inactivation of repression. Phosphomimetic mutants of some of the sites that are predicted targets of Protein Kinase A (PKA) are defective in Bru binding to both itself and Cup, while corresponding phosphosilent mutants have no effect. aa1-146 of Bru that contains these sites can be phosphorylated in vitro by purified PKA. We propose that local phosphorylation of Bru by PKA at the posterior of the oocyte is a likely mechanism for inactivating Bru-dependent translational repression by either of two models.

794B

Dose dependent buffering effects in Drosophila melanogaster. Lina E Lundberg, Per Stenberg, Margarida Figueiredo, Jan Larsson. Dept. of molecular biology, Umeå, Umeå, Sweden.

Chromosomal aneuploidy is a common feature of cancers and involves loss or duplication of chromosomal copies. This normally lethal condition can be tolerated when small regions are affected, but the transcriptional effects and mechanisms that are triggered by these segmental aneuploidies have been elusive. However, as better and more accurate techniques have evolved, more is known about transcriptional responses in these region and it has become clear that the remaining chromosome copy in a haploid (single) region is not unaffected by this state. Rather, the transcriptional output from a single region

is higher than the expected 50% of wildtype level, meaning that compensatory mechanisms stimulate transcription in these regions in response to chromosomal loss, acting to push the level of transcripts from an aneuploid region closer to wildtype level. This mechanism has been termed “buffering”. It should be noted that genome-wide studies inevitably include analyses of non-expressed genes and genes expressed at non-detectable levels; two groups of genes that thus will be scored as fully compensated. By taking these genes into consideration, we have recently shown that this buffering effect is in fact lower than previously reported. To further study this buffering effect and to find out which factors influence the level of buffering, 7 *Drosophila* deficiency stocks were analyzed by microarray, both as single replicates and in pairwise combinations. In addition, 6 wildtype replicates were used to create a model of normal biological variation, an important understanding when examining small effects in genomic expression. We show that buffering is a general mechanism which targets whole regions and that the buffering effect is larger in short regions compared to long regions. On individual gene level, a long gene length is the primary determinant for buffering effect. For short genes, the expression level is more important, where genes with lower expression levels are more buffered. Further, we have discovered that proteolytic genes are upregulated as a general response to aneuploidy.

795C

A Global Transcriptomics Approach Identifies Sex-Specific and Immune-System Changes in Gene Expression and pre-mRNA Splicing Induced by Doa and fne Mutations. Leonard Rabinow[1], Marie-Laure Samson[1], David Sturgill[2], Xia Sun[1], John Malone[2], Yunpo Zhao[1], Brian Oliver[2]. 1) University Paris Sud, UMR 8195, Orsay, France; 2) Laboratory of Developmental Genomics NIDDK, NIH, Bethesda MD USA.

We are using high-throughput sequencing of cDNAs (RNA-Seq) Drosophila melanogaster cDNAs to identify splicing and expression-level targets of the *Doa* and *fne* loci. *Doa* encodes a protein kinase, which among other things phosphorylates SR proteins, influencing alternative splicing. *Doa* mutations affect somatic sex-determination due to aberrant splicing of doublesex. *fne* is one of the three *elav*-family paralogues present in the Drosophila genome. Members of this neuronally-expressed gene family, conserved in all metazoans, bind RNAs to influence diverse processes, including neuronal development, memory and sleep regulation. Flies were raised under controlled conditions of temperature and low-density population for 3 generations, to avoid excessive competition among larvae; constant lighting, to ablate circadian rhythms; and aging for 7 days post-eclosion in mixed-sex cultures to allow for developmental stabilization of transcript levels. Flash-freezing was followed by isolation of fly heads on dry-ice. Poly-adenylated RNAs were prepared from sex-sorted heads of wild-type, *fne* and *Doa* mutants and cDNAs subjected to high-throughput sequencing. Three rounds of sequencing were performed on two independent samples. Potential target genes for *Doa* and *fne* affected either at the expression level or alternative splicing are being validated via RT-PCR and genetic crosses. Importantly, the RNA-Seq results reproduce the aberrant alternative splicing of doublesex transcripts previously described for *Doa* mutants. Further results suggest that DOA kinase represses transcription of several anti-microbial peptide genes, since their expression is dramatically elevated in mutants. Differences in the alternative splicing of several potential *Doa* target loci has also been observed.

796A

Regulation of *twin-of-eyeless*, a *Drosophila Pax6* gene. John Skottheim Honn, Linn Jacobsson, Karin Ekström, Åsa Rasmuson-Lestander. Dept. of Molecular Biology, Umeå University, Umeå, Sweden.

There are two *Pax6* genes in *Drosophila*; *eyeless (ey)* and *twin-of-eyeless (toy)*. They encode transcription factors that are important for the correct development of head structures, a function that is conserved also in humans. Heterozygosity in humans leads to eye abnormalities known as aniridia, homozygous mice are lacking eyes and in severe mutants in *Drosophila* most of the head is missing. This indicates that *Pax6* is not only involved in eye formation and development but in formation of the early brain and central nervous system as well. *toy* is considered to be the first eye specification gene expressed in the regulatory network that governs eye formation and the gene that, in turn, activates *eyeless*. In this study our focus is on how the *toy* gene is regulated since that is still an open question. Toy is expressed very early during development (stage 5, 2-3 hrs after egg laying) and it is conceivable that early transcription factors, like maternally deposited proteins or gap gene products are involved. By using a Toy specific antibody we study the expression of Toy in various mutant backgrounds to find out which genes are involved in the temporal and spatial regulation of Toy. We show, by misexpression and mutant analysis, that the head-specific gene *empty spiracles* alters the expression pattern of Toy in the head region around the visual primordia.

797B

Effect of matrix and nucleocapsid on multimerization of gypsy structural protein Gag. Boris V. Syomin[1,2], Tatjana A. Trendeleva[1,2], Yurii V. Ilyin[2], Vladimir I. Popenko[2]. 1) Russian Institute of Experimental Vet Medicine, Moscow; 2) Institute of Molecular Biology, Russian Academy of Sciences, Moscow, 119991 Russia.

Gypsy is an endogenous retrovirus that was first revealed in Drosophila. Amino acid sequence of the gypsy Gag does not contain a canonical motif known for the majority of vertebrate retroviruses. Moreover, protein translation can theoretically begin with two separated initiation codons located within its unique open reading frame. Therefore there are two theoretically possible variants of Gag gypsy that differ from each other by 41 amino acid residues of the N-terminal. The Gag contains matrix domain in the case of the “long” variant, “short” variant of the Gag lacks matrix. We designed constructs for expression of two variants of Gag polypeptide and investigated the ability of each product to form virus-like particles in the bacterial cell, i.e., in the absence of eukaryotic cell factors. Both variants of structural protein Gag gypsy can form ellipsoid virus like particles with average diameter 27 nm in bacterial cells. However, “short” variant of Gag is preferably synthesized in a eukaryotic cell because this variant of gypsy Gag was detected by anti-Gag antibodies in the preparations of virus-like particles isolated from Drosophila. Expression of an alone capsid domain of gypsy Gag was also sufficient for particle assembly in Escherichia coli. However, particles assembled from the capsid domain were variable in size and displayed much less organization than particles formed by the whole Gag or its deleted mutant containing capsid fused to N-termini (proximal) part of nucleocapsid. The assembly of examined proteins in vitro can be driven through interactions with RNA or single strand DNA oligonucleotide as well.

798C

Molecular mechanism of the miRNA machinery responding to serum deprivation in Drosophila. Pei-Hsuan Wu, Richard Carthew. Northwestern University, Department of Molecular Biosciences, 2205 Tech Drive, Hogan 2-100, Evanston, IL60208.

miRNAs have the remarkable capacity to modulate their target gene expression in response to environmental challenges ranging from various cellular stress to nutritional deprivation, which reflects on its crucial role in numerous cellular processes. Serum deprivation has been shown to alter the function of

miRNAs, but the effect of serum deprivation on the miRNA machinery at the mechanistic level is still poorly understood. We set out to extend the understanding of miRNA response to serum deprivation and its mechanism at the molecular level. By taking advantage of density gradient centrifugation, we found that upon serum deprivation in S2 cells, denser Ago1-associated-complexes rapidly form in addition to the pre-existing Ago1-complexes under the serum-fed condition. Interestingly, the dense Ago1-complexes can be further identified as two pools, one pool being observed as the denser pool than the other. Treating serum-deprived cells with various reagents and drugs targeting candidates of required factors reveals distinct dependence of these Ago1-complexes on the presence of long RNA, membranes, polysomes, and active protein synthesis. Furthermore, the well-characterized and important Ago1-binding partner, GW182, is only detected in the least dense pool of Ago1-complex but not the denser pools. These results suggest that miRISC is a dynamic complex capable of adapting to the environmental stimuli by modifying its assembly and possibly its function, thereby reprograms the global gene expression that a cell calls for when facing extracellular challenges.

799A

Drosophila miR-9a guards organism's homeostasis by regulating stress response protein Dystroglycan. Andriy S Yatsenko, Halyna R Shcherbata. Gene Expression and Signaling, Planck Institute for Biophysical Chemistry, Goettingen, Germany.

MicroRNAs (miRNAs) are small non-coding RNAs that function as negative regulators of gene expression. miRNAs bind to the 3`UTR region of their target mRNAs and lead to translational inhibition. Despite the fact that many miRNAs have been identified in Drosophila their function in regulating development and tissue maintenance is not completely understood. Here we show that miR-9a is required for response to stress. Changing the ambient temperatures and applying energetic stress to miR-9a mutants increases embryonic lethality and shorten lifespan in adults when compared to wild type animals. Interestingly, on elevated temperatures the expression level of miR-9a is decreased and vice versa. In addition we found that miR-9a mutants have higher metabolic rate measured by CO2 production. Dystroglycan (Dg) is a predicted target for miR-9a and has been previously shown to be required for stress response, muscle tissue homeostasis and control of metabolic rate in Drosophila. In the embryo miR-9a and Dg have reciprocal expression patterns and changing the levels of miR-9a affects amount of Dg protein. Since Dg misregulation disrupts tissue homeostasis and increases sensitivity to stress we propose that miR-9a is important for maintaining homeostatic Dg expression levels during development and stress response.

800B

Autoregulation and context-specific regulation of the Yan/Pnt bistable network. Lauren Cote, Jie Zhang, Jemma L. Webber. Univ Chicago, Ben May Dept Cancer Research, Chicago, IL.

Downstream of receptor tyrosine kinase (RTK) signaling, two ETS-family transcription factors Yan and Pnt, a repressor and an activator respectively, are thought to form a bistable switch controlling cell-fate decisions (Graham et al, 2010). RTK signaling induces Yan degradation and thus derepression of target genes, and concomitant Pnt activation and thus activation of target genes. Genome-wide binding profile analysis of the repressor Yan identified large enrichment domains containing multiple peaks over tens of kilobases termed high density regions. Conservation of this chromatin association pattern in *D. virilis* and its presence at multiple developmentally important genes indicate that it might be functionally relevant for proper and robust gene expression. One of the most prominent examples of this high density signature is found at the *yan* locus, leading us to hypothesize that Yan autoregulation via transcriptional repression is important for the function and stabilization of this cell-fate decision network. To test this hypothesis, we are investigating the ability of Yan to repress itself using recombineered constructs which delete various Yan binding regions from the *yan* locus. In addition, we are exploring the ability of the activator Pnt to regulate Yan expression in different developmental contexts. These preliminary results point to autoregulation and developmental context as contributors to the network topology which ensures precise expression of target genes and buffers against intrinsic and extrinsic perturbations.

801C

Bicaudal-C represses *nanos* mRNA in *Drosophila* oogenesis through a direct association with a 3' UTR motif distal to the translational control element. Chiara Gamberi. 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 provide evidence 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 an effect on its polyadenylation state. In *Bic-C* mutants, or when forms of *nos* mRNA that are mutated for the Bic-C interaction site are expressed, 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 elaborate the complexity of post-transcriptional regulation of *nos*.

802A

The DEAD-box RNA helicase, *belle*, provides post-transcriptional control to steroid-triggered responses during *Drosophila* metamorphosis. Robert Ihry[1,2], Arash Bashirullah[1]. 1) Division of Pharmaceutical Sciences, University of Wisconsin-Madison, Madison, WI; 2) Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI.

The steroid-hormone ecdysone, through its receptor heterodimer (EcR/USP), directly induces the expression of a small set of target genes; these "early" response genes in turn induce expression of a larger set of secondary or "late" response genes. Here, we show that post-transcriptional control is critical for late gene induction and the self-limiting nature of the ecdysone-triggered transcriptional cascade. We identified *belle* in a forward genetic screen for mutations that disrupt one ecdysone-triggered process (the destruction of larval salivary glands during metamorphosis) but not other responses to the same pulse of ecdysone. In *belle* mutant larval salivary glands, the ecdysone-triggered induction of *reaper* and *hid* is disrupted even though other ecdysone induced genes are not affected. We show that this defect is due to the failure to translate the early gene *E74A*. We show that, although the *E74A* mRNA is induced in a broad peak, E74A protein is expressed in a very short pulse and that the timing of E74A protein determines the timing of the death response. Moreover, we show that this transient pulse of E74A protein is generated by a feed-forward loop between *E74A* and *belle*. In addition, we show that E74A protein is critical for limiting its own transcription and that of other early response genes, demonstrating its critical role in the self-limiting behavior of the ecdysone-triggered transcriptional cascade. Together our results suggest that post-transcriptional control is critical for the proper function of steroid-triggered transcriptional responses.

803B

Protein interacting with Ttk69 and Sin3A (Pits) acts as a mediator to repress *tailless* expression. Gwo-Jen Liaw. Dept Life Sci, Natl Yang-Ming Univ, Taipei, Taiwan.

Histone deacetylation plays an important role in transcriptional repression. Our previous data showed that Tramtrack69 (Ttk69) formed a repression complex with GAF and Heat Shock Factor (HSF) and that the genetic interaction of *ttk* with *rpd3*, encoding a histone deacetylase, was involved in *tailless* (*tll*) repression. To reveal molecular mechanism of how the Sin3A/Rpd3 complex is recruited by Ttk69, proteins interacting Ttk69 were screened. A protein was found that it interacted with both Ttk69 and Sin3A, called as Protein interacting with Ttk69 and Sin3A (Pits). Pits uniformly distributed in early stages of *Drosophila* embryos. Embryos with reduced maternal *pits*, *sin3A* and *ttk* activities showed a greatly expanded *tll* expression patterns, indicating that these three gene activities work together to repress *tll* expression.

804C

Functional analysis of Blimp-1during pupal developmental stage in *Drosophila*. ABDEL-RAHMAN SAYED SULTAN[1], HITOSHI UEDA[1,2]. 1) The Graduate School of Natural Science and Technology, Okayama University, Japan; 2) Department of Biology Faculty of Science, Okayama University, Japan.

Blimp-1, an ecdysone-inducible transcriptional repressor, has been identified as one of the factors that binds to the promoter region of the *ftz-f1* gene and plays an important role in determining the expression timing of the *ftz-f1* gene. It has been shown that its temporally restricted expression is important for embryonic and prepupal development of *Drosophila*. However, its expression pattern and function during pupal period have not been analyzed. *Blimp-1* mRNA was detected at pupal stage by RT-PCR. We found that the *Blimp-1* gene is expressed during the pupal stage and the expression in female is advancing to that in male. On the other hand, the expression level of *Blimp-1* in male is high comparing to that in female. These results consistence with the reported ecdysone level during pupal stage. To elucidate the functional expression of *Blimp-1* at pupal stage. We examined effects of *Blimp-1* knockdown by using GAL4/UAS system, We found that *Blimp-1* knockdown exhibits morphological malformations in the eye, wing and leg. Interestingly, *Blimp-1* knockdown in the whole body using *Act5C-GAL4* line induces advancing of pupation, delaying of pupal development and inhibition of eclosion.

805A

Role of *Drosophila* retinoblastoma proteins in insulin signaling pathway regulation. Yiliang Wei[1], Pankaj Acharya[2], Liang Zhang[3], William Henry[1], David Arnosti[1]. 1) Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI; 2) Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI; 3) Cell and Molecular Biology Graduate Program, Michigan State University, East Lansing, MI.

Drosophila retinoblastoma family members Rbf1 and Rb2 are orthologs of human retinoblastoma (RB) tumor suppressors, which function as transcriptional co-repressors controlling cell cycle and developmentally regulated gene expression. Our lab has carried out the first ChIP-Seq analysis of Rbf1 in the *Drosophila* embryo. Surprisingly, in addition to conserved cell cycle-regulated genes, Rbf1 was also found to associate with promoter-proximal regions of genes in the insulin signaling pathway. This conserved pathway plays an essential role in controlling animal growth and maintaining cellular and organismal homeostasis. In vitro reporter assays showed that Rbf1 can functionally repress the insulin receptor (InR) promoter, and this repression function appears to be E2F-independent, consistent with the lack of E2F motifs in the promoter. To investigate the physical binding of Rbfs to the InR promoter, we carried out ChIP for Rbf1 and Rbf2 in *Drosophila* S2 cells. Remarkably, these proteins stay associated with the promoter region even when transcription is stimulated by ecdysone treatment or starvation. *rbf1* and *rbf2* knock-down in *Drosophila* S2 cells induced only modest changes in transcript levels of genes in insulin signaling pathway, indicating that other factors, such as FOXO or ligand-bound ecdysone receptor might be required to fully activate the transcription of insulin signaling genes. Altogether, these results show that Rbf proteins may play critical roles in integrating signaling from cell-cycle and growth control inputs by setting sensitivity of the insulin signaling pathway.

806B

The Instability Element of Retinoblastoma Proteins in Drosophila: Functional Overlap between Protein Turnover and Activity. Liang Zhang[1], Nitin Raj[2], Yiliang Wei[3], William Henry[3], David Arnosti[1,3]. 1) Cell and Molecular Biology Program, Michigan State University, East Lansing, MI; 2) Genetics Program, Michigan State University, East Lansing, MI; 3) Dept of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI.

Retinoblastoma (RB) proteins, frequently inactivated in different types of cancers, are pivotal transcriptional corepressors in regulating cell cycle progression and development through their interactions with E2F family transcription factors. Activity and stability of RB proteins are precisely controlled during cell cycle through CDK-mediated phosphorylation and proteasome-dependent degradation. However, the molecular mechanism for degradation of RB family proteins is not well understood. We characterized mutant forms of the Rbf1 protein in Drosophila and identified an instability element (IE) in the C-terminal region. Paradoxically, when the IE is deleted, increased protein levels do not cause enhanced repression activity. Rather, these mutations diminish repression activity of Rbf1, indicating a linkage between Rbf1 activity and instability, similar to the “degron” model of transcriptional activators. By assaying the Rbf1 IE in the context of chimeric GFP proteins, we find that the IE is an independent module which is able to direct the turnover of a heterologous protein through ubiquitylation pathway. More importantly, the IE itself is a repression domain when directly tethered to the promoter, suggesting that the IE may serve as an interaction domain for multiple cofactors linking protein turnover and transcriptional repression. By fusing a single ubiquitin to the inactive Rbf1 mutant, we observed its repression activity being partially restored. We propose that the IE controls Rbf1 stability and activity in a ubiquitylation-mediated pathway.

807C

dNIAM: A chromatin protein that negatively regulates cell proliferation. Olivia E. Jones[1], Diane E. Cryderman[1], Kristen E. Syring[1], Shannon R. Mackey[1], Sara Reed[2], Dawn E. Quelle[2], Lori L. Wallrath[1]. 1) Department of Biochemistry, University of Iowa, Iowa City, IA; 2) Department of Pharmacology, University of Iowa, Iowa City, IA.

Regulation of cell proliferation is a key step in the initiation and management of cancer. In humans, the nuclear factor NIAM (Nuclear Interactor of ARF and Mdm2) associates with chromatin, maintains chromosome stability, and inhibits cellular proliferation independent of the tumor suppressor ARF. We

identified *CG31111* as a potential orthologue of NIAM and designed it *dNIAM*. Both human and fly proteins possess an N-terminal lysine-rich region and C-terminal phenylalanine/tyrosine-rich (FYRN/FYRC) domains, which are exclusively found in proteins that modify chromatin structure. Knock-down of dNIAM in the developing eye caused over-proliferation of eye tissue, demonstrating an anti-proliferation function. Analysis of transgenic flies expressing a dNIAM-GFP showed that the protein localizes to actively transcribed regions of Drosophila polytene chromosomes, including heat shock loci and developmental puffs. Co-localization studies demonstrated a partial overlap with RNA Polymerase II phosphorylated at serine 5, which occurs at the 5' region of genes. Collectively, these data demonstrate a conserved anti-proliferation for dNIAM and suggest a role in transcriptional regulation.

808A

Functional characterization of dmyc downstream promoter element in transgenic Drosophila. Jasmine Kharazmi[1,2], Cameron Moshfegh[1]. 1) Molec Biol Lab, Biotech Ctr Zurich, Zurich; 2) University of Zurich-Jrchel, Switzerland.

Myc is a master regulator of growth and proliferation during animal development. Many signals and transcription factors lead to changes in the expression levels of Drosophila myc, yet no clear model exists to explain the complexity of its regulation at the level of transcription. In this study we used Drosophila genetic tools to track the dmyc cis-regulatory elements. Bioinformatics analyses identified conserved sequence blocks in the noncoding regions of the dmyc gene. Investigation of lacZ reporter activity driven by upstream, downstream, and intronic sequences of the dmyc gene in embryonic, larval imaginal discs, larval brain, and adult ovaries, revealed that it is likely to be transcribed from multiple transcription initiation units including a TATA-less downstream promoter element in conjunction with an initiator within the intron 2 region (Kharazmi, et.al., Gene Regulation And Systems Biology, 2011, in press). Our data provide evidence for a modular organization of dmyc regulatory sequences; these modules will most likely be required to generate the tissue-specific patterns of dmyc transcripts. These data provide a framework for further investigation of the transcriptional regulatory mechanisms of dmyc.

809B

The regulation and function of microRNAs during the maternal-to-zygotic transition in *Drosophila*. Shengbo Fu[1], Chung-Yi Nien[1], Hsiao-Lan Liang[1], Stephen Butcher[2], John Manak[2], Christine Rushlow[1]. 1) Department of Biology, New York University, New York, NY; 2) Department of Biology, University of Iowa, Iowa City, IA.

The maternal-to-zygotic transition (MZT) is a conserved pivotal process in which the transcribed zygotic genome gradually replaces maternally loaded products to control embryonic development. Previously, our lab identified Zelda as a key activator of the early zygotic genome during the MZT of *Drosophila*. Zelda binds specifically to CAGGTAG and related sites, which are over-represented in the upstream regions of early zygotic genes (ten Bosch et al., 2006; De Renzis et al., 2007). By comparing the expression profiles of wild-type and *zelda* mutant embryos, we demonstrated that over 70% of the early-expressed zygotic genes are down-regulated in *zelda* mutants, but also that many maternal transcripts are up-regulated. Moreover, expression of the *miR-309* cluster, the first *Drosophila* zygotic factor shown to be involved in maternal transcript degradation (Bushati et al, 2008), is eliminated in *zelda* mutants. More recently, we showed that Zelda binds to the enhancer region of *miR-309* (Nien et al., 2011). Together, these results hinted that Zelda can function in maternal mRNA degradation by activating early zygotic microRNAs. To further study the activation and function of microRNAs during the MZT, we examined the expression profiles of wild-type and *zelda* mutant embryos using whole genome tiling arrays, and validated several Zelda-regulated microRNA candidates by *in situ* hybridization. Further analysis showed that several of these microRNAs are regulated by a complex transcriptional network. Current studies that focus on the function of these early zygotic microRNAs during the MZT will be presented that demonstrate the significance of the Zelda-microRNA-maternal mRNA network during the MZT.

810C

miRNA function in a stress response in Drosophila S2 cells. Mamiko Isaji, Pei-Hsuan Wu, Richard Carthew. Molecular Biosciences, Northwestern University, Evanston, IL.

Cells are always exposed to environmental stresses, for example, nutrient restriction, oxidative stress, physical stress and also viral infections. Cellular stress responses are very dynamic for allowing cells to effectively counteract and survive. A novel class of genes, termed microRNAs (miRNAs), has recently been implicated in the cellular stress response. However, the relationship between miRNA and stress responses is just beginning to be explored. Accumulating evidences show that the stress conditions can alter the miRNA biogenesis, expression of mRNA targets and the activities of miRNA-protein complexes. In many of these cases, the molecular mechanism has been unclear and need to be investigated further to elucidate these fundamental roles of miRNAs in controlling mRNA regulation during stress. How does stress regulate miRNA activities and how do cells mediate stress responses through miRNAs? For understanding of this question, we observed Ago1-associated-complexes in cell fractionation by density gradient centrifugation in Drosophila S2 cells under serum deprived condition. The Ago1-associated-complexes enriched at lower density fractions under the normal condition, however, under serum deprivation, their enrichment has shifted to higher density fractions, and most of them were observed into the highest fraction which contains abundant membrane structures including endoplasmic reticulum. Moreover, we tried to see the activity of the Ago1-associated-complexes by biochemical experiments including a reporter assay combined with a pulse-chase assay. Our results showed that the Ago1-associated-complexes can react dynamically to environmental condition and miRNA plays an important role for stress responses.

811A

Sensitivity to nicotine: Can a Regulated Mechanim's microRNAs? Ivan Sanchez Diaz, Veronica Narvaez Padilla, René Hernandez Vargas, Enrique Reynaud Garza. Genetica del Desarrollo y Fisiologia Molecular, Instituto de Biotecnologia, Cuernavaca, Cuernavaca, Mexico.

It has been amply demonstrated that of all the compounds in tobacco, nicotine is the molecule responsible for causing addiction. The study of the susceptibility to nicotine and drugs in general, is very complex because addiction depends on both genetic and environmental factors. Drosophila melanogaster has been used to study addiction. Using volatilized nicotine, we isolate a line (L-70) that showed increased sensitivity to nicotine in relation wt. The line L-70 has a P-element (p {GawB}) in the region of the genome designated as 2R:16470008 which corresponds to an intergenic region, near a cluster of micro-RNA 's (mir-310/311/312/313) which become over express by the presence of this insertion in the adult stage. We also show that removing the P-element in this region is restored wild phenotype and levels of micro-RNA's become wt. Interestingly knockout flies of this cluster (Δ 40) show a resistance phenotype compared to control. Based on these data we hypothesized regulation of the response to nicotine in the fly through these mir's. This regulation would be mediated through the inactivation of target the mirs genes.

812B

***mir-11* limits the pro-apoptotic function of its host gene, *dE2f1*.** Mary Truscott[1], Abul Islam[2], Núria López-Bigas[2], Maxim Frolov[1]. 1) Department of Biochemistry & Molecular Genetics, University of Illinois at Chicago, Chicago, IL; 2) Department of Experimental and Health Sciences, Barcelona Biomedical Research Park, Univ Pompeu Fabra, Barcelona, Spain.

The E2F family of transcription factors regulates the expression of both genes associated with cell proliferation, and genes that regulate cell death. The net outcome is dependent on cellular context, and tissue environment. The mir-11 gene is located in the last intron of the Drosophila E2F1 homolog gene, dE2f1, and its expression parallels that of dE2f1. We investigated the role of miR-11, and found that miR-11 specifically modulated the pro-apoptotic function of its host gene dE2f1. A mir-11 mutant was highly sensitive to dE2F1-dependent, DNA damage-induced apoptosis. Consistently, co-expression of miR-11 in transgenic animals suppressed dE2F1-induced apoptosis in multiple tissues, while exerting no effect on dE2F1-driven cell proliferation. Importantly, miR-11 repressed the expression of the pro-apoptotic genes rpr and hid, which are directly regulated by dE2F1 upon DNA damage. In addition to rpr and hid, we identified a novel set of cell death genes that were also directly regulated by dE2F1 and miR-11. While some of these genes displayed increased enrichment for dE2F1 following irradiation, some did not, suggesting a different context for their regulation. Thus, our data support a model in which the coexpression of miR-11 limits the pro-apoptotic function of its host gene, dE2f1, upon DNA damage by directly modulating a dE2F1-dependent apoptotic transcriptional program. Furthermore, dE2F1 and miR-11 may intersect in the regulation of cell death genes in contexts beyond irradiation-induced apoptosis.

813C

Genetic analysis of a pseudogene and its parent gene in Drosophila melanogaster. G. Elizabeth Sperry, Denise V. Clark. University of New Brunswick,

Fredericton, New Brunswick, Canada.

Pseudogenes are generally considered to be non-functional due to mutations. However, some pseudogenes are transcribed and functional. For instance, in flies and mice, some pseudogenes play a role in regulating the expression of closely related genes through the endogenous small interfering RNA (siRNA) pathway. This work focuses on *CR14033*, a pseudogene copy of *CG9203*. Both genes are highly maternally expressed and *CG9203* has a domain associated with DNA repair. Compared to *CG9203*, the *CR14033* coding region has nonsense and frameshift mutations yet it has retained 73% nucleotide sequence identity with *CG9203* across 41% of its transcript length. High throughput sequencing of RNAs bound to AGO2 identified small RNAs with sequences shared between *CR14033* and *CG9203*. Furthermore, the level of intact *CG9203* transcript is enhanced in the male germline of a *Dcr-2* mutant, supporting a role for the siRNA pathway in its regulation (Czech *et al.* 2008). We are examining the functions of *CG9203* and *CR14033* using P element excision to make deletion mutations. Of the 19 independent excisions of $P\{SUPor\text{-}P\}CG9203^{KG05829}$, all but one are viable. Two P element insertions were used to generate excisions from the *CR14033* region. For one P element insertion, $P\{SUPor\text{-}P\}tkv^{KG01923}$, 15 independent excisions were generated and 11 of these have a similar large clear-body 3rd instar larval lethal phenotype. *CR14033* lies in an intron of thickveins, yet we found that the 11 lethal alleles complement a *tkv* allele. For the other P element insertion, $P\{lacW\}l(2)k01302^{k01302}$, 24 independent excisions are all viable when uncovered by a deficiency of the *tkv* region. PCR amplification of genomic DNA from the 11 lethal mutant homozygotes using primers flanking the P element insertion site shows variations in product size. Further mapping of these mutations, combined with sequencing and transcript analysis, is now being done to determine if they are indeed lethal alleles of *CR14033*.

814A

The roles of *Drosophila RNase* Z^L in mitochondrial function and dysfunction. Xie Xie, Veronica Dubrovskaya, Nancy Yacoub, Tara Gleason, Joanna Walska, Edward Dubrovsky. Biological Sciences, Fordham University, Bronx, NY.

Drosophila RNase Z^L (*dRNaseZ*) encodes a member of the ELAC1/ELAC2 protein family with homologs in every living organism. Previously, we showed that dRNaseZ is involved in nuclear and mitochondrial tRNA 3'-end maturation and is essential for viability. Tissue-specific knockdown of dRNaseZ affects cell proliferation in imaginal discs and cell growth in endoreplicating salivary glands. In this study, we analyzed the subcellular localization of dRNaseZ, and identified the functional mitochondrial targeting signal (MTS) and nuclear localization signal (NLS). Using GAL4/UAS system, we ubiquitously expressed a mutant construct lacking MTS ($RNZ^{\Delta MTS}$) in *dRNaseZ* knockout animal. $RNZ^{\Delta MTS}$ larvae arrested their development at the third larval instar and survived for at least 2 weeks with reduced body mass. As expected, these larvae display a strong reduction in mitochondrial activity; however, they have a 3-fold increase in mitochondrial DNA level. Since the mitochondrial genome encodes 22 tRNAs that are transcribed within long polycistronic transcripts separating the mRNA and rRNA genes, precise endonucleolytic cleavages before and after tRNA sequences are required to yield the mature mRNA/rRNA transcripts. Northern blot analysis revealed that mitochondrial specific knockout of *dRNaseZ* affects not only the generation of mature tRNA, but also affects the processing of mitochondrial mRNA and rRNA molecules. We therefore conclude that the mitochondrial dRNaseZ is responsible for the normal functionality of this organelle.

815B

The role of piRNAs in genome stability and germline maintenance in *Drosophila virilis*. Mauricio Galdos[2], Chris Harrison[1], Kim Box[1], Michelle Wickersheim[1], Christine Yoder[1], Jianwen Fang[3], Justin Blumenstiel[1]. 1) Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS; 2) Molecular Biosciences, University of Kansas, Lawrence, KS; 3) Applied Bioinformatics Lab, University of Kansas, Lawrence, KS.

Transposition, natural selection and RNA silencing are three forces that jointly determine transposable element (TE) proliferation. piRNAs are a class of small RNAs that are essential for epigenetic regulation of TEs in animals. They are primarily found in the germline, where they control TE mobilization through a Dicer-independent pathway. In our current study, we investigate the role of piRNAs in protecting the germline using the hybrid dysgenesis system in *Drosophila virilis*. In this system, when male flies with a higher TE copy number are mated with females that have a reduced copy number, progeny become sterile. This is likely due to TE mobilization in the germline. In this particular system, we have found that protective mothers have much higher levels of piRNA abundance corresponding to TEs that mobilize in the progeny, including the telomeric TART elements. We have also found that TEs appear generally destabilized in the inducer strain. Furthermore, we have determined that the degree of hybrid sterility is highly dependent on the dose of inducer chromosomes, either present in the mother, or inherited from the father. These results suggest that genome instability can be an inherent feature that can be transmitted across generations in an epigenetic and dose dependent manner.

816C

U bodies respond to nutrient stress in *Drosophila*. MICKEY BUCKINGHAM, JI-LONG LIU. MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom.

The neurodegenerative disease spinal muscular atrophy (SMA) is caused by mutation of the *survival motor neuron 1 (SMN1)* gene. Cytoplasmic SMN protein-containing granules, known as U snRNP bodies (U bodies), are thought to be responsible for the assembly and storage of small nuclear ribonucleoproteins (snRNPs) which are essential for pre-mRNA splicing. U bodies exhibit close association with cytoplasmic processing bodies (P bodies), which are involved in mRNA decay and translational repression. The close association of the U body and P body in *Drosophila* resemble that of the stress granule and P body in yeast and mammalian cells. However, it is unknown whether the U body is responsive to any stress. Using *Drosophila* oogenesis as a model, this study compares the morphology of U bodies and the U body-P body interaction between well-fed and starved *Drosophila*. Here we show that U bodies increase in size following nutritional deprivation. Despite nutritional stress, U bodies maintain their close association with P bodies. Our results show that U bodies are responsive to nutrition changes, presumably through the U body-P body pathway.

817A

Characterization of in vivo targets of the nuclear RNA-binding protein Lark during oogenesis. Christopher Ferrari[1], Gerard McNeil[1,2]. 1) Doctoral Program in Biology, The Graduate Center, The City University if New York, New York, NY; 2) Department of Biology, York College, The City University if New York, Jamaica, NY.

Early Drosophila development is controlled by maternal gene expression. It is these maternally expressed gene products that are required for development

of the oocyte and establishing the body axes. Many maternal effect genes have been identified and characterized, one of these genes being *lark*. This gene encodes a nuclear RNA-binding protein that has been shown to be essential for oogenesis. Elimination of the *lark*+ maternal component results in visible defects in the organization of the actin cytoskeleton resulting in a 'dumping' defect and female sterility. Since Lark is a nuclear RNA-binding protein it likely functions by regulating RNA splicing or nuclear-cytoplasmic transport. To date, we have currently identified 38 potential RNA targets using a RIP-CHIP approach. *Dmoesin* (*Dmoe*), an actin-binding protein, and *Transportin* (*Trn*), a proposed protein transmembrane transporter, were found to be two potential RNA targets of Lark. We have been particularly interested in *Dmoe* as an in vivo target since it is an actin-binding protein and its protein localization to the developing oocyte is perturbed in *lark* mutants. We have taken an RT-PCR approach to identify potential defects in RNA splicing of *Dmoe* and *Trn* in *lark*[1] mutants. Here we show that expression of *Trn* and *Dmoe* during oogenesis is perturbed in the absence of *lark* maternal expression.

818B

An analysis of maternally contributed mRNAs in early Drosophila embryogenesis and germ cell specification. Michelle A Kowanda[1], Stephanie Yee[1], Eric Lécuyer[2], Paul Lasko[1]. 1) McGill University, Montreal, Canada; 2) Institut de recherches cliniques de Montréal, Montreal, Canada.

Germline specification by the localization of particular mRNAs at the posterior of the embryo ensures the generation of primordial germ cells, also known as pole cells, that later combine with somatic cells to create the gonads. In two screens a number of mRNAs have been found to localize at the posterior of the embryo, some of whose function in pole cell development have not been determined. Additionally, a mechanism of active transport has been found to localize a subset of germ plasm mRNAs to the pole cells. Active transport along astral microtubules creates RNA islands in stage 3 embryos, ensuring that specific mRNAs are incorporated into the primordial germ cells. Interestingly, many well-characterized mRNAs that perform crucial roles in pole cell development are recruited to RNA islands. Stephanie Yee and I have been investigating the localization of 43 D. melanogaster posterior mRNAs in two other Drosophila species, to utilize the conservation of localization as a potential indicator of their requirement in germline development. We found so far that at least 37 of these 43 mRNAs localized at the posterior pole in D. simulans and 25 are localized in D. virilis, with complete overlap between the mRNAs that localize in both species. I have chosen CG18446, CG5292, Bsg25D and gcl for further studies from the list of conserved RNA island localizing mRNAs. Preliminary studies were started on the CG18446 mutant and no pole cell defects have been observed. This research will identify novel Drosophila primordial germ cell specification genes by investigating poorly characterized posterior mRNAs in early embryogenesis.

819C

Genome wide analysis of mRNA sub-cellular localization in embryos and larvae, with a focus on all the *Drosophila* Nuclear Receptors. Ronit Wilk[1,2,3], Jack Hu[1,2], Henry Krause[1,2,3]. 1) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada; 2) Banting and Best Department of Medical Research, University of Toronto, Toronto, ON, Canada; 3) Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada.

Transcript localization controls essential biological processes, including, cell migration, cell polarity, tissue morphogenesis and neuronal function. Thus, the sub-cellular visualization of transcripts *in situ* (in their original place) is an important tool to infer and understand their trafficking, stability, translation and biological functions. Here, we describe the continuation of a genome-wide screen to analyze mRNA localization and expression throughout the development of *Drosophila* embryos (*Lecuyer et al, Cell 131, 2007*) and the beginning of a similar screen in larval tissues. We use a high-resolution fluorescent *in situ* hybridization technique that allows us to determine sub-cellular information of each transcript. This is followed by data annotation and entry into a searchable public database *http://fly-fish.ccbr.utoronto.ca/*. To date, we have screened about 45% of the fly genome in embryonic stages: ~70% of these transcripts are localized sub-cellularly. We have found dozens of novel RNA localization patterns and we see a high correlation between mRNA localization and protein function. As a pilot study to initiate a genome wide screen in larval tissues, we are analyzing all *Drosophila* Nuclear Receptors. Nuclear Receptors are ligand-regulated transcription factors that play important roles in gene regulation and control key metabolic steps and several developmental pathways. *Drosophila melanogaster* has only 18 Nuclear Receptor genes and they all have vertebrate homologues. Despite their function as transcription factors, preliminary findings are revealing a variety of interesting sub-cellular localization events. Our long term goal is to screen the entire *Drosophila* genome for mRNA localization in selected larval tissues and to complete the ongoing embryonic screen.

820A

The role of the NMD pathway in endogenous gene regulation. Alex Chapin, Mark Metzstein. Human Gen, Univ Utah, Salt Lake City, UT.

The nonsense mediated mRNA decay (NMD) pathway functions to degrade mRNA transcripts harboring nonsense mutations. This regulation allows cell to silence mutant alleles that would encode for truncated and possibly toxic proteins. Additionally, NMD also functions to degrade many native transcripts. For instance, the wild-type transcripts of the genes *tra* and *Oda* have been shown to be directly targeted by NMD. Genetic disruption of core NMD components leads to inviability and upregulation of a significant portion of the transcriptome. It is not currently known which of these upregulated genes are direct targets of NMD and whether overexpression of NMD targets is responsible for inviability in NMD mutants. We have taken several approaches to address these two questions. We have performed a tissue-specific rescue screen to look for tissues where NMD function is vital for whole animal viability. These results indicate that in larval stages, NMD function in neuronal tissue is sufficient for viability, suggesting that critical targets of NMD may be of neuronal origin. To identify such neuronal NMD targets, we performed RNA-seq on RNA isolated from L3 larvae harboring a strong hypomorphic allele of the NMD gene *Upf2*. Our data show that a candidate NMD target, *Arc1*, is upregulated > 7 fold in NMD mutants. *Arc1* is expressed in neuronal tissue and is a target of NMD in mammals. Corroborating this result, deficiencies that uncover *Arc1* rescue the subviability of NMD mutants. We are currently examining whether *Arc1* lies downstream of NMD regulation to influence viability. To ask whether *Arc1* and other upregulated genes in our *Upf2* mutant are direct targets of NMD, we performed a mRNA decay timecourse in S2 cells, ± cycloheximide, an inhibitor of translation and NMD. We are currently examining whether the transcripts upregulated in *Upf2* mutants are stabilized by cycloheximide in cell culture. In addition, we are optimizing a transgenic system in vivo to identify direct targets of the NMD pathway by identifying genes whose stabilities are reduced by reintroduction of *Upf2* in a *Upf2* mutant.

821B

A suppression screen for required targets of the nonsense mediated mRNA decay pathway. Jonathan O. Nelson, Mark M. Metzstein. Department of Human Genetics, University of Utah, Salt Lake City, UT.

Nonsense mediated mRNA decay (NMD) is a cellular quality control pathway that degrades mRNA which contain premature termination codons (PTCs). The predominant role of NMD is to identify PTC containing transcripts during and target them for degradation. In addition to its quality control function, NMD targets many native transcripts as a mechanism of post-transcriptional regulation. NMD is required during development for larval viability, possibly due to the misregulation of native NMD targets. Very few genes have been identified as being directly regulated by NMD, and their misregulation fails to explain the requirement for NMD during development. Identifying genes whose regulation by NMD are necessary for proper development, and the function of these genes, may provide insight into important developmental pathways and the role of NMD in these pathways.

To identify genes regulated by NMD we performed a suppressor screen using heterozygous deficiencies. In theory, a deficiency should lower the transcription of mRNAs within the deficiency region by half. Thus, if lethality in an NMD mutant is due to over-expression of a target gene, transcriptional reduction by the deficiency may suppress lethality in the mutant. To perform this screen, we used a strong hypomorphic allele of a core NMD gene, $Upf2^{25G}$, and were able to identify several deficiencies that suppress lethality in this mutant. Assuming that any key target will be over-expressed in NMD mutants, we examined expression levels of genes uncovered by rescuing deficiencies using whole genome RNA expression profiling of $Upf2^{25G}$. Using this procedure we have identified a candidate gene, *Arc1*, which may be a key target of NMD.

Arc1 functions in stress response pathway, and NMD may inhibit *Arc1* expression to prevent an excessive stress response, which may cause lethality. We are currently investigating how NMD regulates *Arc1* expression, through promoter elements or transcript features, in addition to the physiological role for *Arc1* regulation by NMD in the stress response pathway.

822C

Exploring *Drosophila* Genes Involved in the Oxidative Stress Pathway and the Response to Hypergravity. Husein Badani, Oana Marcu, Ravikumar Hosamani, Sharmila Bhattacharya. NASA Ames Research Center, Moffett Field, CA.

In order to investigate the effects of hypergravity on the mushroom body in *Drosophila melanogaster*, it is important to monitor behavioral changes in knockout fruit flies. The brain is known to respond to hypergravity and studies suggest that the neurons in the brain undergo oxidative stress. Genes involved in the oxidative stress pathway are knocked out in neuronal populations of adult flies using the GAL4-UAS system to drive the RNAi for each particular gene. Here, we exposed *Drosophila* to a hypergravity environment by centrifuging the organisms at 3g, and we monitored changes with climbing assays. Results depicted that select experimental samples are affected by hypergravity conditions. Genetic lines that show significant alterations will be further studied using climbing assays and behavior analysis to reveal a complete understanding of the oxidative stress pathway.

823A

Characterization of TSE and its Role in the piRNA Pathway. Arlise P. Andress, Yanxia Bei, Richard Carthew. Molecular Biosciences, Northwestern University, Evanston, IL.

Previous studies have shown that the nuage ensures the repression of transposable elements in female germ cells. The RNA helicase, Spindle-E (SPN-E), localizes to the nuage and affects localization of other nuage components, such as the Piwi protein, Aubergine. It is also essential for the production or stability of piRNAs that silence transposons. In order to gain a better understanding on how SPN-E does this, a combination of immunoprecipitation and mass spectrometry were utilized to identify proteins that co-purify with SPN-E. One of the top copurified proteins is the subject of this presentation. We identified a mutation in the gene encoding this protein and have named it trapped SPN-E (TSE). Mutants are female sterile and show defects in gurken protein localization and oocyte positioning. They also show elevated expression of I-element. Intriguingly, the TSE mutant affects SPN-E protein localization in the ovary. SPN-E protein appears localized to a disorganized nuage and is depleted in other regions of germ cells. We suggest that TSE is required for the proper localization of SPN-E and execution of transposon silencing.

824B

Using functional proteomic approach to study Spindle-E function. Yanxia Bei[1], Bryan Fonslow[2], Arlise Andress[1], John Yates[2], Richard Cathew Cathew[1]. 1) BMBCB, Northwestern Univ, Evanston, IL; 2) Department of Chemical Physiology, SR11 The Scripps Research Institute La Jolla, CA.

Transposable elements impose a serious threat to genomic integrity. Animals have thus evolved a small RNA based piRNA (piwi-interacting RNAs) mechanism to combat the replication and mobilization of transposable elements. Spindle-E (Spn-E) is an RNA helicase whose function is crucial for the germline piRNA synthesis/stability but its molecular function is still unknown. To define Spn-E function in the piRNA pathway, we expressed a functional epitope-tagged Spn-E protein under its own promoter in spn-e mutant flies. We then performed immunoprecipitation followed by MudPIT mass-spectrometry to identify Spn-E interacting proteins. We found that Spn-E associates with some but not all of the known piRNA components. From these results together with the Spn-E localization data in different piRNA mutants, we propose that Spn-E localizes to the nuage, an electron-dense perinuclear structure that is found in all animal germ cells, in a complex that includes a few piRNA components and RNAs. From this proteomic approach, we were also able to identify two top Spn-E interactors as novel components involved in transposable elements silencing. Mutations in genes encoding either of these proteins changed localization of Spn-E and also resulted in the expression of transposable elements protein. One of them, TSE, will be presented in a separate poster, abstract control #: 70643. We are currently investigating how these two proteins interact with Spn-E to suppress transposable elements in Drosophila germ cells.

825C

Germline Silencing of Transposable Elements. Sidney Wang, Kiri Ulmschneider, Sarah Elgin. Biology, Washington University in St Louis, St Louis, MO.

Transposon control is a critical process during reproduction. The PIWI family proteins can use a piRNA-mediated slicing mechanism to suppress transposon activity. In *Drosophila melanogaster*, Piwi is predominantly localized in the nucleus, and has been implicated in heterochromatin formation. Here we use female germline-specific depletion to study Piwi function. Depletion of Piwi leads to infertility and to axis specification defects in the developing egg chambers; correspondingly, widespread loss of transposon silencing is observed. Transposon expression analysis shows that certain transposons require Piwi for silencing but not Aubergine. Piwi-Aub double knockdown results suggest that depending on the target transposons, germline Piwi can function either in the same pathway as Aub or independent of Aub. Germline Piwi does not appear to be required for piRNA production. Instead, Piwi requires Aub for proper nuclear localization. We observe a loss of HP1a and H3K9me2 in germline Piwi-depleted ovaries at the promoter region of

transposons that require Aub for silencing. Germline HP1a depletion leads to a loss of silencing for the same set of transposons. Considering our results and those of others, we infer that germline Piwi functions downstream of secondary piRNA production to promote silencing of some transposons via recruitment of HP1a. On the other hand, germline Piwi could also function in silencing certain transposons independent of Aub; the mechanism there remains unclear. In addition to *piwi*, many other genes have been implicated in piRNA-directed silencing. Two of these, Armi and Squ, have been shown to co-IP with Piwi, and therefore might play a role in Piwi-dependent transposon silencing. Using germline specific knockdown, we are determining whether loss of these proteins results in deregulation of the same transposons as loss of Piwi. We are also examining how the chromatin marks at transposon loci are affected in the knockdowns. Understanding the role of *armi* and *squ* should clarify the mechanism of Piwi-dependent transposon silencing. Supported by NIH GM068388 to SCRE.

826A

The Control of Lipid Metabolism by mRNA Splicing in Drosophila. Nicole M Chichearo[1], Michelle E Warren[3], Robert M Gingras[3], Thomas Carr[2], Timothy Rudolph[2], Justin R DiAngelo[3], Alexis Nagengast[2]. 1) Dept Biology, Widener University, Chester, PA; 2) Dept Biochemistry, Widener University, Chester, PA; 3) Dept Biology, Hofstra University, Hempstead, NY.

The fat body of Drosophila responds to different nutrient conditions and controls overall energy metabolism by regulating long-term storage of triglycerides in structures called lipid droplets. Therefore, the fat body in the fly serves a function similar to the liver and adipose tissues in mammals. Recent genome-wide RNAi screens in Drosophila tissue culture cells have identified mRNA splicing factors such as the Serine-Arginine (SR) domain containing proteins B52 and U2AF-50 as playing a role in lipid droplet formation; their decreased expression results in the production of fewer lipid droplets. Using conditional RNAi knock down experiments under GAL4-UAS control in the fat body of larvae, we have identified several early splicing factors that control lipid storage in vivo on both high and low nutrient food sources. Larvae raised on both food sources demonstrate a visibly lean phenotype and developmental delay with decreased expression of U1-70K, U2AF-50 or U2AF-38 and prp19 in the fat body and this lean phenotype corresponds to a significant decrease in triglyceride levels as measured by quantitative colorimetric assays. Decreased triglyceride levels also are observed in adult females undergoing RNAi of the same splicing factors in the fat body when raised on the high nutrient food. Interestingly, knock-down of the SR protein 9G8 in larval fat body is male lethal on both high and low nutrient food sources but leads to increased triglycerides on low nutrient food and decreased on high nutrient food. To further understand these defects in lipid storage, we are taking a candidate gene approach to identify potentially alternatively spliced genes important for lipid metabolism. Through these experiments we hope to gain insight into the mechanisms underlying tissue-specific splicing in the fat body and how the alternative splicing of important lipid metabolic genes leads to proper fat storage.

827B

A truncated *Drosophila dADAR* mRNA isoform which is evolutionarily conserved but not translated into protein potentially regulates full-length isoform expression during embryogenesis. John A Cook, Lea N Chhiba, Dana L Doctor, Jack C Vaughn. Zoology, Miami University, Oxford, OH.

Adenosine Deaminases Acting on RNA (ADARs) function to deaminate (edit) some adenosines to inosines in selected pre-mRNAs. The *ADAR* gene in *D. melanogaster* (*dADAR*) produces two major mRNA transcript classes, full-length (FL) and truncated (T). The FL-class has been functionally well characterized and encodes a catalytic protein in the adult brain, but an inactive protein in embryos. Virtually nothing is known about the T-class isoform, which in *D. melanogaster* has been fully sequenced, lacks a catalytic domain, contains a complete open reading frame, a stop codon in intron 6, a poly(A)-tail, a canonical poly(A)-signal, and is translated in an *in vitro* system. During fly development, levels of the two mRNA isoform classes are inversely related. Here, we show *via* 3'-RACE and sequencing that the T-class mRNA transcript is present in every *Drosophila* species studied, extending back to those diverging 40 million years ago. In every species studied, the sequenced transcripts terminate in intron 6, have a stop codon, a poly(A)-tail, and a poly(A)-signal. Utilization of Westerns employing a polyclonal anti-dADAR antibody produced using full-length protein shows that the FL-class isoform in *D. melanogaster* encodes a protein with the expected molecular weight at every developmental stage. Quantification of these results shows that the FL-class protein isoform is most abundant between 0-4 h of embryo development, then gradually declines during embryogenesis. Unexpectedly, the T-class mRNA isoform does not appear to encode a protein during any embryonic stage of development in *D. melanogaster*. This was a surprise, since this mRNA isoform class is present in every species studied, and is highly abundant throughout embryogenesis, suggesting that it has a conserved function. Taken together, these observations lead us to hypothesize that T-class *dADAR* mRNA may perform a novel regulatory function, perhaps playing a role in FL-class isoform expression during embryogenesis.

828C

Examining the role of the fat body in the ovarian response to diet. Alissa R Armstrong[1], Leesa LaFever[2], Kaitlin Laws[1], Robert Cole[3], Daniela Drummond-Barbosa[1]. 1) Biochemistry and Molecular Biology, Bloomberg School of Public Health, Baltimore, MD; 2) Division of Experimental Hematology and Cancer Biology, Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, OH; 3) Mass Spectrometry and Proteomics Facility, Johns Hopkins School of Medicine, Baltimore, MD.

Nutritional status is sensed by many tissues and must be coordinated properly for appropriate cellular responses. In many organisms, modulation of fertility by diet ensures that energy and building blocks used to generate gametes are not expended when resources are limited. Our past work showed that *Drosophila* females fed a yeast-free diet have reduced proliferation rates of germline stem cells (GSCs), follicle stem cells (FSCs) and their progeny, increased early cyst death and a block in vitellogenesis. We also showed that insulin, Target of rapamycin (TOR) and ecdysone signaling act in the ovary to mediate these effects. However, it remains largely unclear how the fat body, a nutrient sensitive tissue with storage and endocrine roles, modulates the ovarian response to diet. Preliminary data from our lab revealed that altered TOR signaling or amino acid transport in the fat body affects egg production rates, and we are currently expanding these analyses. We also performed a quantitative iTRAQ proteomics comparison between the fat body from flies fed a yeast-rich versus switched for 12 hours to a yeast-free diet, and identified 27 putative secreted proteins that were either down-regulated or up-regulated. We are currently performing a functional screen of these candidates for a potential role in oogenesis. These studies will shed light on the interaction between the fat body and ovary during the response to diet and may identify specific factors secreted by the fat body that help transmit dietary information to the ovary.

829A

The LEM-D protein Otefin regulates niche signaling cascades to maintain female germline stem cell homeostasis. Lacy J. Barton[1], Belinda S. Pinto[2], Pamela K. Geyer[1]. 1) Dept Biochemistry, Univ Iowa, Iowa City, IA; 2) Whitehead Institute, MIT, Cambridge, MA.

Adult stem cell populations are supported by a specialized microenvironment or niche. The Drosophila female germline stem cells (GSCs) niche balances GSC maintenance and differentiation by tightly regulating the Bone-Morphogenetic-Protein (BMP) pathway. Production of the dominant BMP ligand, Decapentaplegic (Dpp), is transcriptionally controlled by Janus Kinase/Signal Transducer and Activator of Transcription (Jak/Stat) signaling. The LEM Domain (LEM-D) protein, Otefin, is required for both female GSC maintenance and germline differentiation; however the dual nature of these requirements remains poorly understood. Here we show that loss of Otefin results in over-expression of genes encoding Jak/Stat and BMP ligands, despite premature loss of the niche cells which normally produce Dpp. As a result, BMP signaling is activated in germline and somatic cells located far from the GSC niche. These effects block germline differentiation, generating small tumors of ten to fifty GSC-like cells. Further, restricted expression of *otefin* in somatic cells restores Jak/Stat and BMP ligand expression and rescues the block in germline differentiation. Together, these data suggest that Otefin regulates the somatic signaling pathways required for proper niche function. Otefin and other LEM-D proteins are part of an extensive nuclear lamina network that lies beneath the nuclear envelope. Mutations in genes encoding LEM-D proteins cause a collection of age-enhanced diseases which affect the mesenchymal tissues that depend upon resident adult stem cells. Our findings are consistent with emerging evidence indicating that nuclear lamina proteins have important roles in maintaining the homeostasis of adult stem cell populations.

830B

Investigating the Role of Hr39 in the Germline Stem Cell Lineage. Grace H. Hwang, Elizabeth T. Ables, Daniela Drummond-Barbosa. Dept. of Biochemistry and Molecular Biology, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD.

Proper regulation of stem cell maintenance and division coordinated with organismal physiology is important for tissue homeostasis. Steroid hormones, by binding to conserved nuclear hormone receptors, are important systemic regulators of cellular activity in diverse cell types in response to nutrient intake, but little is known about their direct effects on adult stem cells. The steroid hormone ecdysone regulates *Drosophila* ovarian germline stem cell (GSC) maintenance and proliferation via the ecdysone signaling target *E74*; however, additional targets likely also mediate this response. Nuclear hormone receptors, downstream of the ecdysone receptor, are potential candidate targets of ecdysone signaling, but whether ecdysone controls GSC activity through these hormone receptors is unclear. *Hr39* is a nuclear hormone receptor that is a target of ecdysone in larval tissues and has reported roles in the reproductive tract. We have obtained a collection of *Hr39* mutant alleles and are currently testing whether *Hr39* regulates the GSC lineage downstream of ecdysone signaling.

831C

***in vitro* analyses of cellular interactions among germline stem cells, cap cells and escort cells in *Drosophila*.** Yuzo Niki, Takuya Sato, Yusuke Iizumi. Dept. Biology, Faculty of Science, Ibaraki University, Mito, Ibaraki, Japan.

Drosophila germline stem cells (GSCs) and their niche cells comprise one of the best-characterized stem cell systems and are well suited to study of stem cell biology. The elucidation of the cellular and molecular mechanisms responsible for GSC behavior is hindered by the lack of suitable culture systems. Recently, we succeeded in establishing *Drosophila* larval ovarian cell lines of which primordial germ cells (PGCs) and niche cells are coexisted. In addition, we cloned stable cell lines originated from terminal filament and cap cells (named TCAP cells) from these cell lines. We analyzed self-renewal divisions under the living conditions and signal pathways occurring between TCAP and germ cells after co-culturing PGCs or GSCs with TCAP cells. We found that PGCs and GSCs proliferated stably for a long term, while their differentiation was suppressed. The Dpp signal pathway occurs normally as seen GSC/niche *in vivo*, which indicating that TCAP cells retains functional ability of niche cells. TCAP cells express wingless and hedgehog as do cap cells. Furthermore, we analyzed functional ability of escort cell lines (ESC) that we have established. Interestingly, ESC can differentiate male PGCs and GSCs as well as female GSCs *in vitro*. This *in vitro* reconstituted niche system will facilitate further elucidation of the molecular mechanisms responsible for the construction and maintenance of GSCs/niche system during normal development.

832A

PointedP1 connects the establishment and maintenance of intermediate neural progenitor cell fate in *Drosophila* neural stem cell lineages. Derek H Janssens[1,4], Hideyuki Komori[4], Xiao Qi[2,4], Cheng-Yu Lee[1,2,3,4]. 1) Cellular and Molecular Biology Graduate Program; 2) Department of Cell and Developmental Biology; 3) Division of Molecular Medicine and Genetics, Department of Internal Medicine; 4) Center for Stem Cell Biology, Life Sciences

Institute, University of Michigan Medical School, Ann Arbor, MI 48109.

Stem cells in numerous tissues give rise to transit-amplifying cells that divide a limited number of times to produce post-mitotic progeny. Homeostasis of these tissues requires that following stem cell division a fraction of the daughter cells are specified as transit-amplifying cells that go on to maintain their restricted identity. Failure of this process results in unconstrained expansion of the stem cell pool at the expense of differentiated cells. Neural stem cell lineages (Type II Neuroblasts) that produce transit-amplifying cells (intermediate neural progenitors (INPs)) in the *Drosophila* larval brain provide an extremely robust biological system for genetic analysis of this process. Previous studies have identified two classes of genes that are required to functionally distinguish INPs from there parental neuroblasts. In *brat* or *numb* mutants newly born INPs rapidly reacquire a neuroblast identity, thus establishment of an INP fate is dependent on these genes. In *erm* mutants INPs are properly established, but fail to maintain their restricted developmental potential and de-differentiate into neuroblasts. We find that heterozygous mutation of the transcription factor pntP1 dominantly enhances both *erm* and *brat* sensitized mutant backgrounds, indicating pntP1 is involved in both the establishment and maintenance of INP identity. PntP1 protein is enriched specifically within newly born INPs just prior to activation of erm expression. In addition, epistatic analysis reveals that pntP1 functions downstream of brat and likely functions upstream or in parallel to erm. This demonstrates that a signaling network, initiated in the neuroblast, persists within INPs to intrinsically establish and maintain their fate.

833B

The Landscape of Drosophila *cis*-Regulation as Revealed by *EvoPrinter* Analysis. Mukta R. Kundu[1], Alexander Kuzin[1], Tzu-Yang Lin[2], Chi-Hon Lee[2], Thomas Brody[1], Ward F. Odenwald[1]. 1) Neural Cell Fate Determinants, NINDS, Bethesda, MD; 2) Section on Neuronal Connectivity, NICHD, NIH, Bethesda, MD.

To gain insights into the diversity and structural complexity of *cis*-regulatory DNA within intergenic regions that are not nearby flanking genes, we have tested the *cis*-regulatory activities of 20 consecutive conserved sequence clusters (CSCs), identified by *EvoPrint* analysis (Yavatkar et al., 2008, BMC Genomics 9: 106), within a 28kb genomic region beginning 23kb downstream of *ventral veins lacking/drifter* gene and separated from the flanking gene by 67kb. Enhancer/reporter analysis reveals a diversity of *cis*-regulatory functions associated with these CSCs, including CNS, PNS, ventral midline, epidermal and trachea enhancers that are active in the embryo, larva and/or adult. Two of the neural enhancers share conserved POU-homeodomain binding sites, a common signature of late temporal neuroblast network enhancers such as those regulating *castor* and *grainyhead*, and each drives expression in distinct sets of bilaterally symmetrical larval CNS neurons. Another identified enhancer drives reporter expression in the embryonic and larval ventral cord CNS midline glia. Analysis of this CSC revealed the presence of four conserved binding sites for the midline determinant Single-minded. Both tracheal and midline enhancers appear to consist of two sub-modules, revealed by the clustered distribution of repeat sequences within these enhancers and the observation that these modules exhibit DNaseI accessible embryonic genomic DNA (Thomas et al., 2011, Genome Biol. 12: R43) within a restricted region of their CSCs. Based on these findings, we conclude that most of the ~100,000 CSCs within the *Drosophila* genome that flank transcribed DNA represent *cis*-regulatory modules, and that phylogenetic footprinting via *EvoPrinter* is an invaluable tool for the discovery and further analysis of these sequences. We are currently using these observations to test the relationship of DNaseI hypersensitivity to CSC enhancer function.

834C

The *Drosophila* gene *clueless* is required for mitochondrial function and dynamics in larval neuroblasts. Aditya Sen, Vanessa Damm, Rachel Cox. Biochemisty and Mol. Biology, Uniformed Services Univ., Bethesda, MD.

Mitochondria are double membranous organelles responsible for making ATP. They are highly dynamic, changing location, shape and numbers, characteristics that are important to maintain the structure and functional integrity of the organelles, as well as maintain cellular health. There appears to be a link between mitochondrial dysfunction and neurodegenerative diseases, such as Alzheimer's diseases (AD) and Parkinson disease (PD). To study genes important for mitochondrial function and dynamics, we have taken a candidate gene approach and identified and characterized the *Drosophila* gene *clueless* (*clu*). *Drosophila* Clueless protein shares 53% overall identity to its uncharacterized human homolog (KIAA0664), and shows even greater (85%) identity in one of its hypothetical domains, called the 'Clu' domain. In female germ cells, *clu* mutants have mislocalized mitochondria, and are sterile. Because *clu* mutants are uncoordinated and *clu* genetically interacts with *parkin*, we are examining mitochondrial dynamics during neurogenesis in wildtype and *clu* mutant brains. We have found that NBs contain high levels of Clu and large numbers of small, spherical mitochondria. The normal wildtype distribution pattern of mitochondria during the cell cycle is disturbed in *clu* mutant NBs. Mitochondria tend to clump, a phenotype also seen in female germ cells. We are currently creating maternal/zygotic *clu* mutants to determine if they will experience NB loss. To identify Clu's molecular mechanism, we are testing Clu function in S2R+ insect cells and have found that mitochondrial distribution is affected in *clu* knockdown cells, similar to NBs and germ cells. We are carrying out a structure function study using both transgenic flies and knockdown S2R+ cells and have found that the 'Clu' and the'TPR' (tetratricopeptide repeat) domains, are important for Clu function. Due to Clu's high sequence identity among species, we believe that our findings about Clu function and mitochondrial dynamics in neuroblasts could be of general importance for stem cells.

835A

***Klumpfuss* (*klu*) encodes a novel regulator of neuroblast identity during larval brain neurogenesis.** Qi Xiao[1,3], Cheng-Yu Lee[1,2,3]. 1) Department of Cell and Developmental Biology; 2) Division of Molecular Medicine and Genetics, Department of Internal Medicine; 3) Center for Stem Cell Biology, Life Sciences Institute, University of Michigan Medical School, Ann Arbor, MI 48109.

Precise distinction of the daughter cell potential following asymmetric stem cell divisions is essential for preserving the stem cell pool and generating sufficient post-mitotic progeny, but the mechanisms are unclear. We study how the daughter neuroblast and progenitor cell are functionally distinguished during asymmetric divisions of type II neuroblasts in which the basal protein Brain tumor (Brat) plays a central role. We identified *klu* as a dominant suppressor of the ectopic type II neuroblast phenotype in a sensitized *brat* mutant genetic background. *klu* encodes a C2H2 zinc-finger transcription factor, and the Klu protein is detectable in all brain neuroblasts. Neuroblasts in *klu* mutant brains undergo premature differentiation, indicating that *klu* is necessary for maintenance of neuroblast identity. Immature intermediate neural progenitor cells over-expressing Klu fail to acquire restricted potential and assume the progenitor cell fate, and instead, rapidly reacquire the type II neuroblast identity, strongly suggesting that Klu is sufficient to promote the neuroblast fate. Domain analyses reveal that both a novel central domain and the zinc fingers are necessary for Klu to promote the neuroblast fate. This result suggests that

Klu might require additional co-factor proteins to regulate transcription of its target genes. Finally, loss- and gain-of-function analyses indicate that *klu* is epistatic to *brat*. Together, these results lead us to propose that Brat-dependent inactivation of the Klu function provides a precise mechanism to distinguish neuroblasts from intermediate neural progenitors ensuring a steady pool of neuroblasts and rapid generation of post-mitotic progeny during larval brain neurogenesis.

836B

Piwi is a key regulator of the testicular stem cell niche in *Drosophila*. Jacob M. Gonzalez, Haifan Lin. Yale Stem Cell Center, Yale University, New Haven, CT.

The Piwi protein in *Drosophila* regulates a wide range of processes, which include transposon silencing, epigenetic programming, and stem cell self-renewal. Our lab previously showed that Piwi functions are critical for niche signaling and intrinsic mechanisms to promote *Drosophila* ovarian germline stem cell (GSC) division. Here we aim to clarify Piwi's molecular activities in the niche of *Drosophila* testis stem cells. The conventional model for niche function in the *Drosophila* testis is that a group of somatic cells called hub cells produce local signals to promote their adjacent stem cells to self-renew. Piwi is expressed in both the germline and soma, which include the hub cells and somatic cyst stem cell (CySC) lineage. We showed that reducing levels of Piwi in the hub cells via RNAi does not affect GSC maintenance or differentiation. Using cell-type-specific genetic mosaic analysis, we found that Piwi is required autonomously for the maintenance of the two resident stem cell populations in the testis, GSCs and CySCs. Furthermore, we showed that germline defects found in *piwi* mutant testis is rescued by restoring Piwi expression specifically and only in the CySCs, but not in the hub. Interestingly, reducing levels of Piwi in CySCs causes a large expansion of stem cell-like cells. Our findings demonstrate that Piwi functions in CySCs nonautonomously regulate GSC differentiation. We are currently investigating whether Piwi-mediated signaling from CySCs genetically interacts with key signaling pathways, such as JAK-STAT or BMP, known to function in the male niche. Our work highlights the ability of Piwi to simultaneously serve as a cell-autonomous regulator for one type of stem cell (i.e. GSC) and as a niche-signaling regulator from another type of stem cell (CySC). In addition, it illustrates a novel facet of the complicated nature of the stem cell niche: one type of stem cell can serve as the niche signaling cells for a second type of stem cell in the same organ to coordinate the self-renewal divisions and differentiation of adult stem cells.

837C

Asrij maintains the hematopoietic stem cell niche, controls blood cell homeostasis and is required for *Drosophila* immunity. Rohan J. Khadilkar, Vani Kulkarni, Srivathsa M.S., Maneesha S. Inamdar. Molecular Biology and Genetics Unit, JNCASR, Bangalore, Karnataka, India.

The lymph gland is the hematopoietic organ that orchestrates the second wave of hematopoiesis in *Drosophila*. A small group of signaling cells which form the hematopoietic stem cell niche (Posterior Signaling Center) help in maintaining the hematopoietic progenitors in the medullary zone thereby regulating hematopoietic differentiation in the cortical zone. The maintenance of the stem cell niche, precursor hemocytes and the differentiated hemocytes is controlled by a host of conserved factors and signaling pathways but the mechanisms that modulate and integrate these signals are poorly understood. The conserved endocytic protein Asrij maintains the Stem Cell Niche and controls differentiation during *Drosophila* lymph gland hematopoiesis. *asrij* null mutants have a reduced niche, leading to loss of Domeless expression in the medullary zone and causing quiescent hemocyte precursors to differentiate prematurely. Conversely, excess of *asrij* in the lymph gland causes expansion of the niche and reduces differentiation. Niche maintenance is regulated by Serrate-mediated Notch signaling. Further *asrij* null mutants also show increase in crystal cell number, suggesting aberrant Notch signaling. Hence we investigated the status of Notch in *asrij* null mutants. *asrij* null mutants show endocytic retention of Notch Intracellular Domain, resulting in a gain-of-function phenotype and excess specification of crystal cells. Interestingly *asrij* null mutants also had reduced pro-phenoloxidase (PO) activity which could be rescued by crystal cell rupture. This suggests that endocytic function of *asrij* is required for PO release. Taken together our data suggests a role for Asrij in modulating signals that maintain the hematopoietic stem cell niche thereby affecting hemocyte differentiation.

838A

Control of *Drosophila* female germline stem cell niche formation by insulin signaling. Chun-Ming Lai, Hwei-Jan Hsu. Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan.

Stem cells are a small group of cells embedded in tissues with the capacity to produce differentiated cells for replenishing lost cells in fast-turnover or damaged tissues, thereby maintaining tissue homeostasis. Stem cells reside in the stem cell niche, a specialized microenvironment, which provides both physical contact and tissue-intrinsic signals to control stem cells. Stem cells and tumor cells share similar features of self-renewal and proliferation; interestingly, a hypothesis of a latent tumor cell niche for tumor initiation is also proposed. The regulation of the stem cell niche itself, however, is poorly understood. The *Drosophila* ovary is an excellent system to study stem cell biology, because of its ease of manipulation and well-characterized germline stem cells (GSCs) and their niches. The GSC niche formed by terminal filament cells and cap cells houses two to three GSCs. We have previously shown that insulin/insulin-like growth factor (IGF) signals mediate the effect of diet to directly control GSC niche cell survival. It is unclear, however, if insulin/IGF signaling also controls the formation of the GSC niche. To examine this, we diminished the expression of the insulin receptor in the somatic cells of developing ovaries using *UAS-RNAi* lines driven by specific *GAL4* drivers. Compared to the controls, flies that grew from insulin receptor-suppressed larvae produced fewer progeny two days after eclosure and thereafter. In addition, we also observed that those flies carry small ovaries which are composed of fewer ovarioles, functional units of ovaries, suggesting that insulin/IGF signaling controls the formation of terminal filament cells known to play a role in subdividing the developing ovary into ovarioles. If insulin signaling controls cap cell formation and GSC recruitment, and the mechanisms underlying these processes will be further investigated. Nevertheless, our results have provided new insights into the role of nutritional inputs on stem cell niche formation, and that may eventually reveal therapeutic intervention for cancer.

839B

Activin signaling affects niche formation and stem cell establishment in the larval gonad through interaction with Ecdysone signaling. Tamar Lengil, Lilach Gilboa. Biological Regulation, Weizmann Institute of Science, Rehovot, Israel.

The step-by-step formation of a three-dimensional organ is one of the most complex processes within a developing organism. In particular, coordination between proliferation and differentiation of the various cell types within the organ must be achieved. How such coordination is accomplished is largely

unknown. We are using the development of the ovary in the fruit fly Drosophila melanogaster to study organ formation. During the larval growth period, ecdysone signaling coordinates the proliferation and differentiation of two distinct cell populations: primordial germ cells (PGCs), the precursors for germ line stem cells (GSCs), and the precursors for their somatic niches. We demonstrate that Activin signaling is also required for soma-germ line coordination by modulating the ovarian response to ecdysone. Removing the type I Activin receptor, Baboon (Babo), or its down-stream effector Smox from the ovarian soma by RNAi results in significantly smaller gonads, with underdeveloped niches. Conversely, somatic over-expression of the constitutively active receptor (Babo*), results in larger ovaries, and precocious niche formation. PGCs also differentiate precociously in Babo* ovaries. Babo* expressing ovaries resemble ovaries in which the Ecdysone pathway is precociously activated. These gonads also demonstrate early precocious expression of Broad-Z1 isoform, a major target of the Ecdysone pathway. Similarly, removal of Smox or Babo from the ovarian soma by RNAi results in a significant decrease in Broad-Z1. Our results suggest a role for Activin signaling in niche formation and PGC differentiation. They also demonstrate how temporal cues might converge with growth and differentiation cues to coordinate these two processes.

840C

Characterization of the Follicle Stem Cell Niche. Pankaj G. Sahai-Hernandez, Todd G Nystul. Anatomy Dept., UCSF, san Francisco, CA.

Stem cells are responsible for maintaining different tissue types in a multicelullar organism. Stem cells are located in a specialized microenvironment termed a niche, which provides the appropriate signals for homeostasis. The follicle stem cells (FSCs) in the Drosophila ovary have been an informative model for investigating epithelial stem cell biology in vivo. Previous studies have identified several signaling pathways, including wingless, BMP and hedgehog, as well as cell adhesion complexes such as adherens junctions and integrins that are necessary for FSC maintenance. However, little is known about whether there is a cellular component to the FSC niche. FSCs directly contact the neighboring escort cells, a stromal cell population that regulates the first stages of germ cell development. FSCs must coordinate with escort cells and germ cells to ensure that new follicle cells are produced at the proper time and place, but it is unclear what role escort cells play in FSC niche function. To better understand the cellular architecture of the FSC niche, we characterized the shape and position of escort cells in the FSC niche region and investigated the role that these cells play in the production of niche signals. Our results suggest that the FSC niche receives signals from several sources and must be frequently remodeled to accommodate the passage of germ cell cysts through the germarium.

841A

magu is required for germline stem cell self-renewal through BMP signaling in the Drosophila testis. Qi Zheng[2,3], Yiwen Wang[1], Eric Vargas[1], Stephen DiNardo[1,3]. 1) Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2) Department of Biology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA; 3) Penn Institute for Regenerative Medicine, University of Pennsylvania, 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. It is known that the BMP and JAK-STAT pathways are necessary for the maintenance of GSCs in the testis (Kawase et al., 2004; Kiger et al., 2001; Schulz et al., 2004; Shivdasani and Ingham, 2003; Tulina and Matunis, 2001). However, our recent work strongly suggests that BMP signaling is the primary pathway leading to GSC self-renewal (Leatherman and DiNardo, 2010). Here we show that magu controls GSC maintenance by modulating the BMP pathway. We found that magu was specifically expressed from hub cells, and accumulated at the testis tip. Testes from magu mutants exhibited a reduced number of GSCs, yet maintained a normal population of somatic stem cells and hub cells. Additionally, BMP pathway activity was reduced, whereas JAK-STAT activation was retained in mutant testes. Finally, GSC loss caused by the magu mutation could be suppressed by overactivating the BMP pathway in the germline.

842B

The centrosome positioning checkpoint monitors centrosome interaction with cortical Bazooka. Mayu Inaba[1,2], Yukiko Yamashita[1,2]. 1) Center for stem cell biology, Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Cell and Developmental Biology, School of Medicine, University of Michigan.

Asymmetric cell division is widely utilized by many adult stem cells to balance self-renewal and generation of differentiated, short-lived cells. Mitotic spindle orientation relative to the surrounding tissue is a strategy utilized by many adult stem cells to divide asymmetrically. We recently demonstrated that GSCs with misoriented centrosomes do not enter mitosis until their centrosomes are re-oriented (Cheng et al.), pointing to the presence of a checkpoint mechanism that monitors correct interphase centrosome orientation prior to mitosis (the centrosome orientation checkpoint)(Inaba 2010, Yuan 2011). In this study, we demonstrate a novel function of a polarity protein Bazooka/Par-3 in the centrosome orientation and its checkpoint. Bazooka is localized at the hub-GSC interface forming a small “patch“, which closely associates (“engages”) with the apical centrosome in the late G2 cells right before mitotic entry. Overexpression of Baz, which leads to even cortical localization of Baz, results in ectopic engagement of centrosome and defect in the centrosome orientation checkpoint. Serine151 residue of Bazooka becomes highly phosphorylated in a Par-1 dependent manner, correlating with the association with the centrosome during late G2 phase. We further provide the evidence supporting that the correct orientation of the centrosome is monitored as the engagement between the Bazooka patch and the apical centrosome.

843C

Zfrp8/PDCD2 a new stem cell gene. Ruth Steward, Neha Changela, Svetlana Minakhina. Waksman Inst, Rutgers Univ, Piscataway, NJ.

The lymph gland is the hematopoietic organ of Drosophila. Drosophila hematopoiesis shows many similarities to human hematopoiesis and virtually all genes and pathways functioning in Drosophila blood formation also have an essential role in vertebrate hematopoiesis. We have shown that the embryonic and first instar larval lymph gland contains a few cells that have similar characteristics as vertebrate hematopoietic stem cells (HSCs). We have further found that the highly conserved Zfrp8/PDCD2 gene has an essential function in HSCs, but that it is dispensable in more mature cells. This work led us to study the function of Zfrp8 in other stem cells and we find that it is also required in both germline and somatic stem cells in the Drosophila ovary. Consistent with our findings, the vertebrate homolog PDCD2 has been shown to be essential for the viability of mouse embryonic stem cells. We propose that PDCD2/Zfrp8 has a conserved role in stem cells of different species.

844A

The role of the adiponectin receptor homolog in Drosophila melanogaster oogenesis. Kaitlin Laws[1], Leesa LaFever[2], Daniela Drummond-Barbosa[1,3]. 1) Department of Biochemistry and Molecular Biology, Division of Reproductive Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2) Division of Experimental Hematology and Cancer Biology, Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, OH; 3) Department of Environmental Health Sciences, 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 modulate 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 and obtained *CG5315* hairpin lines for RNAi, and are in the process of analyzing the 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.

845B

Elucidating the mechanism of asymmetric division within the epithelial follicle stem cell niche. Angela Castanieto, Todd Nystul. University of California San Francisco, San Francisco, CA.

Adult stem cells are maintained through the action of a specialized microenvironment, or niche, that facilitates asymmetric division. Detailed studies of the Drosophila male and female germline stem cell niches have provided valuable insight into the mechanism by which asymmetric stem cell division occurs in vivo. However, less is known about somatic stem cell niches in Drosophila and it is unclear whether somatic stem cells use a similar mechanism to self-renew. We used the epithelial follicle stem cells (FSCs) in the Drosophila ovary as a model for understanding the mechanism of asymmetric division in an epithelial stem cell. Precisely two follicle stem cells per germarium reside in defined locations against the basement membrane at the anterior edge of the tissue. Loss of components of the Bone morphogenetic protein (Bmp) pathway from FSCs causes rapid loss from the niche, indicating that this pathway is required for self-renewal (Kirilly et al, 2005). We found that loss of components of the Epidermal Growth Factor Receptor (EGFR) pathway causes early follicle cell differentiation defects. These findings have led us to investigate the involvement of the Bmp and EGFR pathways in the regulatory mechanism of FSC asymmetric division. Here we present our data in support of a model in which Bmp and EGF signaling promote asymmetric FSC division through regulation of follicle cell differentiation and cell polarity. These findings will further our understanding of asymmetric division in an epithelial niche.

846C

Apontic controls somatic stem cell numbers in the testis by inhibiting the JAK/STAT signaling pathway. Michelle Starz-Gaiano, Archana Murali, Kathryn Bus. Biological Sciences, University of Maryland Baltimore County, Baltimore, MD.

The molecular mechanisms governing adult stem cell maintenance within a microenvironment are incompletely understood, and are a central issue in regenerative biology. The Drosophila testis provides an ideal context for examining the genetic and molecular signals that balance stem cell self-renewal and differentiation. In the testis, germ line stem cells (GSCs) and somatic cyst stem cells (CySCs) are maintained by their association with niche cells, called the hub. Several laboratories have shown that activation of the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway is necessary maintaining both types of stem cells. We have found that Apontic (APT), a transcription factor and STAT signaling feedback inhibitor, is highly expressed in the somatic cells of the testis. 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*. In ovaries, *apt* mutant cells display altered adhesion and morphological properties, and parallel changes may explain delayed CySCs differentiation in the testis. Thus, additional CySCs in *apt* mutants may permit GSCs self-renewal by acting as a secondary niche or altering the architecture of the microenvironment. We propose that APT acts cell-autonomously in a genetic circuit to maintain CySC by restricting STAT signaling and Zfh-1 expression to cells in the niche. It may also act non-autonomously to organize the GSCs within the distal tip of the testis. This suggests a complex interplay between different types of stem cells maintain the appropriate number of each near the niche.

847A

Trio regulates midgut stem cell proliferation and differentiation. Longze Zhang, Heinrich Jasper. Biology Department, University of Rochester, Rochester, NY.

Somatic stem cells are critical for tissue renewal and maintenance. In recent years, studies on Drosophila midgut stem cells have provided important new insights into the regulation of stem cell proliferation and differentiation, as well as the role of stem cells in maintaining tissue homeostasis. In this study, we have identified a function for the RhoGEF protein Trio as a critical regulator of stem cell activity and function in the Drosophila intestine. Trio was identified in a screen for genes expressed specifically in the intestinal stem cell (ISC) lineage. It is expressed in both ISCs and the immediate daughter cell, the enteroblast (EB), but not in other cell types of the posterior midgut. Using MARCM, as well as ISC and EB-specific Gal4 drivers, we found that trio has distinct roles in ISCs and EBs. In stem cells, trio controls proliferation, being both sufficient and required for ISC division. At the same time, our data suggest that trio is required to sustain Delta expression in ISCs. In EBs, trio regulates cell fate. Trio has been implicated in N signal transduction in neurons, mediating non-canonical signaling events downstream of the N receptor. We are currently testing whether Trio acts in a similar fashion in EBs, thus regulating differentiation of EBs into ECs and/or enteroendocrine cells. As trio is conserved from Caenorhabditis elegans to humans, our study has the potential of providing new insight into the regulation of stem cell function in both invertebrates and vertebrates, including humans.

848B

FlyExpress: A Platform for Discovering Co-expressed Genes via Comparative Image Analysis of Spatial Patterns in Drosophila Embryogenesis. Michael E. McCutchan[2], Sudhir Kumar[1,2], Stuart J Newfeld[1,2]. 1) School of Life Sciences, Arizona State Univ, Tempe, AZ; 2) Center for Evolutionary Medicine and Informatics, Biodesign Institute, Arizona State Univ, Tempe, AZ.

Images containing spatial expression patterns illuminate the roles of different genes during embryogenesis. Overlaps in expression patterns are frequently an initial clue to genetic regulatory interactions. FlyExpress is a web resource that facilitates the discovery of putatively interacting genes during Drosophila embryogenesis. It contains a library of >100,000 standardized expression images from >4500 genes from two high throughput sources (BDGP and FlyFISH) and from >2,600 peer-reviewed publications. All images have been uniformly oriented, aligned, and scaled allowing direct comparison by gene across stages and anatomical views. FlyExpress provides tools to automatically identify co-expressed and, thus potentially co-regulated, genes by searching for other genes with similar expression profiles. Our search tool directly compares expression pattern images and emulates biologists' practices of manual inspection. The image atlas can now be searched for fly genes that are homologenes of other species, and results can now be exported to CSV files for offline browsing and analysis. In addition, FlyExpress provides global views of gene activity across each developmental stage through Genomewide-Expression-Maps (GEMs). GEMs are two-dimensional heat maps that synthesize individual spatial patterns into genomic summaries. By simple point-and-click, one can query GEMs directly to produce a list of genes expressed or not expressed in any region of the embryo or to display all publications reporting gene expression at any given embryo coordinate. Users can also create GEMs for their own list of genes. Therefore, the FlyExpress platform is designed to meet needs of biologists to identify genes with similar expression patterns, judge the biological relevance of these matches within the complexities and subtlety of the developmental process, generate novel gene interaction hypotheses, and visualize genome-scale summary of gene expression relevant to development.

849C

Machine Learning Approaches for Drosophila Expression Image Analysis. Lei Yuan, Cheng Pan, Shuiwang Ji, Sudhir Kumar, Jieping Ye. Arizona State University, Tempe, AZ.

Today, more than a hundred thousand images of spatial patterns of gene expression have recently become available in a canonical model organism (Drosophila melanogaster) for understanding how a single cell, through gene expression and interaction, transforms into a complex organism. Efficient and accurate analyses of these images will provide the next generation of scientists biological insights into gene functions, interactions, and networks. Currently, many tasks in biological image analysis including the developmental stage and term annotation are conducted manually by domain experts. This manual practice does not scale with the continuously expanding collection of images, and it proves to be a major impediment in making discoveries. Therefore, we developed novel computational methods for the automated annotation of expression images. For stage annotation, we obtained a collection of about 5000 images annotated with precise stages by expert biologists. Gabor filters were adopted for feature extraction, and sparse structure of the feature space was exploited using a group lasso formulation for predicting the developmental time at the stage level. In the context of developmental term annotation, we employed state-of-the-art sparse learning techniques to construct robust representation of the expression images, overcoming the limitations of prior schemes. The proposed computational systems achieve promising results for both developmental stage and term annotation tasks.

850A

Targeted Gene Conversion, an efficient method to engineer endogenous genes. Manasi Apte[1], Victoria Moran[1], Richard Kelley[2], Victoria Meller[1]. 1) Biological Sciences, Wayne State University,MI; 2) Molecular and Human Genetics, Baylor College of Medicine,TX.

Engineering *Drosophila* genes at their endogenous location is quite challenging. Techniques such as ends-in and ends-out recombination are widely used but labor intensive. We have tested a relatively simple and efficient genome engineering technique that we call 'Targeted Gene Conversion'. This multi-step approach starts with creation of a template P-element containing the engineered target sequence and a phenotypic marker, such as w^{+mC}. The template is moved close to the site of desired change by targeted transposition, using an existing P-element as a target. Once the template is in place, it is re-mobilized to create a dsDNA break. If gap repair occurs using a sister chromatid template, a gene conversion that substitutes the engineered template for sequence near the break site can result. w^{+mC} is eliminated when repair utilizes homology between the engineered target sequence and the region surrounding the break site. Our technique requires the presence of a P-element in the vicinity of the site to be engineered. However, our preliminary studies suggest that sites 0.5 to 1kb from an existing P-element may be engineered with reasonable efficiency. As a proof of principle, we have introduced a tag of six tandem MS2 loops into the *roX1* gene. *roX1* is a long non-coding RNA that assembles with the Male Specific Lethal (MSL) proteins to facilitate X-chromosome dosage compensation in male flies. MS2 loops interact with MS2 coat protein (MCP) in the MS2 bacteriophage. MS2 loop tagged RNAs can be visualized with an MCP-GFP fusion protein. We found that 10% of re-mobilized chromosomes that had lost w^{+mC} had incorporated MS2 loops into the endogenous *roX1* gene. The 322 bp MS2 loops are over 400 bp from the point of insertion, suggesting that repair tracts capable of incorporating large amounts of non-homologous sequence occur frequently. To assess the generality of this technique, we are currently engineering mutated and tagged versions of the autosomal *CTCF* gene.

851B

Comparing TALENS with Zinc Finger Nucleases in Drosophila. Kelly J. Beumer[1], Michelle Christian[2], Jon Trautman[1], Daniel F. Voytas[2], Dana Carroll[1]. 1) Dept Biochem, Univ Utah, Salt Lake City, UT; 2) Dept GCD, Univ Minnesota, Minneapolis, MI.

Introduction of a double-strand break (DSB) in chromosomal DNA stimulates repair by recombination in the vicinity of the break. We have previously shown that zinc finger nucleases can be used to create targeted breaks in the Drosophila genome that are repaired either through non-homologous end joining, or through homologous recombination. However, design of effective ZFNs remains complicated and somewhat empirical. Recently, a new class of DNA binding domains, transcription activator-like effectors (TALE), has been described and used to target the FokI nuclease (TALENs). Each TALE repeat binds a single base pair in the DNA target, thereby simplifying design, and there seem to be fewer context effects than with zinc fingers. We are testing the function of TALENs in Drosophila, and comparing their use and effectiveness to our previously described ZFNs. We will report on TALENs targeting the *ry* and *y* genes of Drosophila.

852C

Epitope labeling of histidine decarboxylase in *Drosophila melanogaster*. Benjamin Fair[1], Marc Vander Vliet[2], Stephanie Payne[3,4], Martin Burg[2,3]. 1) Biology, Grand Valley State Univ., Allendale, MI; 2) Biomedical Sciences, Grand Valley State Univ., Allendale, MI; 3) Cell & Molecular Biology, Grand Valley State Univ., Allendale, MI; 4) Biology, Johns Hopkins Univ., Baltimore, MD.

Histidine decarboxylase (HDC) plays a critical role in the synthesis of histamine, a central and peripheral nervous system neurotransmitter used by invertebrates. Past attempts to create antisera that recognize HDC *in vivo* have not produced satisfactory results. While some HDC antisera have been made in other organisms, they appear not to be useful across species, including *Drosophila melanogaster*. As a result, little is known about the localization or biochemistry of HDC in the fly. It has been suggested that HDC undergoes a complex maturation process, undergoing cleavage at both the N- and C- termini of the protein. We report an approach that allows a functional HDC protein to be examined *in vivo* using internal epitope tagging. A genomic fragment that had been previously shown to contain a completely functional *Hdc* gene was modified by a PCR-mediated insertion of an epitope tag, 6x-HIS, into the protein coding region of the *Hdc* gene at specific sites. The location of these tags in the protein structure was selected to be in regions of the mature HDC protein which likely would not affect its function, based on comparisons of the structure of DDC from other species with the HDC protein sequence. Each *Hdc* transgene containing a 6X-HIS tagged *Hdc* gene was transformed into Hdc^{JK910} mutant flies that normally have little to no histamine or HDC activity. Results indicate that while one of the epitope tags appears to disrupt *Hdc* function (indicated by a lack of histamine staining in the CNS), a 6X-HIS tag in a different location of the HDC protein structure appears to have no disruptive effect on *Hdc* function (indicated by normal histamine staining in the CNS). Assuming other epitopes can be used that may be easier to detect in tissue; this approach should enable further studies into the biochemistry and cell biology of HDC *in vivo*.

853A

Fluorescent fusion protein knockout mediated by anti-GFP nanobody. Oguz Kanca, Emmanuel Caussinus, Markus Affolter. Cell Biology, Biozentrum of University of Basel, Basel, Basel-Stadt, Switzerland.

Disruption of protein function has been a central approach in modern biology. Diverse methods such as RNA interference and morpholinos are routinely used in order to knock down protein function. Nevertheless, those approaches target the RNA encoding for the protein, thus the removal of protein function depends on the turnover rate of existing target protein. Here we present a new genetic method, named deGradFP, for direct and fast depletion of target Green Fluorescent Protein (GFP) fusions. deGradFP uses the highly conserved ubiquitination pathway to deplete the target proteins, making it applicable on any eukaryotic model system. Moreover since it targets GFP fusion protein the knock down can be monitored during live imaging through disappearance of GFP signal. For many targets it is a ready to use technique as GFP protein trap stocks are being generated through community efforts in Drosophila (http://flytrap.med.yale.edu/index.html, http://www.flyprot.org/) and in zebrafish (http://kawakami.lab.nig.ac.jp/ztrap/).

854B

Construction of Drosophila strains expressing affinity-tagged Ubiquitins: Investigating the regulation of Epsin by ubiquitination in Notch signaling cells. Kristin D. Patterson, Janice A. Fischer. Molecular, Cell and Developmental Biology, The University of Texas at Austin, Austin, TX.

Ubiquitin (Ub) is a 76 amino acid polypeptide that may be attached covalently to other proteins and regulate their activities. Proteins may be mono-ubiquitinated, or they may be attached to a Ub chain in which several Ub moieties are linked together through one of the seven lysine residues found in Ub itself. Mono-ubiquitination or polyubiquitination with a particular type of chain linkage affects the fate of the modified protein in different ways. The Drosophila genome has four genes that encode Ub. We are engineering the fly genome so that most, if not all, of the endogenous Ub is affinity tagged. The tags do not affect the Ub function, and they will enable purification of any ubiquitinated substrate. We will demonstrate the utility of the tagged Ub fly lines by purifying and analyzing Ub-Epsin. Extracts from fly stocks that express tagged substrate and tagged Ub will be the starting material for tandem affinity purification (TAP). The purified Ub-substrate will be analyzed by mass spectrometry to identify the site and type of Ub linkage. The primary role of Epsin in the developing fly is to activate Notch cell signaling by facilitating ligand endocytosis into the signaling cell. Fly eyes with too much or too little active Epsin have developmental defects that recapitulate Notch pathway gene mutant phenotypes. Ubiquitination of Epsin, and its deubiquitination by the enzyme Fat facets, regulates Epsin activity. Two models have been proposed to explain the inactivity of Ub-Epsin: Ub-Epsin may be targeted to the proteasome for degradation or ubiquitination may prevent the association of Epsin with ligand at the plasma membrane. We will begin to distinguish between these mechanisms and others by determining the sites and types of Ub linkages on purified Ub-Epsin.

855C

Microarray-based Capture of Novel Expressed Cell type-specific Transcripts (CoNECT) to annotate tissue-specific transcript isoforms. Xiaojing Hong[1], Harshavardhan Doddapaneni[2], Matthew Rodesch[3], Heather Halvensleben[3], Raghu Metpally[1], Todd Richmond[3], Bolei Fu[1], Thomas Albert[3], J Robert Manak[1,2,4]. 1) Dept of Biology, Univ of Iowa, Iowa City, IA; 2) Carver Center for Genomics, Univ of Iowa, Iowa City, IA; 3) Roche NimbleGen, Madison, WI; 4) Dept of Pediatrics, Univ of Iowa, Iowa City, IA.

Faithful annotation of tissue-specific transcript isoforms is important not only to understand how genes are organized and regulated, but also to identify potential novel, unannotated exons of genes which may be additional targets of mutation in disease states or while performing mutagenic screens. We have developed a microarray enrichment methodology followed by long-read next generation sequencing for identification of transcript isoforms expressed in *Drosophila* ovaries and the testes. These studies have identified a large number of novel transcript isoforms, including over 2,300 novel 5' exons/extensions, over 900 novel 3' exons/extensions, 282 novel internal exons, 1011 internal exon extensions and 23 gene fusions. Additionally, we identified both germline-specific transcription start sites and splicing events. As has been suggested in other studies, we find that the testis transcriptome harbors a significantly higher number of novel transcript isoforms than the ovary, indicating that a more diverse transcriptome is required for male germ cell development. Finally, comparing our enrichment data set with tiling array analysis, we demonstrate that microarray enrichment 1) is able to capture both highly expressed as well as low-expressed genes, 2) is quantitative in terms of gene expression levels, and, 3) is able to capture a large number of transcripts which cannot be identified by microarray analysis. These studies introduce an efficient methodology for cataloguing transcriptomes in which specific classes of genes or transcripts can be targeted for capture and sequence, thus reducing the significant sequencing depth normally required for accurate annotation.

856A

Developing a quantitative, cellular resolution morphology and gene expression atlas for *Drosophila* embryogenesis: 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) Lawrence Berkeley Natl Lab, Berkeley, CA; 2) Electrical Engineering and Computer Science, UC Berkeley, Berkeley, CA.

Animals comprise of dynamic 3D arrays of cells which differ from each other in histological type, shape, size, location, and other characteristics. We are creating a quantitative, digital, cellular resolution map of morphology and gene expression for multiple stages during *Drosophila* embryo development. We have previously published a VirtualEmbryo (http://bdtnp.lbl.gov/Fly-Net/), a digital morphology and gene expression map of cellularizing blastoderm. However, the late embryo is structurally much more complex, having estimated 40,000 cells, over 70 cell types and the major larval organs. To accurately capture this complexity, we have made major improvements in both our image acquisition and image analysis strategies. We are initially focusing on the morphological richness of stage 16 embryos with the goal of assigning all cells in an embryo image to specific tissue types. To enable this, we have developed feature detection algorithms that accurately detect a single location or a set of points by learning their appearance from given fiducial coordinates within multiple embryos stained for nuclear DNA. This will eventually allow us to assign cells in each image to any of multiple tissue types without having to label each embryo with large numbers of probes of genes expressed in individual cell types. The registered data from multiple embryos can then be used for quantitative comparisons of gene expression and morphology data. As part of this work, we have developed a visualization tool, FlyAnnotator, which the user can employ to place defined fiducial points within the 3D data and explore cellular resolution maps of the embryo.

857B

Efficient phenotypic analysis using unfixed, uncoated adult Drosophila for scanning electron microscopy. Nicholas J. Tardi, Kevin A. Edwards. Biological Sciences, Illinois State Univ, Normal, IL.

Projects featuring repetitive phenotypic analysis of insects, such as mutant screens, quantitative genetics, and taxonomic studies, could be greatly facilitated by a simpler approach to SEM. Here, we have applied Low Vacuum SEM to wild type and mutant Drosophila, and demonstrate that high quality ultrastructure data can be obtained easily using minimal preparation. Adult flies, frozen live for storage, were mounted on EM stubs with carbon cement and directly imaged with no chemical treatment or sputter coating. The key imaging parameters were identified and optimized, including pressure, spot size, accelerating voltage, working distance, and exposure time. Potential artifacts, including apparent water droplets, variations in chamber pressure, charging, and sample dehydration were investigated. We conclude that our optimized protocol is well suited to large scale imaging of eyes, wings, bristles, and other adult structures.

858C

OpenSPIM - an open hardware project to bring Selective Plane Illumination Microscopy to the hands of the Drosophila researchers. Pavel Tomancak, Peter Pitrone, Johannes Schindelin. MPI-CBG, Dresden, Germany.

Selective Plane Illumination Microscopy (SPIM) is an emerging technique that promises to revolutionize developmental biology by allowing *in toto* imaging of large samples with high-resolution. Several spectacular, proof of principle set-ups, capable of recording Drosophila embryogenesis at cellular resolution have been introduced over the past few years.

We are particularly interested in using SPIM to record patterns of gene expression during embryogenesis using live gene expression reporters based on the FlyFos system (transgeneome.mpi-cbg.de). SPIM imaging of Drosophila embryogenesis takes by definition as long as the embryo develops and therefore in order to achieve at least medium throughput the only reasonable way to go is to employ several SPIM set-ups in parallel.

We have recently developed a low-cost, open access SPIM set-up designed specifically for imaging of expression patterns in Drosophila embryogenesis. We will document all details necessary to build this so called, **OpenSPIM**, via a publicly accessible wiki. We are also working with the Journal of Visual Experiments (JoVE) to record the assembly and operation of the OpenSPIM set-up. The microscope is driven using **MicroManager** from within **Fiji** (Fiji Is Just ImageJ; fiji.sc) an Open Source image analysis platform for which we developed advanced algorithms for SPIM image processing. Thus we merge the concept of Open Source software and hardware into a single integrated imaging solution. We hope that the OpenSPIM will nucleate a community of open hardware 'makers' that will continue to develop the set-up for the imaging needs of Drosophila research and beyond.

Finally, the current OpenSPIM prototype has been designed to fit into a cabin suitcase (the "*SPIM in a briefcase*" concept) and so if custom regulations permit it, we will bring the set-up to the meeting for live demonstration of its capabilities.

859A

Morphogen gradients quantified by sub-single embryo RNA-seq. Peter A. Combs[1], Michael B. Eisen[2,3]. 1) Biophysics Grad Group, UC Berkeley, Berkeley, CA; 2) Department of Molecular and Cell Biology, Univ California, Berkeley, CA; 3) Howard Hughes Medical Institute, Univ California, Berkeley, CA.

Genome-scale techniques have been invaluable at illuminating multi-gene interactions at the expense of spatial information; conversely, any conventional technique that respects spatial dependence works for only a handful of genes at a time. This is particularly troublesome for studying *Drosophila* patterning, which has detailed and precise spatial dependence among a network of genes. Previous work from our lab has shown that single *Drosophila* embryos provide ample material for RNA-seq. Here I extend this work to quantify gene expression within a spatially-restricted region of a single embryo. I used a combination of cryo-sectioning and Illumina sequencing to measure gene expression along the anterior-posterior axis of single *D. melanogaster* embryos. The sample preparation protocol was specifically optimized for small volume samples, and yielded enough cDNA to perform high density sequencing of 60 micron thick samples. Our analysis focuses on the expression of the gap gene morphogens along the AP axis.

860B

New Tool in the lab: a Robotic System to process *Drosophila* samples. Joana Branco[1], António Lopes[1], João Salgado[1], Rui Cortesão[2], Jorge Batista[2], Nuno André Faustino[1]. 1) Gene PreDiT, SA Núcleo 4 - Lote 4-A, Ed. Biocant II 3060-119 Cantanhede Portugal; 2) Institute of Systems and Robotics Electrical and Computer Engineering Department, University of Coimbra 3030-290 Coimbra Portugal.

To overcome some of the constraints due to Human-handling of *Drosophila* samples, we have developed a robotic system to process adult flies, and extract its material for subsequent assays. Currently our robotic system allows preparation of samples for wet weight measurement, protein, lipids and sugar

assays, but it can also be easily adapted for DNA extractions as well. The robotic system comprises a device to grind the flies, a scale (under an anti-vibratic plate) to weight them and a mechanism to remove major debris (through 2 sequential centrifugations). All of these components are incorporated in a single platform system that in the end will provide the material in a 96-well plate. We believe that the robotic system here presented represents a major advantage for laboratories because not only it improves quality and consistency in the samples, avoiding Human- errors and variability, but because it also releases personnel to be devoted to other tasks or projects in the lab.

861C

Accessing fly data from the modENCODE project: modMine, GBrowse and dataset search. Sergio Contrino[1], Daniela Butano[1], Seth Carbon[2], Adrian Carr[1], Fengyuan Hu[1], Ellen Kephart[2], Paul Lloyd[2], Rachel Lyne[1], Marc Perry[3], Peter Ruzanov[3], Richard Smith[1], E.O. Stinson[2], Radek Stepan[1], Julie Sullivan[1], Alex Kalderimis[1], Zheng Zha[3], Suzanna Lewis[2], Gos Micklem[1], Lincoln Stein[3]. 1) university of cambridge, cambridge1.Department of Genetics, University of Cambridge, Cambridge, UK; 2) Lawrence Berkeley National Laboratory; Genomics Division, Berkeley, CA, USA; 3) Ontario Institute for Cancer Research, Toronto, ON, Canada.

The goal of the modENCODE project is to provide the biological research community with a comprehensive encyclopedia of genomic functional elements in the model organisms *C. elegans* and *D. melanogaster*. Its data span the main domains of genomic functions, including gene structure, mRNA and ncRNA expression profiling, transcription factor binding sites, histone modifications, chromatin structure and origins of DNA replication. **modMine** is the main gateway to the project data, and allows researchers to navigate easily through modENCODE experiments, to view and export data and to perform sophisticated, ad hoc queries. External data are incorporated in modMine to complement modENCODE data, including genome annotations from FlyBase and WormBase, Gene Ontology annotations, physical and genetic interactions, and details on proteins, protein domains and orthology. modMine is based on the InterMine data warehousing system and allows users, among other things, to create and analyse lists of data items. **GBrowse** is a genome browser giving a graphical view of all of the datasets created by modENCODE, plus a number of reference tracks such as annotated genes from FlyBase. It is integrated within modMine or accessible independently for all the Drosophila species used in the project. **Dataset Search**: a faceted search index to find and download specific data set from the project. Datasets can be filtered by organism, category of experiment, experimental factors, technique, target elements, etc. An FTP site is also available to download datasets. All the tools illustrated are available from www.modencode.org.

862A

Organically Modified Silica nanoparticles are biocompatible and can be targeted to Drosophila neurons in vivo. Shermali Gunawardena[1,2], Farda Barandeh[1], Phuong-Lan Nguyen[1], Rajiv Kumar[1], Gary Iacobucci[1], Michelle L Kuznicki[1], Andrew Kosterman[1], Earl J. Bergey[2], Paras N. Prasad[2], Shermali Gunawardena[1,2]. 1) Biological Sciences, SUNY at Buffalo, Buffalo, NY; 2) 2Institute of Laser, Photonics and Biophotonics, The State University of New York at Buffalo, Buffalo, NY, 14260.

The application of nanotechnology in biological research is beginning to have a major impact leading to the development of new types of tools for human health. One focus of nanobiotechnology is the development of nanoparticle based formulations for use in drug or gene delivery systems. However most of the nano probes currently in use have varying levels of toxicity in cells or whole organisms and therefore are not suitable for in vivo application or long-term use. Here we test the potential of a novel silica based nanoparticle (organically modified silica, ORMOSIL) in living neurons within a whole organism. We show that feeding ORMOSIL nanoparticle suspension to Drosophila has no effect on viability. ORMOSIL nanoparticles penetrate into living brains, neuronal cell bodies and axonal projections. In the neuronal cell body, nanoparticles are present in the cytoplasm, but not in the nucleus. Strikingly, incorporation of ORMOSIL nanoparticles into the brain did not induce aberrant neuronal death or interfere with normal neuronal processes. Our results in Drosophila indicate that these novel silica based nanoparticles are biocompatible and not toxic to whole organisms, and has potential for the developed of long-term applications.

863B

pΔTubHA4C: a new versatile vector for constitutive expression in *Drosophila*. Barbara Perez[1,2,4], Stephanie Arcia[1,3,4], Yan Zhang[4], Pedro Fernandez-Funez[4], Diego Rincon-Limas[4]. 1) Undergraduate Dept of Microbiology; 2) McNair Scholar; 3) HHMI/Science for Life Program; 4) Dept of Neurology, University of Florida, Gainesville, FL.

Control of gene expression relies on a variety of strategies, including gene overexpression, gene rescue, and gene silencing. The binary UAS/GAL4 system has become a popular choice for genetic manipulation. However, there are cases in which ubiquitous expression is desirable independently of GAL4. Traditionally, constitutive expression has been achieved by cloning cDNAs under control of ubiquitous promoters such as the Actin5C or Hsp70 genes. Unfortunately, the Actin5C promoter displays heterogeneous expression and the Hsp70 promoter requires heat induction, which may affect certain experiments. In a search for more homogeneous expression, the promoters of α1-Tubulin, Armadillo or EF-1αF1 were isolated. Since the first intron of these genes contain essential regulatory information, they were used together with the first exon containing the ATG and a fragment of the second exon for ligation of the cDNA. Thus, expression under these promoters requires the creation of a fusion with their first amino acids. Additionally, exon 2 contains few suitable restriction sites, complicating cDNA cloning. To overcome these limitations we created pΔTubHA4C. This plasmid was designed for expression of cDNAs in flies under control of a simplified Tubulin promoter. For this, we cloned and fused the critical regulatory regions of the promoter and intron 1 of α1-Tubulin, producing a shorter, optimized Tub promoter (880 nt). Then, we incorporated an optimized polylinker to offer flexible cloning options. Finally, we 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. We have cloned the LacZ gene under control of both the ΔTub and unmodified α1-Tubulin (2.6 Kb) promoter to compare their strengths and spatial regulation in cell culture and transgenic flies. The properties and advantages of the new pΔTubHA4C vector will be presented.

864C

The FlyMine Project: New Developments and Interoperation. Julie Sullivan, Daniela Butano, Adrian Carr, Sergio Contrino, Hu Fengyuen, Alex Kalderimis, Rachel Lyne, Mike Lyne, Richard Smith, Radek Stêpán, Gos Micklem. Genetics Dept, University of Cambridge, Cambridge, UK.

FlyMine is a well-established, open-source database of genomic, expression and protein data for Drosophila species. A powerful and flexible query

interface to these data enables users to perform arbitrary and complex queries across the data either via a web or a programming interface. Queries and sets of results can be saved for use in further queries or for feeding to analysis programs. A sophisticated list upload and analysis facility enables users to work with many genes at a time. FlyMine loads data from over 30 different sources, including mRNA expression data from BDGP, interactions, pathways and RNA_seq expression data from the modENCODE (www.modencode.org) project. We will describe the data and analysis available through FlyMine, with a focus on new and soon-to-be available features. These include a Genomic Region Search, which allows users to search for features mapped to particular genomic regions, new analysis widgets, in particular providing statistical enrichment analysis and interaction visualisation, new export formats and developments to web services. In addition, FlyMine is entering an exciting new phase of development which allows users to easily view and query data from other model organisms. An NHGRI-funded initiative to deploy the software FlyMine is built with (InterMine) at several major model organism databases (MODs) is well under way with InterMine already up and running for SGD (yeast) and RGD (rat), with ZFIN (zebrafish), MGI (mouse) and WormBase (worm) in development. Through this collaboration researchers will be able to see relevant information from another model organism without leaving FlyMine and transfer a list of genes for further analysis. Further information and documentation can be found on the FlyMine website (www.flymine.org).

865A

The TRiP: The Transgenic RNAi Project at Harvard Medical School. D. Yang-Zhou[1], L. Holderbaum[1], J. Ni[1,4], L. Liu[1,4], S. Kondo[1,5], R. Tao[1], L. Jiang[1], Y. Hu[1], R. Sopko[1], A. Miller[1], S. Randklev[1], M. Foos[1], S. Ball[1], B. McElvany[1], I. Flockhart[1], S. Mohr[1], N. Perrimon[1,2], L. Perkins[1,3]. 1) Dept. of Genetics, HMS, Boston, MA; 2) HHMI; 3) MGH, Boston, MA; 4) Tsinghua, China; 5) DGRC, Japan.

The *Drosophila* Transgenic RNAi Project (TRiP) started in 2006. The initial goals of the TRiP, funded by NIH/NIGMS in 2008, were to improve *in vivo* transgenic RNAi methods and generate RNAi stocks for the community. We have optimized vectors, the VALIUM series, for introducing RNAi into the genome. The first generation of vectors, VALIUM1 and VALIUM10 proved effective for transgenic RNAi (Ni et al., 2008. Nature Methods; Ni et al., 2009. Genetics). The second generation of the TRiP vectors, VALIUM20 and VALIUM22, expresses short hairpins through the microRNA pathway. Data have shown that small hairpin RNAs (shRNAs) are much better reagents than long double stranded RNAs (dsRNAs) as they are more effective in somatic tissues and work in the male and female germlines (Ni et al., 2011; unpublished results). We have generated >6,000 fly stocks (including 3,000 shRNA stocks) that are openly available from the BDSC. The TRiP reagents are extremely popular, as evidenced by the high volume of TRiP stocks requested from the BDSC and the growing number of research citations. With NIH funding from our recent successful competing application, we will continue to generate shRNA stocks. Our goal is to generate at least 1 fully validated, transgenic line for each fly gene. In addition, we are generating a digital “Red Book” for all the RNAi lines generated at the TRiP. To create this community resource, we will perform a number of validation experiments, including qPCR, collect community information on existing stocks, and make this information available and searchable online. The community is invited to review the list of available TRiP stocks, and/or contribute to the RNAi "Red Book" on our website (http://www.flyrnai.org/TRiP-HOME.html). The TRiP is also accepting nominations of any gene(s) of interest that are not yet available.

866B

Metabolomic characterization of *Sod1* null flies using Liquid Chromatography/Mass Spectrometry. Jose M Knee, Thomas J.S. Merritt. Department of Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario, Canada.

Mass spectrometry is a powerful and fast developing tool in metabolomics, providing researchers with a highly sensitive and accurate method for quantification of a broad array of small molecule metabolites. The field is, however, still in its infancy and well-established methods and protocols are not broadly available. We are developing such protocols for liquid chromatography/mass spectrometry (LC/MS) using oxidative stress and cytosolic superoxide dismutase gene (*Sod1*) in *Drosophila melanogaster* as a model system. SOD1 is involved in reactive oxygen species scavenging and Sod1 mutants accumulate both ROS and products of ROS damage. Our initial work is targeting these “known” metabolites to allow us to develop and optimize sample preparation and chromatography protocols. Using known standards, fly homogenates, and spiked-homogenates, we are investigating different homogenization buffers, filtering and sample clean-up techniques and chromatography formats including both C18 and Hydrophilic Interaction Liquid Chromatography (HILIC). Subsequent work will use these protocols to expand our investigation to a broader, currently unknown, suite of metabolites. This combination of targeted and discovery metabolomics is allowing us to develop methods and protocols while investigation both SOD1 function, oxidative stress in general, and biological networks and their response to genetic alterations.

867C

Causes and Consequences of Genetic Background effects: Re-integrating genetic background into mutational analysis. Ian M. Dworkin. Dept Zoology, Michigan State Univ, East Lansing, MI.

In genetic analysis, it is well known that the observed phenotype is not only a function of a given mutant allele, but also the influences of the genetic background in which it occurs, and the environment in which the organism is reared. Yet, in most genetic analyses such influences are often removed from consideration (via studying in a single environment in an isogenic background), or worse, ignored. When such influences are explicitly considered, it is usually only the consequences on the focal mutation that are examined; without consideration of the ordering of allelic effects (allelic series), pleiotropy or epistasis. I will present work from the lab that has utilized genetic, genomic and bioinformatic approaches to investigate the causes and consequences of genetic background effects for series of mutations in *scalloped*, *vestigial* and other genes that influence the development of the wing. Results demonstrating the significant influence of genetic background on epistatic interactions, the ordering of allelic series and transcriptional profiles in the wing imaginal disc will be discussed. Results from our work to map the modifiers causing the background dependent differences, including re-sequencing efforts will be presented. These results will be discussed within the context of a broadening appreciation for the influence on genetic background effects, and how it can significantly aid our understanding and interpretation of genetic analysis.

868A

Different Patterns of H3K27me3 in Four *Drosophila* Species Revealed Through ChIP-seq. Robert Arthur[1,2], Matthew Slattery[2], Rebecca Spokony[2], Jennifer Zebia[2], Lijia Ma[2], Xiaochun Ni[1,2], Sarah Suchy[2], Nicolas Negre[2], Joelle Perusse[2], Ilya Ruvinsky[1,2], Kevin White[1,2]. 1) Ecology and Evolution, University of Chicago, Chicago, IL; 2) Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL.

Epigenetic regulation exercises profound effects on gene expression throughout development by creating tissue-specific transcriptional profiles. Despite the significance of epigenetic regulation in guiding development, the evolutionary processes governing it remain obscure. One means of epigenetic regulation is through the posttranslational modification of histones. Histone 3 lysine 27 trimethylation (H3K27me3) is a histone modification often associated with gene silencing and thought to be maintained by cis-regulatory modules called Polycomb response elements (PREs). Although H3K27me3 is a crucial epigenetic regulator, very little is known about its genome-wide evolution. We are investigating patterns of H3K27me3 conservation and divergence through chromatin immunoprecipitation and sequencing (ChIP-seq). We have gathered data in four species of *Drosophila*: *melanogaster*, *simulans*, *yakuba*, and *pseudoobscura*. For each species, two developmental stages were assayed: the embryo, from 0-4H; and the white prepupal stage. Comparison of the four species ChIP-seq results reveals that patterns of H3K27me3 are strongly conserved. Genes with H3K27me3 signatures overlapped substantially between different developmental stages both within and between species. In addition, we observed examples of conserved exon-specific H3K27me3. We plan to examine how conservation of this crucial histone modification relates to gene expression (as measured by RNAseq), as well as conservation of the underlying methylated sequence and the most proximal PRE. We are also extending this method to examine other epigenetic signals in a greater number of species and developmental stages. ChIP-seq assays against other factors, including those bound to PREs, will enable a better grasp of how epigenetic marks are created and maintained, both developmentally and evolutionarily.

869B

Correlating gene expression patterns with gene, protein, and RNA interaction networks in Drosophila. Thilakam Murali, Russell Finley. Ctr Molecular Medicine and Genetics, Wayne State Univ Med School, Detroit, MI.

A systems-level understanding of cellular processes requires analysis of the emerging flood of data on how genes and their products (RNAs and proteins) interact. We developed DroID, the Drosophila Interactions Database (www.DroIDb.org), to be a comprehensive resource for interaction data, including transcription factor-gene (TF-gene) interactions, microRNA-gene (miR-gene) interactions, genetic interactions, experimentally determined protein-protein interactions (PPI) and PPI predicted from data in other organisms (interologs). DroID also has genome-wide expression and localization data that can all be used to search and filter interaction networks. The majority of the interactions in DroID, however, are determined by methods that are independent of gene expression patterns in vivo; i.e., the data are a composite of potential interactions. To help identify general characteristics of gene networks that operate in different spatiotemporal contexts, we are correlating the interactome data with recent tissue-specific and developmental stage-specific expression data from FlyAtlas and the modENCODE project. Our broad aim is to understand how the interactome is modified by gene expression patterns. Initially, we ranked gene expression on a scale from tissue- or stage-specific to ubiquitous. Interestingly, ubiquitously expressed genes have about ten times more interactions among themselves than non-ubiquitously expressed genes. In addition, the tissue- or stage-specific genes were found to interact more with ubiquitously expressed genes than among themselves or with the other non-ubiquitous genes. The genes that were neither ubiquitous nor tissue-specific tend to interact to a lesser degree among themselves but more with the ubiquitous and tissue-specific genes. These findings are consistent with a hub of widely expressed genes to which are attached various tissue or stage specific genes. To facilitate additional analyses of gene expression and interactome data we present an approach to filter interaction networks based on normalization of expression data.

870C

Prediction of Orthologous Gene Function: Experimental Verification of I_D Test Results. Anna James[1], Sudhindra R. Gadagkar[1], Ellen D. Tarr[2], Gerald B. Call[3]. 1) Department of Biomedical Sciences, College of Health Sciences, Midwestern University, Glendale, AZ; 2) Department of Microbiology & Immunology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ; 3) Department of Pharmacology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ.

The Disparity Index (I_D) statistic of Kumar and Gadagkar (2001) measures the observed difference in evolutionary (e.g., nucleotide substitution) patterns between two orthologous molecular sequences, and the associated Monte Carlo test assesses the statistical significance of any observed difference. Thus, the (I_D) test can be used to determine the homogeneity or heterogeneity of nucleotide/amino acid substitution pattern for a given gene between two species after they have diverged from a common ancestor. While a homogeneous substitution pattern signifies that the given gene is still essentially the same between the two species, a heterogeneous pattern implies that the evolutionary constraints have diverged between the two species for that gene. A reasonable inference from a heterogeneous result is that the two orthologous genes have also diverged in function. In this collaboration among three labs (a computational lab, a fly lab and a worm lab), we test the accuracy and reliability of the (I_D) test, by assaying function in the area of general anesthesia response

(sensitive/resistant) in *Drosophila melanogaster* genes based on orthologous genes with known anesthesia response in *Caenorhabditis elegans*, and vice versa. Preliminary results (after assaying seven genes in fly based on known response in worm) are mixed, with four accurate and three incorrect predictions. However, there are approximately 20 genes to be assayed between the two species, and the results after assaying all of them will be presented at the Fly Meeting.

871A

Vibration-sensing circuitry of Drosophila larva with synaptic resolution. Albert Cardona[1,2], Casey Schneider-Mizell[2]. 1) HHMI Janelia Farm, Ashburn, VA; 2) Institute of Neuroinformatics, University of Zurich and ETH Zurich, Switzerland.

The ventral nerve cord (VNC) of Drosophila larva contains central pattern generating circuits that underlie locomotion, and receives afferent axons from somatosensory neurons. With a reduced number of neurons, the VNC offers us a uniquely approachable model system of somatosensory information processing and motor control. The arbors of sensory neurons that project into a VNC segment, and of its motorneurons, have been characterized with light microscopy (Merritt and Whitington, 1995; Landgraf et al., 1997). Interneurons are largely unknown. Here, we present the neuronal circuitry downstream of the 8+8 chordotonal vibration-sensing sensory neurons of one abdominal segment of first instar, with their 468 interneuronal synaptic partners. We reconstructed the circuitry from an electron microscopy volume that includes a little over one entire abdominal segment of a first instar larva. We used the software TrakEM2 to skeletonize all neurons of interest and annotate their synapses. We analyzed the network and found a subset of interneurons that accrue the lionshare of input from the chordotonals. About half of these interneurons are highly connected and constitute a segmental sensory integration module with clear inputs from the dorsal motor neuropils, and from across the midline.

872B

The Rabome of *Drosophila melanogaster*. Sebastian Dunst, Marko Brankatschk, Andreas Sagner, Beate Brankatschk, Marie Hannusa, Tom Kazimiers, Pavel Tomancak, Suzanne Eaton. MPI-CBG, Dresden, Germany.

Rab family GTPases are major regulators of intracellular membrane trafficking. Disruption of Rab-mediated transport is implicated in several inherited human disorders. The *Drosophila* genome encodes 23 Rab proteins with clear vertebrate homologues. However, no functions have been as yet ascribed to the majority of Rab family members. Here, we present a novel resource to elucidate the role of each individual Rab protein for intracellular vesicle trafficking. Generated by homologous recombination, our Rab library allows profound *in vivo* studies on endogenous expression levels and various knock-down approaches. We systematically profiled and annotated a complete set of *rab* alleles harboring EYFP fusions at the endogenous loci in a tissue-wide manner including larval salivary gland, wing imaginal disc, gut, fat body and brain. In these tissues, we examined their subcellular localizations and protein amounts by quantitative PCR, high-resolution fluorescence microscopy and quantitative Western blotting. We see not only complete tissue specificity of some Rabs, but also differences in quantitative levels and localization. We predict that these differences contribute to the differentiated function of tissues. We further generate a publicly accessible online database that serves as a platform to share our data among the *Drosophila* community. To understand functional requirements for each Rab protein, we are depleting them in a tissue- and time-specific manner by 3 methods: (i) by excision of rescuing transgenes in null mutant backgrounds, (ii) by RNAi targeted against the EGFP tag and (iii) by cleavage of Rabs engineered to contain a TEV protease cleavage site. We will demonstrate how our Rab protein library can be utilized for biochemical and genetic screens intended to identify cargo and additional regulatory components of intracellular compartment classes. The comparison of payload dependent trafficking routes in different cell types during *Drosophila* development will shed light on the formation and function of specialized tissues.

873C

Knockdown of bicoid interrogates models for combinatorial control of gene expression. Max V. Staller, Zeba Wunderlich, Meghan D. Bragdon, Kelly B. Eckenrode. Dept Systems Biology, Harvard Medical School, Boston, MA.

In animals, cis-regulatory elements integrate information from multiple bound transcription factors to control target gene expression. The Drosophila anterior/posterior patterning network offers a uniquely well characterized system for studying combinatorial control of gene expression. Genetic perturbations helped uncover the topology of this network, yet each connection was identified in isolation and we lack an quantitative understanding of how inputs work together to pattern outputs. The gap genes take input from maternal cues and extensively cross regulate each other; by removing one maternal input we can quantify this cross regulation in a perturbed embryo. We took advantage of a new shRNAi system to knock down bicoid in blastoderm embryos. We quantitatively measured the expression patterns of relevant gap genes and pair rules genes 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, enabling us to compare average expression of our selected genes in each cell. This atlas captures both primary and secondary effects of bicoid knockdown, enabling us to disentangle direct from indirect effects. We use these data to test simple mathematical models that accurately predict expression in wild type for their ability to predict perturbed patterns. Specifically, we compare the performance of models that assume independent contributions from each input transcription factor with those that include interactions between inputs. We discuss how quantitative measurement of gene regulatory networks in perturbed embryos can refine our understanding of how patterning informations flows through these networks and their component cis-regulatory functions.

874A

Systematic Characterization of Genetic Interactions in Tumorigenesis using Combinatorial RNAi in Drosophila Melanogaster. Xiaoyue Wang, Jennifer Moran, Kevin White. Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL.

Tumorigenesis is a multi-step process driven by the accumulation of a series of genetic mutations in normal cells. Recently a tremendous amount of genetic alterations in tumors have been uncovered owing to the advances in high-throughput genomics technologies. However, it is still challenging to identify the “driver” mutations and understand how those mutations act jointly to define the course of cancer development. We plan to use combinatorial RNAi in Drosophila Melanogaster to characterize genetic interactions between potential cancer genes. We have developed a system to generate multiple gene knockdowns in flies in a high-throughput manner. We have also identified hundreds of combinations of genes that co-occur in tumors from cancer genome sequencing data as candidate gene pairs to test for interactions. We are now testing genetic interactions by comparing the observed phenotypic effects of combinatorial RNAi of two genes with the predicted effects based on their respective single-RNAi effects. We will further characterize the

mutations that have synergistic interactions in tumorigenesis and explore the potential of using cooperative mutational patterns as diagnostic and prognostic biomarkers for cancer.

875B

Region-specific interpretation of MAPK signaling in the *Drosophila* embryo. Yoosik Kim[1], Antonina Iagovitina[1,2], Dimitri Papatsenko[3], Keisuke Ishihara[1], Kate M. Fitzgerald[1], Bart Deplancke[2], Stanislav Y. Shvartsman[1]. 1) Department of Chemical and Biological Engineering and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544, USA; 2) Ecole Polytechnique Fédérale de Lausanne, School of Life Sciences, Institute of Bioengineering, Station 15, 1015 Lausanne, Switzerland; 3) Department of Developmental and Regenerative Biology, Black Family Stem Cell Institute, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029-6574, USA.

Terminal regions of *Drosophila* embryo are patterned by the highly conserved Mitogen Activated Protein Kinase (MAPK) pathway which induces several genes, relieving their repression by Capicua, a uniformly distributed repressor. The levels of activation signal for the pathway at the anterior and posterior poles of the embryo are essentially the same. Yet, the expression patterns of most of genes induced MAPK signaling at the termini display a pronounced anterior-posterior asymmetry. We present an experimentally based theory that can explain this asymmetry in the expression domains of *tailless* (*tll)* and *huckebein* (*hkb*), the two genes essential for the terminal patterning. The expression patterns of *tll* and *hkb* overlap at the posterior pole, with the expression domain of *hkb* fully covered by a broader domain of *tll*. In contrast, the expression domains of these two genes do not overlap at the anterior pole. We propose that this asymmetry is generated by a complex mechanism that involves multiple graded signals, an incoherent feedforward loop, and multiple enhancers. Based on this mechanism, we can explain the wild-type expression patterns of *tll* and *hkb* and their changes induced by variations in the distribution of the Bicoid, Dorsal, and MAPK activation gradients.

876C

Natural Variation in Olfactory Discrimination in the Drosophila Genetic Reference Panel. Gunjan H. Arya[1,2], Michael M. Magwire[2,3], Yazmin L. Serrano Negron[1,2], Trudy F. C. Mackay[2,3], Robert R. H. Anholt[1,2,3]. 1) Dept. of Biology, NC State Univ, Raleigh, NC; 2) W. M. Keck Ctr. for Behavioral Biology, NC State Univ, Raleigh, NC; 3) Dept. of Genetics, NC State Univ, Raleigh, NC.

Most organisms depend on chemical signals for survival and reproduction. To gain insights in the genetic underpinnings of natural variation in olfactory behavior we measured behavioral responses to 14 odorants in 168 lines of the Drosophila Genetic Reference Panel. Genetic variation among individuals within each line is minimal, whereas genetic variation among the lines reflects the variation of the population from which they were derived. Whole genome sequences have been obtained for these lines and 4,672,297 single nucleotide polymorphisms (SNPs) have been identified. We observed substantial variation in olfactory behavior for all 14 odorants with broad sense heritabilities ranging from 0.11 to 0.35. Genome-wide association analysis identified 2,537 SNPs associated with variation in olfactory behavior towards at least one odorant at a nominal $P < 10^{-5}$. Polymorphisms associated with variation in responses to different odorants do not necessarily reside in olfactory receptors (although some chemoreceptor transcripts are also implicated), but cover a range of gene ontology categories, with apparent overrepresentation of genes associated with functions of the nervous system. Thus, variation in behavioral responses to odorants depends on their perceptual integrated neural representations. Gene ontology analysis using R-Spider (www.bioprofiling.de) showed a network of 74 interconnected genes with overrepresentation of categories that include axon guidance, cell adhesion, intracellular signaling pathways, cytoskeletal organization and EGFR signaling. In addition, a second network of four genes was centered on *Notch*. Thus, SNPs that give rise to subtle variations in neural connectivity contribute to genetic variation in olfactory perception and individual differences in odor discrimination. Supported by NIH grants GM059469 and GM045146.

877A

Label-Free Imaging of Lipid-Droplet Intracellular Motion in Early Drosophila Embryos Using Femtosecond Stimulated Raman Loss Microscopy. Wei Dou[1], Delong Zhang[2], Yookyung Jung[3], Ji-Xin Cheng[2,3], David Umulis[1,3]. 1) Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN; 2) Department of Chemistry, Purdue University, West Lafayette, IN; 3) Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN.

Lipid droplets are complex organelles that exhibit highly dynamic behavior in early Drosophila embryo development. Imaging lipid droplet motion provides a robust platform for the investigation of shuttling by kinesin and dynein motors, but current imaging methods are either destructive or deficient in resolution and penetration to study large populations of droplets in an individual embryo. Here we report real time imaging and quantification of lipid-droplet motion in live embryos using a newly developed technique termed femtosecond stimulated Raman loss microscopy (fSRL). To quantify intra-embryonic lipid-droplet transport, we applied fSRL microscopy to image living Drosophila embryos from the syncytial blastoderm stage through early gastrulation. Our results show that lipid droplets provide the major contrast captured by fSRL imaging of the embryo, which also provided higher resolution and deeper penetration depth than other existing techniques. Furthermore, fSRL proved to be capable of long-duration, high-resolution imaging on the order of hours to track the dynamic evolution of lipid droplets in vivo with minimal photo-damage to the embryos that proceed with normal development. Using fSRL, time-lapse images of the embryo in vivo at both the organism and cellular levels were acquired, and quantitative analysis of lipid-droplet intracellular motion by the tracking of single droplets shows both a time and space dependence for the speed and turning rate for droplet motion. Based on the tracking results, we simulated droplet motion using a velocity-jump model. The model yielded droplet net distributions that agreed well with experimental observations without any model optimization or unknown parameter estimation, demonstrating the sufficiency of a velocity-jump process for trafficking dynamics of lipid droplets in early fly embryos.

878B

Dpp Signaling Activity Requires Pentagone to Scale with Tissue Size in the Growing Drosophila Wing Imaginal Disc. Fisun Hamaratoglu[1], Aitana Morton de Lachapelle[2,3], George Pyrowolakis[4,5], Sven Bergmann[2,3], Markus Affolter[1]. 1) Growth and Development, Biozentrum, University of Basel, Basel; 2) Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland; 3) Swiss Institute of Bioinformatics, Lausanne, Switzerland; 4) Institute for Biology I, Albert-Ludwigs-University of Freiburg, D-79104 Freiburg, Germany; 5) Centre for Biological Signaling Studies (BIOSS), Albert-Ludwigs-University of Freiburg, D-79104 Freiburg, Germany.

The wing of the fruit fly, Drosophila melanogaster, with its simple, two-dimensional structure, is a model organ well suited for a systems biology approach. The wing arises from an epithelial sac referred to as the wing imaginal disc, which undergoes a phase of massive growth and concomitant patterning during larval stages. The Decapentaplegic (Dpp) morphogen plays a central role in wing formation with its ability to co-ordinately regulate patterning and growth. Here, we asked whether the Dpp signalling activity scales, i.e. expands proportionally, with the growing wing imaginal disc. Using new methods for spatial and temporal quantification of Dpp activity and its scaling properties, we found that the Dpp response scales with the size of the growing tissue. Notably, scaling is not perfect at all positions in the field and the scaling of target gene domains is ensured specifically where they define vein positions. We also found that the target gene domains are not defined at constant concentration thresholds of the downstream Dpp activity gradients P-Mad and Brinker. Most interestingly, Pentagone, an important secreted feedback regulator of the pathway, plays a central role in scaling and acts as an expander of the Dpp gradient during disc growth.

879C

Screening for recessive suppressors of ectopic Wnt/Wg signaling in the Drosophila eye. Fabian H. Jenny[1,2], Monika Hediger Niessen[1], Carla Baenziger[1], Corinna Schuett[1], Luca Mariotta[1], Konrad Basler[1]. 1) Institute of Molecular Life Sciences, University of Zurich, Zurich, Zurich, Switzerland; 2) Zurich Ph.D. Program in Molecular Life Sciences, Life Science Zurich, Zurich, Zurich, Switzerland.

In the past, several screens for dominant Wnt/Wg signaling components were performed leading to the identification of a number of key components. Since only few recessive screens were performed, we initiated EMS screens for recessive components on chromosome arms 3L and 3R. In the first screen on chromosome arm 3L we used a sev-wg construct to ectopically expression wg and on the 3R screen a sev>y+>wg flpout cassette, which increased the lines viability significantly. Both setups, the latter one in combination with eyeless induced Flippase, lead to a small/rough eye phenotype. Mutagenized animals were screened for suppression of this phenotype and several candidate lines could be established. On 3L candidates were mapped conventionally with recombination mapping. This led to the discovery of wntless, an important factor in Wg secretion. On 3R nine of 32 candidates are being mapped with a whole-genome re-sequencing (WGS) approach. The gene identification and analysis is ongoing.

880A

The Genetic Basis for Natural Variation in Alcohol Sensitivity in Drosophila. Tatiana V. Morozova[1,3], Michael M. Magwire[2,3], Trudy F.C. Mackay[2,3], Robert R.H. Anholt[1,2,3]. 1) Dept. Biology; 2) Dept. Genetics; 3) M. W. Keck Center for Behavior Biology, NCSU, Raleigh, NC.

Alcohol abuse and alcoholism are significant public health problems, but attempts to elucidate genetic risk factors in human populations have been hampered by difficulties in quantifying alcohol-related phenotypes; obtaining large sample sizes; co-morbidity of alcoholism with other neuropsychiatric disorders; and population admixture. *Drosophila melanogaster* presents a powerful model system to dissect the genetic underpinnings for alcohol related phenotypes from which evolutionarily conserved aspects can be extrapolated to human populations. We used 167 wild-derived inbred lines of the Drosophila Genetic Reference Panel (DGRP) to measure alcohol sensitivity and induction of tolerance. Genome-wide association (GWA) analyses identified polymorphisms associated with both phenotypes. In parallel, we made reciprocal crosses of two DGRP lines that were extremely sensitive or resistant to ethanol, assessed alcohol sensitivity in the F2, and retained the 10% most sensitive and resistant males and females for QTL mapping. We developed 96 evenly spaced SNP markers for single fly Illumina genotyping to map QTLs affecting ethanol sensitivity using composite interval mapping. We detected several sex-specific QTLs on the X chromosome and chromosome 3R. These regions encompass genes with SNPs associated with alcohol sensitivity identified by the GWAS study. These candidate genes can be verified further with mutational analysis or targeted gene disruption through RNAi knock-down. Supported by NIH grants AA016560 and GM045146.

881B

Direct Quantification of Transcriptional Regulation at the Single Gene Level. Heng Xu, Anna Sokac, Ido Golding. Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX.

Recent advances in RNA labeling and imaging techniques have enabled the precise quantification of transcription kinetics at a single gene locus, in both live and fixed samples. However, it has not been possible to directly relate the observed kinetics with their causative events, namely the binding of transcription factors at the gene's regulatory region. We are currently developing a fluorescence-based method aimed at simultaneously measuring the number of transcription factors bound at the vicinity of a gene and the resulting mRNA synthesis, both at the level of an individual gene copy in a single nucleus. The method combines high-resolution fluorescence confocal microscopy, automated image segmentation and statistical analysis of multiple stochastic events. We have begun to apply our new method to the study of hunchback (*hb*) transcriptional regulation by multiple transcription factors during early embryo development.

882C

Phenotypic Plasticity of the Drosophila Transcriptome. Shanshan Zhou, Terry Campbell, Eric Stone, Trudy Mackay, Robert Anhot. Dept Biology, North Carolina State Univ, Raleigh, NC.

Phenotypic plasticity is the ability of a single genotype to produce different phenotypes in response to changing environments. We assessed variation in genome-wide gene expression and four fitness-related phenotypes of an outbred Drosophila melanogaster population under 20 different physiological, social, nutritional, chemical and physical environments, and compared the phenotypically plastic transcripts to genetically variable transcripts in a single environment. The environmentally sensitive transcriptome consists of two transcript categories, which comprise ~15% of expressed transcripts. Class I transcripts are genetically variable and associated with detoxification, metabolism, proteolysis, heat shock proteins, and transcriptional regulation. Class II transcripts have low genetic variance, and show sexually dimorphic expression enriched for reproductive functions. Clustering analysis of Class I transcripts reveal a fragmented modular organization, and distinct environmentally-responsive transcriptional signatures for the four fitness-related traits. Our analysis suggests that a restricted environmentally-responsive segment of the transcriptome preserves the balance between phenotypic plasticity and environmental canalization.

883A

Transcriptional mechanisms that compensate for the cost of bistability. Alistair N. Boettiger[1], Jacques Bothma[2,3], Michael Perry[3], Michael Levine[3]. 1) Chemistry and Chemical Biology, Harvard University, Cambridge, Ma; 2) Biophysics Grad. Group, UC Berkeley, Berkeley CA; 3) Molecular and Cell Biology, UC Berkeley, Berkeley CA.

Bistable switches govern a variety of cellular and developmental processes, including sporulation in Bacillus subtilis and rhodopsin expression in the ommatidia of adult flies. Yet, they are inherently costly since a small change in the levels of a signaling molecule or transcription factor can produce a catastrophic change in gene expression and cell identity. Here we explore the transcriptional mechanisms responsible for the reliable deployment of the bistable switch controlling the establishment of the sharp mesoderm/ectoderm boundary in the early Drosophila embryo. We introduce an automated analysis to simultaneously track many hundred thousand individual snail mRNA transcripts in separate cells of whole mount embryos. With this method, we find wildtype mesodermal cells are able to transcribe snail and a surprisingly rapid rate, within a factor of two of the theoretical limit.

Transgenic expression experiments with snail show that this bistable regulatory architecture is exquisitely sensitive to molecular noise, and that small quantitative differences in the levels of early snail mRNA resolve into large qualitative differences in embryo morophology and cell fate. We present evidence that the rapid rate of transcription of snail in wildtype embryos minimizes gene expression noise for snail resulting from promoter switching. This rapid and precise expression arises through the use of both paused polII and dual enhancers, and is essential for the formation of a sharp and accurately positioned mesoderm-ectoderm boundary.

884B

Topological Dynamics of the Gap Gene System in *Drosophila Melanogaster*. Lena Panok[1,2], Konstantin Kozlov[6], Svetlana Surkova[6], Vitaly Gursky[7], John Reinitz[1,3,4,5]. 1) Department of Ecology and Evolution, University of Chicago; 2) Department of Applied Mathematics and Statistics, and Center for Developmental Genetics, Stony Brook University, Stony Brook, New York; 3) Department of Statistics, University of Chicago; 4) Department of Molecular Genetics and Cell Biology, University of Chicago; 5) Chicago Center for Systems Biology, University of Chicago; 6) Department of Computational Biology, Center for Advanced Studies, St. Petersburg State Polytechnical University, St. Petersburg, Russia; 7) Theoretical Department, The Ioffe Physico-Technical Institute of the Russian Academy of Sciences, St. Petersburg, Russia.

Pattern formation in *Drosophila Melanogaster* is a robust developmental process. The parallels between robustness in biological systems and the mathematical concept of structural stability suggest that we analyze development with the aid of tools from dynamical systems. In this poster we present a dynamical model that agrees with the properties of a biological system studied, and analyze what geometric structure(s) ensure the stability of the system. Our analysis was carried out by smoothly varying the concentration of two maternal factors and determining how both stable and unstable equilibria points responded. To further understand how our system responded to variation of upstream maternal factors (Bicoid and Caudal) we carried out a bifurcation analysis. The goal of such analysis is to give a global understanding of the behavior of the system with respect to these parameters. The parametric portrait, which subdivides [Bicoid]-[Caudal] space into regions with different dynamics is also computed. Finally four different mechanisms of pattern formation are presented. Our ongoing work is to understand Kr mutants.

885C

New Applications of Synthetic DNA Technology: Testing the Combinatorial Effects of Co-Occurring Cancer Genes through RNAi Double Knockdown. Jennifer R. Moran, Xiaoyue Wang, Kevin P. White. Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL.

The freedom to synthesize DNA molecules that do not exist in nature opens up exciting new experimental possibilities. The IGSB Recombineering Core Facility is now able to synthesize DNA molecules up to 1 kb in length through the annealing and extension of commercially-available synthetic oligos. These products may then be joined using a One-Step Isothermal Assembly reaction to form DNA molecules up to 10 kb in length, or cloned directly into expression vectors, attB integration vectors, or other vehicles. We have developed a system that permits production of complex RNAi constructs through in vitro DNA synthesis. We can readily create a double-shmiR (short hairpin micro-RNA) construct that enables us to test the combinatorial effects of multiple gene knockdown in a high-throughput manner in Drosophila. These constructs, based on two shmiRs in a single transcript, are functional in Drosophila. We are beginning to test combinations of candidate "cancer genes" identified through cancer sequencing projects using this method.

886A

Design and validation of novel gene regulatory functions by perturbing existing cis-regulatory elements. Ben Vincent[1], Tara Martin[1], Garth Isley[2], Zeba Wunderlich[1], Meghan Bragdon[1], Kelly Eckenrode[1], Nick Luscombe[2], Angela DePace[1]. 1) Department of Systems Biology, Harvard Medical School, Boston, MA; 2) European Bioinformatics Institute, Cambridge UK.

Transcriptional regulation is critical for the specification of individual cell types and the control of complex cellular processes. In animals, cis-regulatory elements (CREs) encode gene regulatory functions (GRFs) that integrate inputs from upstream regulators and output precise spatio-temporal expression patterns. Despite recent advances in the identification of individual CREs and their component transcription factor binding sites, it remains difficult to predict how regulatory sequence variants will affect GRF output. To address this problem, we build new GRFs by perturbing existing CRE sequences. The *even-skipped* (*eve*) locus is an ideal system for this approach: its expression pattern is produced by a set of known CREs; the expression patterns of upstream regulators have been measured at cellular resolution; and the binding preferences of these regulators are known. By fitting functions that relate the concentration of upstream regulators to expression output, we predict novel GRFs that are feasible within this system. The parameters of these models reflect the role of regulating TFs, and therefore predict how modifications to existing CRMs could produce new GRFs. We have predicted that it is possible to construct a GRF which outputs stripe 2 and stripe 5 by modifying the minimal stripe 2 CRE. We have constructed transgenic lines containing perturbed minimal CREs upstream of a LacZ reporter and have determined output at cellular resolution with fluorescent in situ hybridization and 2-photon imaging. Our results suggest that the 7-stripe *eve* expression pattern may be encoded in many different ways, and we are exploring this flexibility by applying this general approach to other novel GRFs.

Poster Full Abstracts - Educational Initiatives

Poster board number is above title. The first author is the presenter

887B

Adapting the "Fly Lab" for primary research in the genetics classroom. Derek M. Dean, Luana S. Maroja. 59 Lab Campus Drive, Dept. of Biology, Williams College, Williamstown, MA 01267.

The “Fly Lab” is time-honored by genetics instructors as a way to teach genetic analysis in a hands-on fashion. In its classic form, this educational module involves crossing *Drosophila* that are mutant for several genes on the same chromosome to wild type flies, assessing the F1 flies to help determine the mode of inheritance of each mutation, performing a standard three point test cross, then mapping the mutations relative to each other in the F2 generation. For decades, the Fly Lab has been a reliable way to help students grasp Mendel's laws and the mapping of genetic mutations. While the Fly Lab generally involves mapping well-characterized genes, we modified this exercise to allow students to map a mutation that has not been ascribed to a specific gene. We selected *wavy*^{2}, a classic, X-linked, adult morphology mutation with a robust phenotype that is easily scored by students. In the classroom, we mapped *wavy*^{2} relative to five *P*-element insertions at known sites within the putative gene region. These efforts significantly narrowed down the number of candidates for the *wavy* gene while effectively fulfilling our primary educational goals. In this poster, we present the results of our experiment as well as an assessment of the educational value of this module. We are also performing preliminary tests on mutations in several other unknown genes. Here we aim to identify other alleles that can be mapped with our procedure, cross these alleles into appropriate genetic backgrounds, and encourage other educators to join the larger project by making these strains freely available.

888C

An Inquiry-Based Approach to Teaching Undergraduate Students Advanced Molecular Genetics. Jason E. Duncan, Biol350 Molecular Genetics 2011, Biol350 Molecular Genetics 2012. Department of Biology, Willamette University, Salem, OR, 97301.

Research experiences are not only central in training undergraduate students in discipline-specific techniques, but also in fostering the development of science process skills including hypothesis formulation, experimental design, data interpretation, problem solving and scientific writing. I have designed a research-methods course, Biol350 Molecular Genetics that engages students in faculty-mentored research through the identification and characterization of mutant alleles of *Drosophila* genes required for axonal transport, previously identified in a large-scale mutagenic EMS screen. Employing a unique nested approach that addresses the severe time-restrictions of a short 15-week semester, students simultaneously participated in both a collaborative gene mapping group project and an independent molecular and phenotypic analysis of identified mutations. In the span of a single semester, seven students were able to map, identify the lesion, and carry out a phenotypic analysis associated with mutant alleles of seven genes including the *peroxisome biogenesis factor 1* gene (*Pex1*^{WU1}), the ubiquitin specific protease genes *fat facets* (*faf*^{WU2}) and *non-stop* (*not*^{WU3}), the transcription factor *single-minded* (*sim*^{WU4}), the cofilin phosphatase gene *slingshot* (*ssh*^{WU6}), the protein O-mannosyltranferase gene *rotated abdomen* (*rt*^{WU5}), and a gene that encodes a pericentrin-like protein, *cp309* (*cp309*^{WU7}). A summary of the results of the work performed on these mutant alleles will be presented, as will a detailed description of the course and an assessment of the impact it has on the development of science process skills and student attitudes towards research.

889A

The Genomics Education Partnership (GEP): Comparative Analysis of the Drosophila Dot Chromosome by Undergraduate Students. SCR Elgin[1], W Barshop[1], H Yuan[1], M Burg[2], C Coyle-Thompson[3], J DiAngelo[4], D Johnson[5], C Jones[6], L Kadlec[7], SC Silver Key[8], NP Kokan[9], G McNeil[10], A Nagengast[11], DW Paetkau[12], K Saville[13], S Smith[14], J Stamm[15], M Wawersik[16], L Zhou[17], D Lopatto[18]. 1) Washington U MO; 2) Grand Valley State U MI; 3) CSU-Northridge CA; 4) Hofstra U NY; 5) George Washington U DC; 6) Moravian C PA; 7) Wilkes U PA; 8) NC Central U NC; 9) Cardinal Stritch U WI; 10) York/CUNY NY; 11) Widener U PA; 12) St Mary's C IN; 13) Albion C MI; 14) Arcadia U PA; 15) Evansville IN; 16) William & Mary VA; 17) U Pittsburgh PA; 18) Grinnell C IA.

An effective method for teaching science is to engage students in doing science. The GEP, a group of faculty from over 70 primarily undergraduate institutions, provides students with the opportunity to participate in AY genomics research. GEP undergraduates have improved the draft sequence of the Muller F element (Dot chromosome) and portions of the D element from multiple Drosophila species, creating manually curated gene models for these improved regions. Analyses of the D. melanogaster, D. erecta, D. mojavensis and D. grimshawi F elements show higher repeat density, larger gene size, and significantly lower codon bias compared to reference euchromatic domains. D. mojavensis has the highest repeat density among F elements studied, which partially accounts for the larger banded region (1.7Mb vs 1.2 Mb in D. melanogaster). Despite numerous gene rearrangements, most genes on the D. melanogaster F element remain on F elements. Analysis of 8 'wanderer' genes found in a euchromatic domain in at least one species shows that these genes typically adopt the properties of their local environment, with interesting exceptions. The carefully sequenced and annotated domains generated by GEP students provide a high quality resource for these and other analyses. We find engaging students in research rewarding for both faculty and students, and invite other faculty to join us (see http://gep.wustl.edu; next workshop 6/2012). Support: HHMI grant 52005780 & NIH R01 GM068388 to SCRE.

890B

Integration of Transmission Genetics and Molecular Biology in a Genetics Lab Course Using Drosophila Neurologic Mutants. Pat C. Lord, Erik C. Johnson. Dept Biol, Wake Forest Univ, Winston-Salem, NC.

All biology majors must take Genetics and Molecular Biology (BIO213) at WFU. The lab is currently divided into two major sections. One section is focused on classical transmission genetics using historic Drosophila mutants. The second section uses various E.coli lacZ mutants which students test to determine the mutation at the nucleotide level to gain hands-on knowledge of molecular biology. We have recognized several major problems with our current lab including that students see no connection between transmission genetics and molecular biology techniques. In addition, the current lab does not mirror how we use genetics and molecular biology in our research lab. We have designed a new genetics lab course which is intended to be more question driven and better connect transmission genetics and molecular biology. The lab uses wild type and several neurologic mutants. In the first weeks of labs, students will identify their mutant based on its performance in behavioral assays. Once they have identified the mutant behavior, they will use deficiency mapping to determine a chromosomal map location for the gene. Using bioinformatics, students will identify a candidate gene. Then using RNA isolated from their wild type and mutant flies and primers they have designed, they will use RT-PCR to create cDNAs that span the mRNA of their candidate gene. These cDNAs will then be sequenced. To optimize success, we will manipulate their PCR reactions to ensure that they generate cDNAs and also provide sequence data to enhance success. Once students have their sequence data from mutant and wild type cDNAs, they will use bioinformatics to determine the

mutation in their candidate gene. They will also determine how the mutation would affect the function of the protein encoded by their candidate gene. Their results will be reported in a poster format at the end of the semester. Ultimately, this new lab format should enable students to better connect the role of transmission genetics and molecular biology as well as better replicate how these two fields are integrated in research labs.

891C

Mapping and cloning recessive wing mutants and dominant bristle mutations in an undergraduate course. Eric P Spana, Arun Augustine, Ruvi Chauhan, Rupen Desai, Gabriella Dimarco, Benjamin Hoover, Angela Jiang, Tony Jiang, Ben Joseph, Arjun R Khanna, Temistocles Molinar Jr, Lily Pham, Carter Suryadevara, Allison Umfress, Nikolaos A Valilis, Kristie Vu, Eli Wilber, Yi Dong, Jason Klein, Arun Sharma. Department of Biology, Duke University, Durham, NC.

Adult visible mutations are valuable tools in Drosophila research for understanding developmental mechanisms and genetic mapping. Our class used complementation tests to molecularly localize eight adult visible mutations with available stocks that are not cloned and display the phenotype as described. To begin, we examined four recessive wing mutants. *curvi* (*cui*) mutants display upward curvature at the distal end of the wing. Our complementation crosses revealed a cytological region of 120 kb spanning 28F1-29B1 (~31 genes)—almost 7 map units from the FlyBase genetic map position. *vesiculated* (*vs*) mutants have a bubble at the distal end of the wing. Complementation mapping localized *vs* to 6C1-6C6 (~160kb). *waxy* (*wx*, 2-69.7) mutants display wings with rippled posterior margins. Unexpectedly, deficiencies between 48E to 52D (~ 4Mb) fully complemented *wx*. *wavy* (*wy*) exhibits transverse waves in the third posterior cell of the wing. We mapped *wavy* to a 20 kb region encoding one transcript (IP3K2) at 11E-12A. We also examined four dominant bristle mutants. *Bristle* (*Bl*, 38B5-9) demonstrates thicker bristles that are approximately half the length of wild type. Failure to complement was not observed from 37F-40A. *Kinked* (*Ki*, 83D-E) mutants display shortened, twisted bristles and hairs. We observed no complementation failures between 83C1-84A6. Pin^{Yt} (60C6-D1) possess thin, fragile yellow-tipped thoracic bristles. We screened deficiencies from 56F-60E and none failed to complement Pin^{Yt} or Pin^{1}. *Prickly* (*Pr*) mutants have short/absent notal macrochaetes and we localized *Pr* to a 17kb region containing 4 genes in the *E(spl)* locus. We demonstrated that genetic mapping and cloning is an effective method of educating undergraduate students in experimental genetics.

892A

Darwin Synthetic Interview and Horse Feet - Teaching Evolution through engagement and interactivity. John A. Pollock, David J. Lampe. Biological Sci, Duquesne Univ, Pittsburgh, PA.

A Gallup Poll in 2009 found that 4 in 10 American's believe in evolution. Furthermore, 45% or the respondents could not associate Charles Darwin with evolution. In response to this crisis of understanding, we created learning tools for informal science education, which have also transitioned to curriculum in public schools. The Darwin Synthetic Interview was developed through collaboration between Duquesne University and the Entertainment Technology Center (ETC) at Carnegie Mellon University, and uses the ETC's synthetic interview technology to engage users in a conversation with Charles Darwin. An actor portrays Darwin at age 50, the year he published On the Origin of Species. The display features 199 questions distilled from 1,000 interviews with patrons of all ages. These answers, based on his own writings, explore Darwin the man, his personality, hobbies and beliefs. We learn about the principles of evolution and we hear what Darwin had to say about public reactions to On the Origin of Species. The software also contains interviews with modern experts from science, theology, religion and law who provide insight into Darwin's continuing impact on some of today's most debated topics. Usage data indicates that over 126,000 questions were asked of Darwin over a few months, with the most frequent including 'What are you famous for?' 'What did you do for fun as a kid?' 'How fast does evolution happen?' 'Do you believe in God?' Patron surveys found that 76% (n=3,120) 'would recommend the exhibit to a friend," and that 69% (n=3,038) felt that they "learned something." Furthermore, 65% (n=2,814) found that Darwin's answers were 'Good' to 'Excellent.' We have also developed four meticulously restored replicas of horse feet, dating as far back as 60 million years. Fabricated through an innovative process of digital modeling and 3-D printing we take million-year-old fossils and create durable and identical copies of the bones. Soft tissue of the hoof was sculpted on to the bones, based on trace fossils. These show how the horse's foot has adapted to the changing climate of North America.