54th Annual Drosophila Research Conference

Late Abstracts

Marriott Wardman Park
Washington, DC
April 3-7, 2013

Sponsored by The Genetics Society of America
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966A

Regulation of AJ dynamics during apical-basal polarization by DE-Cadherin phosphorylation. Yi-Jiun Chen, Lynn Huang, Juan Huang, Yang Hong. Cell Biology, University of Pittsburgh, Pittsburgh, PA.

Apical-basal polarization in epithelia divides the cell into distinct apical and basal domains demarcated by cellular junctions such as tight junction (TJ), adherens junction (AJ), and septate junction (SJ). Such polarization process is controlled a conserved group of so-called polarity proteins. To date, it remains unknown how junctional complexes may be regulated by polarity proteins to establish and maintain the polarity. Using quantitative fluorescence recovery after photobleaching (FRAP) assays, we discovered that polarizing epithelial cells in early Drosophila embryos had much faster biosynthetic turn-overs of DE-Cad and β-Catenin (two key components of AJ) than polarized epithelial cells in late embryos. By systematically eliminating the conserved Ser residues on DE-Cad intracellular tail, we found the non-phosphorylatable DE-Cad mutants are indeed lethal. Immunostaining analysis and biochemical assays showed the interaction between β-Catenin and non-phosphorylatable DE-Cad mutant is weaker than wild type (WT). However, FRAP assays showed that the biosynthetic turnover of non-phosphorylable DE-Cad mutants are faster than WT in polarizing and polarized cells. Interestingly, the non-phosphorylatable DE-Cad mutant could be rescued by fusing α-Catenin (α-Cat) at the C-terminus of DE-Cad and the biosynthetic turnover of such non-phosphorylatable DE-Cad::αCat fusion mutant becomes slower than WT. Although it has been showed that DE-Cad likely does not interact with α-Catenin directly, our data suggest that α-Catenin, when covalently bound to DE-Cad, can substitute the β-Catenin function in forming functional and stable AJ complexes.

967B

Spectrin Tetramerization is Dispensable for *Drosophila* Development. Graham Thomas[1], Mansi Khanna[1,2], Sandra Harper[3], David Speicher[3]. 1) Departments of Biology and of Biochemistry and Molecular Biology, Penn Sate, University Park, PA; 2) Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA; 3) Wistar Institute, Philadelphia, PA.

Spectrin is a giant rope-like F-actin cross-linking protein best known for its structural role in the structure of the human erythrocyte, where it forms a fishing net-like network with short actin filaments at the plasma membrane (Spectrin Based Membrane Skeleton; SBMS) that is anchored to integral membrane proteins. Spectrins are heterotetramers of two α and two β chains, and two α|β dimers bind to form the F-actin cross-linking tetramer. Thus, network formation depends on F-actin binding *and* spectrin tetramer formation. In non-erythroid tissues the structure of SBMS is unclear and spectrins have roles both at the plasma membrane and during protein transport through the endomembrane system. Zygotic α-spectrin mutants die as early first instar larvae, and α-spectrin is known to be important for neurogenesis, synaptic function and oogenesis. Dogma suggests that spectrin tetramer formation is central to its function, and previous report in the fly (Deng et al. 1995. J. Cell Biol 128:71) described an α-spectrin tetramerization mutation (R22S) with a temperature sensitive phenotype and concluded that spectrin network formation was necessary for development. In recent biochemical experiments we characterized the K_d's for tetramerization for the wild-type spectrin chains as well as the R22S allele. We find that wild-type spectrins tetramerize with nanomolar K_d's, but that α-spectrin^{R22S} exhibits no detectable binding to β-spectrin at any temperature, and has a ~1000x increase in K_d with β_H-spectrin. However, we find that α-spectrin null flies can be rescued to adulthood by α-spectrin^{R22S} at the permissive and non-permissive temperatures. Rescued flies exhibit low fecundity, suggesting a role for network formation in adult *tissue physiology*; however, we conclude that α|β-spectrin network formation is *not* crucial for adult *development*.

968C

Dusky-like functions late in wing hair morphogenesis. Lukasz F. Sobala[1,2], Paul N. Adler[1]. 1) University of Virginia, Charlottesville, VA; 2) Lodz University of Technology, Lodz, Poland.

Dusky-like is a membrane-anchored protein and a structural component of chitin-based cuticle. During the time of cuticle deposition it is found in bristles (Nagaraj, R. & Adler, P. N. Development 139, 906-916 (2012).), trichomes (hairs) and denticles (Fernandes, I. et al. Developmental Cell 18, 64-76 (2010).). It contains a ZP (zona pellucida)-domain, first found in proteins that make up the coat of oocytes. A knock-down (kd) of dyl by RNAi resulted in a very short stub-bristle phenotype that is associated with a defect in chitin deposition and the collapse of extended bristles (Nagaraj & Adler, 2012.). Recently we found a kd in pupal wing cells results in severely thinned/split/collapsed hairs that also displayed abnormal planar polarity. The abnormal morphology phenotypes were not detected until late in hair development around the time when chitin deposition was first detected. Chitin deposition was abnormal in the kd hairs, thus, like the bristle phenotype the hair phenotype appears to be due to a failure in cuticle deposition. The kd phenotype appears to be completely cell autonomous consistent with Dyl being an integral membrane protein. Over-expression (oe) of dyl resulted in branched hairs that were distinctly different from those seen with the kd. Interestingly, our initial results suggest a non-autonomous component to the oe phenotype. We have identified Chitinase 6 as a candidate gene that functions along with dyl as a Cht6 kd produces weak versions of all of the dyl phenotypes. By comparing the phenotypes associated with a dyl kd and a kkv mutation (kkv encodes the cuticle chitin synthase) we established that dyl has targets other than kkv for cuticle deposition.

969A

Investigating the role of germ plasm components in mitochondrial localization. Beate Herrmann, Julia Sauerwald, Thomas Hurd, Ruth Lehmann. Developmental Genetics, NYU School of Medicine, New York, NY.

In many animal species formation and specification of germ cells depend on the localization of specific maternally provided RNAs and proteins, termed the germ plasm. In Drosophila, the germ plasm is localized at the posterior pole of the embryo; there, approximately 90 minutes after fertilization it induces germ cell formation. Germ plasm is comprised of many components. Chief among these is Oskar, which is both necessary and sufficient for germ cell formation. Another component of the germ plasm previously reported to be required for germ cell formation is the mitochondrial 16S large ribosomal RNA (referred to as the 16S mtrRNA hereafter). Previous experiments by others have demonstrated that the 16S mtrRNA (1) concentrates at the posterior pole of early embryos, and (2) is required for germ cell formation. Here we show that the 16S mtrRNA is indeed enriched at the posterior pole of the embryo, and so too are mitochondria. This localization of the 16S mtrRNA and mitochondria to the embryo posterior requires Oskar. Surprisingly, in contrast to previous experiments we also find that the enrichment of the 16S mtrRNA and mitochondria is not required for germ cell formation, raising the interesting possibility that the localization of mitochondria might by required for some other function, such as to ensure faithful transmission of mitochondrial DNA to subsequent generations.

970B

Identification of *Gap69C* and *Atg2* as genetic modifiers of the eye pigmentation gene *garnet*, which encodes a subunit of the AP-3 complex required for normal biogenesis of lysosome-related organelles. Imilce A. Rodriguez-Fernandez, Esteban C. Dell'Angelica. Human Genetics, University of California, Los Angeles (UCLA), Los Angeles, CA.

Hermansky-Pudlak syndrome (HPS) is a human genetic disorder characterized by defects in pigmentation and platelet aggregation due to malformation of melanosomes and platelet dense granules, which are lysosome-related organelles (LROs). HPS type-2 arises from mutations in a subunit of Adaptor Protein-3 (AP-3), and is also characterized by abnormalities in the LROs of cells of the innate immune system. AP-3 is an evolutionarily conserved, heterotetrameric protein complex that mediates signal-dependent protein sorting from endosomes to lysosomes and LROs. Mutations in the *garnet* (*g*) gene from*Drosophila melanogaster*, encoding one component of AP-3, cause eye pigmentation defects due to abnormal biogenesis of the LRO known as the pigment granule. To identify genetic modifiers of the function of AP-3 in the fly eye, the hypomorphic g^2 mutant line was crossed with 213 lines of the Bloomington Deficiency Kit (DK) carrying deficiencies covering most regions of chromosomes 2, 3 and 4. The initial screening focused on identifying by visual inspection those chromosomal regions that in hemizygous form modified g^2 pigmentation. Secondary screening and validation uncovered four distinct deletions in chromosomes 2 and 3, which in heterozygous form partially suppressed the g^2 phenotype by increasing red pigmentation by over 50%. Fine mapping of two of these regions revealed that the*Gap69C* and *Atg2* genes are modifiers of AP-3 activity. The product of *Gap69C* gene has homology to the human Arf GTPase-activating protein (GAP) 1, therefore encoding a potential regulator of Arf GTPases. *Atg2* encodes a protein involved in autophagy. The identification of genetic modifiers of AP-3 would help elucidate additional components of the cellular machinery involved in LRO biogenesis.

971C

Sec24 is required for *Drosophila* axis elongation. Gillian Siegal[1], Dominique Förster[2,3], Stefan Luschnig[2], Jennifer Zallen[1]. 1) Dept Developmental Biol, MSKCC, New York, NY; 2) Institute of Molecular Life Sciences, Zurich, Switzerland; 3) Max Planck Institute of Neurobiology, München-Martinsried, Germany.

Elongation of the *Drosophila* body axis relies on dynamic patterns of intracellular trafficking to ensure the proper localization of proteins that contribute to cell rearrangement. We found that one component of the protein transport machinery, the COPII vesicle subunit Sec24, regulates protein localization during embryonic development. *sec24* mutant embryos exhibit severe defects in axis elongation, characterized by aberrant cell shapes and reduced cell rearrangement. Preliminary evidence suggests these defects may result from a disruption in the subcellular localization of the myosin II motor protein. Sec24 is required for the localization of the transmembrane proteins Neurotactin and Crumbs, but is not required for the localization of E-cadherin, Notch or Echinoid, consistent with previous evidence that Sec24 homologs bind different cargo proteins in COPII vesicles in vitro. As Neurotactin and Crumbs are not required for germband extension, we are currently investigating potential roles of other putative Sec24 cargo proteins in mediating the elongation defects in sec24 mutants. Furthermore, *sec24* and the second Sec24 paralog *stenosis (sten)* show distinct requirements in epithelial morphogenesis, suggesting partially non-overlapping roles of these COPII coat proteins in cargo export from the ER. Our analysis of *sec24* mutants demonstrates that Sec24-mediated protein transport is required for *Drosophila* axis elongation and provides an opportunity to investigate the role of Sec24 in the cargo selectivity of COPII vesicles *in vivo*.

972A

Characterization of a novel UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase that is essential for viability in Drosophila. Suena Ji, E Tian, Kelly Ten Hagen. Developmental Glycobiology Section, LCDB, NIDCR/NIH, Bethesda, MD.

Mucin-type O-linked glycosylation is abundant in the extracellular matrix and is initiated in the Golgi by the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase family (pGalNAc-T in mammals and PGANT in Drosophila), which adds a GalNAc sugar to serines or threonines of secretory and membrane-bound proteins. In Drosophila, there are nine PGANT isoforms (PGANT1-8, PGANT35A) and three putative PGANT isoforms. Members of this family show substrate peptide sequence preferences and unique spatial and temporal expression during development. CG30463 is one putative PGANT isoform based on sequence conservation. In previous studies, CG30463 is differentially expressed in the embryo and in imaginal discs of larvae. Knockdown of CG30463 via RNA interference (RNAi) in vivo results in lethality, indicating that

CG30463 is an essential gene. However, the catalytic activity of the enzyme encoded by CG30463 had not been identified previously. Here, we demonstrate that CG30463 encodes a functional O-glycosyltransferase, PGANT9. Purified PGANT9 protein transferred GalNAc onto both peptide and glycopeptide substrates in vitro. Additionally, over-expression of PGANT9 in cell culture increased HPA-reactive glycosylation in vivo. Interestingly, tissue-specific knockdown of PGANT9 in vivo showed several phenotypes, including reduced salivary gland size, abnormal digestive system formation, and irregular eye cell arrangement. Our studies demonstrate that the essential gene CG30463 is a functional O-glycosyltransferase and implicate its activity in the proper development of the salivary gland, digestive system and eye in Drosophila.

973B

The Unfolded Protein Response Selectively Targets Active Smoothened Mutants. William J. Bodeen[1,3], Suresh Marada[1,4], Daniel Stewart[1], Young-Goo Han[2], Stacey Ogden[1]. 1) Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN; 2) Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN; 3) Presenting Author; 4) Primary Author.

The Hedgehog signaling pathway, an essential regulator of developmental patterning, has been implicated as playing causative and survival roles in a range of human cancers. The signal transducing component of the pathway, Smoothened, has revealed itself to be an efficacious therapeutic target in combating oncogenic signaling. However, therapeutic challenges remain in cases where tumors acquire resistance to Smoothened antagonists, and also in cases where signaling is driven by active Smoothened mutants that exhibit reduced sensitivity to these compounds. We recently described a set of novel Drosophila Smoothened mutants that, like the human oncogenic mutant SMOM2, induce robust ligand-independent Hedgehog pathway activity. Their expression during Drosophila development triggered pronounced Hedgehog gain of function phenotypes, despite the mutant proteins being predominantly ER-localized. We hypothesize that the prolonged ER retention of these active mutant proteins is the likely result of mutation-induced conformation shifts causing them to be engaged by ER quality control machinery. In this study, we attempted to exploit this biology, and demonstrate that deregulated Hedgehog signaling driven by active Smoothened mutants is specifically attenuated by ER stressors that induce the Unfolded Protein Response (UPR). We show that upon UPR induction, active Smoothened mutants are targeted by ER-associated degradation, resulting in attenuation of inappropriate pathway activity. Accordingly, we find that the UPR agonist thapsigargin attenuates mutant Smoothened-induced phenotypes in vivo in Drosophila. Wild type Smoothened and physiological Hedgehog patterning are not affected, suggesting that UPR modulation may provide a novel therapeutic window to be evaluated for targeting active Smoothened mutants in disease.

974C

The gene *hindsight* functions in association with the Notch signaling pathway in multiple contexts. Yang Du, Brittany Baechler, Minhee Kim, Bruce Reed. Biology, University of Waterloo, Waterloo, Ontario, Canada.

The gene *hindsight* (*hnt*/*peb*/*CG12212*) encodes a C2H2-type zinc finger transcription factor orthologous to the human Ras Responsive Element Binding Protein 1 (RREB1). Named for its embryonic lethal phenotype, *hnt* null mutants fail in the morphogenetic process of germ band retraction due to premature death and dissociation of the amnioserosa. In addition, *hnt* is associated with a complex and dynamic expression pattern during embryogenesis and has been implicated in a variety of developmental processes throughout development. Despite a wealth of information regarding *hnt* mutant phenotypes, the fundamental role of *hnt* remains unclear. We have used the GAL4/UAS system of inducible gene expression to perform a microarray analysis of stage 12-14 embryos over-expressing *hnt*. Among many genes having altered levels of expression we identified a number of genes associated with regulation of the Notch signaling pathway. We have confirmed that *hnt* up-regulation leads to a dramatic up-regulation of *Dpax2* (*sv*/*spa*/*CG11049*) in the developing peripheral nervous system. In addition, we have found that *hnt* is implicated in the regulation of a number of processes associated with Notch signaling, including the maintenance of stem cells in both the larval and adult midgut.

975A

Regulation of Notch signal by a lysosomal associated transmembrane protein. Kazuya Hori, Anindya Sen, Tom Kirchhausen, Spyros Artavanis-Tsakonas. Dept Cell Biol, Harvard Med Sch, Boston, MA.

The Notch signaling pathway defines a conserved mechanism that regulates cell-fate decisions in metazoans. Signaling is modulated by a complex genetic circuitry, which includes several molecular players that affect different steps in receptor activation, trafficking and ligand-receptor interactions. To extend the repertoire of such modulators, we performed a genome-wide screen to indentify genes involved in endosomal/lysosomal degradation of Notch, mediated by the molecular synergy between the ubiquitin ligase, Deltex, and the single non-visual β-arrestin in *Drosophila*, Kurtz. We identified CG14767, a lysosomal associated transmembrane protein (LAMP), which physically interacts with Notch pathway components including Notch and Deltex. Proteomic analysis of CG14767 predicts its interaction with Su(dx) and several components of membrane trafficking machinery. Loss of CG14767, in the presence of Deltex overexpression, results in tissue specific downregulation of Cut, one of targets of the Notch signal. Interestingly, CG14767 co-localizes with Notch in Rab7 positive vesicles, indicating its potential role in late endosomal processing of Notch receptor. Taken together, these data unveil a role of a lysosomal associated transmembrane protein in modulating Notch signaling providing novel insights into the fate of Notch receptor in late endosomal/lysosomal compartments.

976B
JNK-Fos-Jun direct axon injury responses in 3 steps. Li Chen, Melissa Rolls. Penn State Univ, University Park, PA.
Proper injury responses are critical for neurons to survive and recover following an axon injury caused by stroke and trauma. By live imaging Drosophila peripheral neurons, we previously found that axon injury activates a protective response before axon regeneration. To investigate how protection and regeneration are coordinated on the molecular level, we focused on JNK and its downstream transcription factor Fos and Jun. In contrast to the assumed linear pathway model, our results revealed that JNK, Fos and Jun act sequentially in 3 steps of axon injury response. Step 1 and 2 are defined as protection at 8h and 24h after axon injury, which depends on JNK and Fos, respectively. Step 3 is to exit protection and initiate axon regeneration, and it requires inactivation of JNK and Fos in accompany with activation of Jun. Based on these results, we propose a 3-step model of axon injury response that relies on a timely controlled JNK, Fos and Jun. The rapid but transient increase in the activity of JNK and Fos together with a delayed but persistent increase in Jun activity is key to generate the full spectra of axon injury response.

977C
Additional members of the polypeptide GalNAc transferase family are essential for viability in Drosophila. Duy Tran[1], Liping Zhang[1], Ying Zhang[2], E Tian[1], Lesley Earl[3], Kelly Ten Hagen[1]. 1) Developmental Glycobiology Unit, NIDCR, NIH, Bethesda, MD; 2) Department of Microbiology, Immunology and Cancer Biology, University of Virginia, Charlottesville, VA; 3) NCI, NIH, Bethesda, MD.
Mucin-type O-glycosylation represents a major form of post-translational modification that is conserved across most eukaryotic species. This type of glycosylation is initiated by a family of enzymes (GalNAc-Ts in mammals and PGANTs in Drosophila) whose members are expressed in distinct spatial and temporal patterns during development. Previous work from our group demonstrated that one member of this family is essential for viability and another member modulates extracellular matrix composition and integrin-mediated cell adhesion during development. To investigate whether other members of this family are essential, we employed RNA interference (RNAi) to each gene in vivo. Using this approach, we identified 4 additional pgant genes that are required for viability. Ubiquitous RNAi to pgant4, pgant5, pgant7 or the putative glycosyltransferase CG30463 resulted in lethality. Tissue-specific RNAi was also used to define the specific organ systems and tissues in which each essential family member is required. Interestingly, each essential pgant had a unique complement of tissues in which it was required. Additionally, certain tissues (mesoderm, digestive system, tracheal system) required more than one pgant, suggesting unique functions for specific enzymes in these tissues. Expanding upon our RNAi results, we found that conventional mutations in pgant5 resulted in lethality and specific defects in specialized cells of the digestive tract, resulting in loss of proper digestive system acidification. In summary, our results highlight essential roles for O-glycosylation and specific members of the pgant family in many aspects of development and organogenesis.

978A
IDGF2 protects cells from toxics agents through its ability to bind specific carbohydrate moiety. Vaclav Broz[1,2], Lucie Kucerova[1,2], David Smith[3], Richard David Cumingsem[3], Michal Zurovec[1,2]. 1) Biology Centre AS CR, Entomology Institute, Ceske Budejovice, Czech Republic; 2) University of South Bohemia, Faculty of Science,Ceske Budejovice, Czech Republic; 3) Emory University, Atlanta, GA, USA.
IDGFs (Imaginal Dish Growth Factors) are a family of chitinase-like proteins secreted into the hemolymph from the fat body and hemocytes. IDGFs lost the hydrolytic activity of true chitinases but they are still able to bind carbohydrate moieties. Their exact role is unknown but in cell culture IDGFs stimulate cell viability and survival. The crystal structure of IDGF2 was elucidated by Varela et al. 2002. IDGF2 possess a unique shaped carbohydrate binding domain which is different from chitinases and other chitinase-like proteins. We used the baculovirus and the Schneiders S2 cells expression systems to produce recombinant IDGF2 protein. We conducted a glycan array screen to find the binding partner of IDGF2. We established a functional in vitro assay based on the ability of IDGF2 to protect Drosophila Cl8+ cells against the toxicity of adenosine or 2-deoxyadenosine. We measured the cell viability and survival by MTS-Test. In the present study, we have identified Galα1-3(Fucα1-2)Galβ1 carbohydrate moiety as the preferred binding partner of IDGF2. Unlike other known chitinase-like proteins, IDGF2 does not bind to N-acetylglucosamine. Furthermore, we proved that the function of IDGF2 depends on its binding ability. Free Galα1-3(Fucα1-2)Galβ1 trisacharide in excess is partially able to block the effect of IDGF2 in our in vitro assay.

979B
The HIPPO Kinase promotes SCALLOPED cytoplasmic localization independently of WARTS in a CRM1/EXPORTIN1-dependent manner in Drosophila. Julie Cagliero[1], Antoine Forget[2], Enrico Daldello[3], Joel Silber[1], Alain Zider[1]. 1) Univ Paris Diderot, Sorbonne Paris Cité, Molecular Oncology team, IJM, UMR 7592 CNRS, 75205 cedex 13 Paris, France; 2) Equipe signalisation, developpement et tumeurs cérébrales Campus universitaire d'Orsay Batiments 110-111-112, Orsay, France; 3) Equipe Biologie de l'ovocyte Université Pierre et Marie Curie 9, Quai St Bernard 75252 Paris cedex 05, France.
SCALLOPED (SD) is a transcription factor characterized by a TEA/ATTS DNA binding domain. To activate transcription, SD must interact with its co-activators, including YORKIE (YKI) or VESTIGIAL (VG). YKI is the downstream effector of the Hippo signaling pathway that plays a key role in the control of tissue growth. The core components of this pathway are two kinases, HIPPO (HPO) and WARTS (WTS), which negatively regulate the activity of the SD/YKI complex, retaining YKI in the cytoplasm. We previously showed that HPO kinase can also reduce SD/VG transcriptional activity in Drosophila S2 cells. We further

investigated the relationship between the SD/VG complex and the Hippo pathway. We show here that HPO over-expression suppresses over-growth induced by SD/VG in vivo during Drosophila development. Using S2 cells, we show that HPO promotes the translocation of SD to the cytoplasm in a CRM1-dependent manner, thereby inhibiting the induction of SD/VG target genes. Using RNAi-mediated depletion of yki and a mutant SD protein unable to interact with YKI, we demonstrate that HPO regulates SD localization independently of YKI. This function requires HPO kinase activity, yet surprisingly, not its downstream effector kinase WTS. Taken together, these observations reveal a new and unexpected role of HPO kinase in the regulation of a transcription factor independently of YKI.

980C

Antagonistic feedback loops regulate neuronal and glial cell fate decisions. Florian Sieglitz[1], Till Matzat[1], Helen Neuert[1], Yeliz Yuva-Aydemir[2], Christian Klämbt[1]. 1) Institute for Neurobiology, University of Münster, Münster, Germany; 2) Department of Neurology, University of Massachusetts Medical School, Worcester, MA, USA.

During development the strict spatial and temporal control of differentiation is often initiated by the activation of different receptor-tyrosine-kinases (RTKs). This must then be transformed into a well balanced activity level of cytoplasmic signaling cascades. Here we demonstrate at the example of glial and neuronal differentiation processes in the developing Drosophila compound eye, that normal RTK signaling requires two antagonistic feedback loops, Sprouty and Rau. Whereas Sprouty suppresses Ras-signaling, a so far unknown positive feedback loop mediated by the Ras-association domain containing protein Rau sustains Ras activity. Controlled by the COUP-transcription factor Seven-up, this antagonistic signaling module acts during both EGF- and FGF-receptor mediated cell fate decisions. Taken together, our findings reveal a novel signaling module required for the faithful maintenance of RTK signaling.

981A

DNA damage-induced tumorigenic behaviour of *Drosophila* epithelial cells. Andres Dekanty[1], Lara Barrio[1], Marco Milan[1,2]. 1) IRB Barcelona, Barcelona, Spain; 2) ICREA.

DNA damage can be induced by environmental factors, such as ionizing radiation (IR), or normal metabolic processes inside the cell. The ability of a cell to sense and respond to DNA damage is essential for genome stability and unrepaired lesions are proposed to increase the likelihood of tumor formation. In *Drosophila* epithelial tissues, resistance to cell death upon IR maintains damaged cells in the epithelium which drives JNK-dependent tumor-like growth. Here we present experimental evidence supporting the proposal that this tumorigenic behaviour is a consequence of sustained DNA damage. We first show that IR induced tumorigenic growth is independent of p53 and that it is enhanced by mutations in DNA damage response genes affecting the cell cycle arrest or DNA repair. The cellular and molecular mechanisms underlying IR induced tumor-like growth resemble those ones caused by the cooperative action of chromosomal instability and resistance to cell death, characterized by the loss of apical-basal polarity, E-cadherin delocalization, cell delamination, basement membrane degradation and hyperplastic growth. Altogether, our results reinforce the proposal that DNA damage contributes to tumor-development and unravel a pro-tumorigenic action of radiation therapies.

982B

Function of dRYBP protein in apoptosis during development. Sol Fereres, Rocío Simón, Ana Busturia. Centro de Biologia Molecular Severo Ochoa (UAM-CSIC), Nicolás Cabrera 1, 28049, Madrid, Spain.

All organisms seek for homeostasis to reach and maintain healthy development and life. Apoptosis is an evolutionary conserved biological process required both for proper development and for elimination of potential dangerous cells. We are studying the function of the dRYBP (drosophila Ring and YY1 binding protein) protein, which contains an ubiquitin binding domain, during Drosophila development. The results will be discussed in the context of the role of this protein promoting cell survival versus apoptosis.

983C

***Mabiki*-mediated cell death for excessive cells caused by extra copies of *bicoid*.** Kentaro M. Tanaka[1], Aya Takahashi[2], Naoyuki Fuse[3], Toshiyuki Takano-Shimizu-Kouno[4]. 1) Department of of Biological and Medical Sciences,Oxford Brookes University, Oxford, United Kingdom; 2) Department of Biological Sciences, Tokyo Metropolitan University, Tokyo, Japan; 3) Department of Biophysics, Kyoto University, Kyoto Japan; 4) Drosophila Genetic Resource Center, Kyoto Institute of Technology, Kyoto, Japan.

Organisms are subjected to various stresses and perturbations, but can still achieve normal development. As such an example, compensatory cell death is seen in expanded prospective head region of embryos from mothers carrying extra (six) copies of *bicoid* (6x*bcd*). However, the mechanism of this stress-induced cell death was not fully explored. Here, we conducted two types of genome-wide screenings to identify genes induced by this stress condition: a deficiency screening for haplo-insufficient genes in 6x*bcd* condition and a microarray analysis of changes in gene expression between normal and 6x*bcd* conditions. Two genomic regions showed haplo-insufficiency in 6x*bcd* and 11 genes showed more than two-fold up-regulation in the 6x*bcd* embryos suffering from head expansion. From these results, we identified a candidate gene, named *Mabiki* (*Mabi*), encoding a transcription factor whose function is unknown. *Mabi* was up-regulated in the expanded head region of 6x*bcd* embryos. Knockdown of the*Mabi* expression significantly reduced the hatchability in 6x*bcd*, suggesting that the higher expression of *Mabi* is required in normal development of 6x*bcd* embryos. Ectopic expression of *Mabi* caused

reduction of an organ size via cell death that was not blocked by P35. These findings, together with previous observation, suggest that in addition to canonical caspase-dependent cell death, *Mabi*-mediated cell death ensures normal development of 6x*bcd* embryos.

984A

Polyploidization and cell fusion facilitate tissue repair in adult tissues. Vicki Losick[1], Don Fox[2], Allan Spradling[1]. 1) Howard Hughes Medical Institute, Department of Embryology, Carnegie Institution for Science, Baltimore, MD; 2) Department of Pharmacology and Cancer Biology, Department of Cell Biology, Duke University Medical Center, Durham, NC.

Replacing tissue cells lost during injury contributes to successful wound repair in many organisms. Since most adult Drosophila tissues lack active stem cells, wound-induced cell production would require reactivating mitotic cell cycling in quiescent tissue cells. However, we found that mitotic proliferation was not induced by a puncture wound to the adult Drosophila abdominal epithelium. Instead, we identified polyploidization as a novel cellular response to tissue damage. Similar to the wounded larval epidermis, local epithelial cells fuse to form a large syncytium whose membranes reepithelialize the lesion by moving under the scar. In addition, cells both within the syncytium and surrounding the wound reentered the cell cycle and grew into large polyploid cells whose increased size may help to stabilize tissue forces on the wounded region. Cell fusion and polyploidization appear to be coordinated, since blocking either pathway alone is not sufficient to inhibit reepithelialization. Hippo pathway signaling is required to initiate adult epithelial polyploidization via the endocycle. Damage to the hindgut pylorus also triggered the induction of polyploid cells during tissue repair. Our results support the view that induction of polyploidy represents an important mechanism of tissue repair that can function in the absence of cell division.

985B

The Cohesion Protein SOLO Associates with SMC1 and is Required for Homolog Synapsis, recombination and Centromere Orientation in Drosophila Female Meiosis. Rihui Yan, Bruce McKee. Dept Biochem, Cell, Molec Biol, Univ Tennessee, Knoxville, TN.

Cohesion between sister chromatids is mediated by cohesin, a conserved protein complex, and is essential for proper chromosome segregation in both mitosis and meiosis. Meiosis-specific cohesin subunits have been identified in most eukaryotes and shown to play critical roles in several aspects of meiotic chromosome segregation, including homolog synapsis and recombination, stabilization of chiasmata, and mono-orientation of sister centromeres during the first meiotic division. solo encodes a cohesion protein previously shown to be required for mono-orientation and centromere localization of cohesin in Drosophila male meiosis. In this study we show that solo is also required for female meiosis. solo phenotypes include severely reduced homolog recombination frequencies, defects in assembly and maintenance of synaptonemal complex (SC), premature loss of centromere cohesion, and high frequencies of both sister chromatid and homolog nondisjunction. SOLO colocalizes with the cohesin component SMC1 on centromeres and chromosome arms and physically interacts with SMC1 in vivo. Furthermore, loss of SOLO disrupts SMC1 localization on centromeres and chromosome arms. Our studies demonstrate that SOLO is closely associated with meiotic cohesin and plays essential roles in multiple events required for proper chromosome segregation.

986C

Locating the *Modifier of Segregation Distorter* in *Drosophila melanogaster*. Sam J. Craven, Lucas Sanor, Janna R. McLean. Biological Sciences/Physical Sciences, Olivet Nazarene University, Bourbonnais, IL.

Segregation Distorter (*SD*) is a well-studied meiotic drive system in *D. melanogaster* that distorts normal Mendelian inheritance patterns by inhibiting the development of sperm containing the wild-type allele. Several genes are thought to be involved with the distortive effects associated with *SD*, but the bulk of research efforts have focused on only a few of these genes. Little is known about the location or function of one particular *SD*-related gene, *Modifier of SD* [*M(SD)*], and a detailed knowledge of this gene would be required for a comprehensive understanding of the system as a whole. For this reason, isolating possible locations for this gene became the focus of our research, which we initiated by testing various deletions along a suspect segment of the 2nd chromosome for the establishment of distortive offspring ratios when crossed with a distortion-sensitive standard stock. This led to the discovery of a few regions of DNA that cause significant distortion when deleted, and there are numerous genes of known and unknown functions that exist within these regions. Six of these genes (*saxophone*, *torso*, *lin19*, *Kdm4A*, *CanB2* and *CG30497*) were tested for distortion-inducing function by testing mutations in these genes for their ability to cause distortion. Only *Kdm4A* showed distortion, but not as much as that caused by the deletion of the entire segment.

987A

Chromatin Insulator Bodies are Nuclear Stress Structures that Form in Response to Osmostress and Cell Death. Todd Schoborg[1], Ryan Rickels[1,2], Josh Barrios[1,3], Mariano Labrador[1]. 1) Department of Biochemistry & Cellular & Molecular Biology, University of Tennessee, Knoxville, TN; 2) Stowers Institute for Medical Research, Kansas City, MO; 3) Department of Biological Sciences, Louisiana State University, Baton Rouge, LA.

Chromatin insulators assist in the formation of higher order chromatin structures by mediating long-range contacts between distant genomic sites. It has been suggested that insulators accomplish this task by forming dense nuclear foci termed insulator bodies that result from the coalescence of multiple protein-bound insulators. However, these structures remain

poorly understood, particularly the mechanisms triggering body formation and their role in nuclear function. Here we show that insulator proteins undergo a dramatic spatial reorganization into insulator bodies during osmostress and cell death, leading to a large reduction in DNA-bound insulator proteins that rapidly repopulate chromatin upon return to isotonicity. CP190 is a master regulator of insulator bodies, with its BTB domain alone sufficient to drive their formation. Our findings suggest insulator bodies are novel nuclear stress structures while establishing a model system to study the dynamics of nuclear body behavior and new intriguing aspects of genome and nuclear organization.

988B

Asymmetric histone inheritance in Drosophila male germline stem cells. Vuong Tran, Cindy Lim, Jing Xie, Xin Chen. Johns Hopkins University 3400 N Charles St. Baltimore, MD 21218.

Asymmetric cell division is an effective strategy used by stem cells to both self-renew and to generate a daughter cell which will undergo differentiation. Epigenetic information is thought to contribute to maintain stem cell identity. However, a long-standing question has been whether and how stem cells retain their epigenetic information. Here we report the direct evidence that pre-existing histone H3 is selectively retained in the male germline stem cells (GSCs) during the asymmetric divisions. The asymmetric histone inheritance occurs in GSCs but not in symmetrically dividing progenitor cells. Furthermore, if GSCs are genetically manipulated to divide symmetrically, the asymmetric histone inheritance mode is lost. In contrast to canonical histones, the histone variant H3.3 does not exhibit this asymmetric pattern during GSC divisions. Thus, our studies provide the first direct evidence that stem cells retain preexisting canonical histones during asymmetric cell divisions in vivo, which may contribute to maintain their epigenetic memory.

989C

Investigations into the CHD1 chromatin remodeling factor. Shawn A. Ali, Michael A. Erb, Nikhila K. Janakiram, Alison H. Lerner, Reed Stein, Lakshmi V. Bugga, Kelsey A. Schmidt, Jennifer A. Armstrong. W.M. Keck Science Department, Claremont McKenna, Pitzer and Scripps Colleges, Claremont, CA.

Chromodomain, Helicase, DNA binding protein 1 (CHD1) is an ATP-dependent chromatin remodeler important for histone deposition during transcription. Interest in CHD1 has recently piqued with discoveries that CHD1 is required for maintaining the pluripotency of embryonic stem cells in mice and is a common deletion in prostate tumor cells. However, a clear mechanism accounting for CHD1's complex functions remains elusive. Building on previous research, which shows CHD1 co-localizes with all active genes on *D. melanogaster* polytene chromosomes and yet antagonizes repressive features of chromatin, we are investigating the role of CHD1 in counteracting gene silencing using $brown^{Dominant}$ (bw^{D}), a heterochromatic insertion into the brown gene that silences the wild type *brown* (bw^{+}) allele on the homologous chromosome. We are also utilizing the high-resolution technique of chromatin immunoprecipitation (ChIP) to visualize CHD1 localization on the *CrebA* gene in salivary glands. Preliminary results show that CHD1 binds within the body of the gene, consistent with reports identifying a role for CHD1 in transcription elongation and with our immunofluorescence studies of polytene chromosomes.

990A

Profiling Genomic and Epigenetic Changes during Immortalization of Drosophila Embryonic Cells. Mary-Lee Dequeant[1,4], Delphine Fagegaltier[2,4], Amanda Simcox[3], Gregory J. Hannon[2,4], Norbert Perrimon[1,4]. 1) Dept Gen, Harvard Med Sch, Boston, MA; 2) Cold Spring Harbor Laboratory, NY; 3) Ohio State University, Columbus, OH; 4) STARR Cancer Consortium.

One of the most vexing problems in cancer biology is deciphering the roles of genetic and epigenetic events that accompany transformation. We have developed a method to derive new transformed cell lines from embryonic Drosophila cell types that express constitutively activated forms of selected oncogenes. Using this method, we generated RasV12-expressing cell lines and performed a time-course microarray analysis to characterize the changes that these undergo during their establishment. Two main events were observed: 1. a progressive up-regulation of Polycomb Group (PcG) gene expression, suggesting that cells undergo major epigenetic changes; and 2. a change in the activity of most signaling pathways, indicating an ongoing process in which cells are "adjusting" the activity of their signaling networks in response to the oncogene. We propose to broadly document the genetic and epigenetic changes that occur as cells, with different constitutive oncogenic backgrounds (for example we were able to derive cell lines from embryos with loss of the tumor suppressor genes, Pten, warts/lats alone as well as in combination with RasV12) go through the transformation process. These studies will provide a comprehensive view of how genomes, with specific oncogene and tumor suppressor-activated signaling pathways, coordinate their genetic and epigenetic responses towards a transformed state. One exciting outcome would be to associate the activity of a specific oncogene or tumor suppressor with a limited combination of genomic, epigenetic, or signaling changes, since these might hint at possible therapies. Given our experience in regulating signaling via RNAi and the ability to derive cell lines from genetically manipulated flies, we can directly test hypotheses that emerge and translate our results to human lines with similar oncogenic alterations.

991B

INVESTIGATING THE ROLE OF CYTOSKELETAL REGULATORS IN TUMOURIGENESIS. Helena E. Richardson, Kirsten Allan, Peytee Khoo. Cell Cycle/Development,Res Div, Peter MacCallum Cancer Ctr, Melbourne, Victoria, Australia.

Primary brain tumours, including medulloblastoma and neuroblastoma, account for approximately 20% of all childhood

malignancies. Alterations in the cellular architecture, cell mobility and proliferation have all been shown to be factors in tumourigenesis. Changes in filamentous (F)-actin levels can alter cellular dynamics and cause inappropriate tissue growth. However, it is not known if actin cytoskeleton remodelling contributes to brain cancers. To determine if altering the actin cytoskeleton can induce tumour formation, we have manipulated the expression levels of components of the Rho signalling pathway in Drosophila brain and eye neuro-epithelial cells. Over-expression of an activator of Rho-family GTPases (RhoGEF2) in the larval brain led to a reduction in brain size, with decreased cellular proliferation and altered F-actin. In the eye neural-epithelium RhoGEF2 cooperates with activated Ras85D to result in invasive tumours, which depends on the Rho1-Rok-Myosin II pathway (1,2). In the brain nueral-epithilium inhibition of JNK-mediated apoptosis in the RhoGEF2 animals; restored brain size and led to an expansion of the neuro-epithelial cell population. This over-growth was associated with a partial loss of polarity. We are currently investigating whether tumourigenic cooperation is observed between oncogenes, known to be mutated in medulloblastoma, and the components of the Rho1 pathway in Drosophila brain and eye neuro-epithelium. This research has the potential to provide greater insight into the mechanism of action of cytoskeletal regulators and to discover novel therapeutic approaches by which to diagnose and target paediatric brain cancers. References: (1) Brumby et al., 2011,Genetics May;188(1):105-25. (2) Khoo et al., 2013, Disease Models & Mechanisms Jan 11. [Epub ahead of print].

992C

The polarized trafficking of polycystin-2 in Drosophila sperms. Weizhe Li, Stacey Cook, Terry Watnick. Medicine, university of Maryland, Baltimore, MD.

Cilia, a rod-like projection from cell surface is recently emerging as a critical organelle involved in many severe human inherited diseases including Polycystic Kidney Diseases, Joubert syndrome, Bardet-Biedl syndrome, etc. The Autosomal Dominant Kidney Disease (ADPKD) is a common inherited disease that affects 1 to 1000 people and usually results in kidney failure. The patients with ADPKD are suffered from progressive renal cyst formation. The ADPKD is caused by two genes, polycystin-1 and polycystin-2 by which the proteins encoded were found located on the primary cilia besides endoplasmic reticulum (ER) and plasma membrane. The lost of cilia itself can cause cyst formation in kidney. Polycystin-2 is putatively thought as a non-selective cation ion channel and act as a sensor for urine flow in kidney. But how polycystin-2 is transported to cilia is still largely unknown. Given the high homology of polycystin-2 in human and fruit fly, we took the advantage of unparalleled genetic tools of *Drosophila* to investigate the trafficking of polycystin-2 on cilia. In *Drosophila*, polycystin-2 is enriched at the very tip of sperm tails, a kind of motile cilia. By tracing the dynamic localization of polycystin-2 in *Drosophila*testis, we found that polycystin-2 was diffusely localized at the distal part of sperm tails right after the completion of individualization. Then, polycystin-2 was found to be concentrated at the very tip of sperm tails. After that, polycystin-2 was found localized only on one side at the tails of mature sperms. A sperm protein was identified to be critical for the polarized localization of *Drosophila* polycystin-2 at sperm tails.

993A

curcumin, a potent phytochemical for the treatment of Huntington's disese using Drosophila as a model system. Namita Agrawal, Anjalika Chongtham, Nidhi Paliwal. Dept of Zoology, University of Delhi, Delhi, India.

Huntington's disease (HD), a late-onset, progressive, autosomal dominant disorder caused by expansion of a homopolymeric polyQ tract within the huntingtin (Htt) protein.. HD is characterized by neuropsychiatric symptoms, generalized motor dysfunction and cognitive decline. The mandate to date is to find the preventive agents that has the capability to ameliorate the disease symptom with no or minimal side effect. To circumvent the side effects caused by pharmaceutical drugs, we have tested a well known phytochemical Curcuma longa (curcumin) in transgenic Drosophila model engineered to express mutant human genes of neurodegenerative disease like Huntington disease (HD). This model has been proven to be excellent models of these largely dominant human diseases by mimicking most of the disease symptoms, such as late onset, reduced longevity, neurodegeneration, and impaired motor function. We established a dose regimens of curcumin to assess its effectiveness on Drosophila HD model and our findings indicate that 10uM concentration of the curcumin significantly suppress disease symptoms without any side effect and prevents disease from progression if fed early during development. Our data clearly indicates that curcumin can be a potential drug for the treatment of HD pathogenesis in patients with maximum benefit and no side effect.

994B

Neurodegeneration and Altered Lipid Metabolism. Hannah Gordon, Anthea Letsou. Department of Human Genetics, University of Utah, Salt Lake City, UT.

Fatty acids perform key functions in cell biology by acting as membrane components, energy substrates, and signaling molecules. In addition to these functions, studies have revealed a strict requirement for a balance in lipid species, where incorrect regulation or overloading of certain lipid species can lead to their accumulation to toxic levels. The key to this duality of lipid dosage lies in the enzymatic tuning of ratios of particular lipid species with respect to chain length and branching. We have identified and characterized double bubble (dbb), a gene encoding an acyl-CoA synthetase (ACS) that is required for maintenance of adult CNS. 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 single mutants displays neurodegenerative phenotypes that are variably expressed and incompletely penetrant. Neurodegenerative phenotypes in bgm dbb double mutants, however, are more severely expressed and fully penetrant, thus providing a clearer platform to characterize the roles of ACS proteins and

lipid metabolism in neuropathology. The specific lipid alterations that result from the absence of these two ACS proteins, as well as the primary mechanism by which altered ACS activity leads to neurodegeneration is not currently understood. Answers to these questions will result in a greater understanding of the roles and toxicity potential of lipids in neuronal cell types in an in vivo model. Moreover, mutations in homologous proteins in this pathway are associated with the human diseases adrenoleukodystrophy (ALD), which is characterized by neurodegeneration. Here we show validation of this Drosophila double mutant as a model for ALD-a disease for which there is no treatment. Our studies show that neurodegeneration co-occurs with a behavioral defect and an abundance of very long chain fatty acids, both of which are shared phenotypes with humans. We expect future experiments with our fly model of ALD to lead to insight into disease mechanisms and ultimately to therapeutic options.

995C
Glia-Mediated Neurodegeneration in the Drosophila melanogaster CNS. Ivan J. Santiago, Amandeep Kaur, Rosa Mino, Elena Kaplan, Joanna Palacio, Israel Nnah, Tadmiri Venkatesh. Biology, City College of New York, Bronx, NY.
Proper development, function and maintenance of the central nervous system (CNS) are reliant on the intricate relationship between glia and neurons. Glia cells possess a variety of key functions such as maintaining homeostasis, providing neurons with trophic support and the uptake and recycling of neuronal debris. Disruptions in glial function have been implicated in neurological disorders. Despite their obvious importance in the CNS, glia cells remain much less characterized than their neuronal counterparts. With its wide array of genetic tools and its low glia-to-neuron ratio, Drosophila melanogaster presents itself as a useful model organism in studies of glial characterization. Rap/Fzr, an activator of the Anaphase Promoting Complex/Cyclosome (APC/C), a E3 ubiquitin ligase, is the Drosophila homolog of the mammalian Cdh1. Previous studies have shown that proper function of the APC/C regulates glial differentiation in the CNS (Kaplow et al., 2008). Our studies show that targeted pan-glial expression of Rap/Fzr/Cdh1 results in the reduction of glia number in the CNS of 3rd instar larvae. Adult Drosophila with reduced number of glia cells exhibit progressive age-dependent phenotypes such as temperature-sensitive paralysis and vacuolization of the brain, as well as significant lifespan reduction. Employing glia subtype specific GAL4 drivers, we have identified Astrocyte-like glia as a critical cell type in neuroprotection. Targeted expression of Rap/Fzr to Astrocyte-like glia results in temperature-sensitive paralysis and lifespan reduction. These neurodegenerative phenotypes suggest a vital role for Astrocyte-like glia in neuroprotection.

996A
VitaminK2 is a Mitochondrial Electron Carrier that Rescues Pink1 Deficiency. Melissa Vos[1], Giovanni Esposito[1], Janaka N. Edirisinghe[2], Sven Vilain[1], Dominik M Haddad[1], Jan R Slabbaert[1], Stefanie Van Meensel[1], Onno Schaap[1], Bart De Strooper[1], R Meganathan[2], Vanessa A Morais[1], Patrik Verstreken[1]. 1) VIB, center for the biology of disease, KU Leuven, Leuven, Vlaams Brabant, Belgium; 2) Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, US.
We identified Heixuedian (Heix), the fly orthologue of UBIAD1, in a genetic screen for ethylmethane sulphonate (EMS) induced modifiers of pink1. Pink1 is a mitochondrial kinase and pink1 mutations cause mitochondrial defects in flies and mice. In humans, UBIAD1 localizes to mitochondria and converts the natural plant compound vitamin K1/phylloquinone to vitamin K2/menaquinone (MK-n). Vitamin K2 was best known as a cofactor in gamma-carboxylation, involved in blood coagulation in humans, but in bacteria it serves as an electron carrier in the membrane. However, whether vitamin K2 can exert a similar electron carrier function in eukaryotic cells is unknown. Here, we show that vitamin K2 is necessary and sufficient to transfer electrons in Drosophila mitochondria. Heix mutants show severe mitochondrial defects that are rescued by vitamin K2 (MK-4) and we find that similar to ubiquinone, vitamin K2 transfers electrons downstream of a Drosophila ETC Complex to result in more efficient ATP production in an intact mitochondrial preparation. We thus not only identify Heix and its product vitamin K2, as modifiers of the pink1 mutant phenotype, but we also demonstrate that vitamin K2 serves as an electron carrier that aids in maintaining normal ATP production within mitochondria.

997B
The effect of two diets on the QTLs for triglyceride concentration in third instar larvae in *Drosophila melanogaster*. Sean R Mendez, Andrea Moss, Katie Bray, Laura K Reed. Biological Sciences, University of Alabama, Tuscaloosa, AL.
Metabolic syndrome is a collection of symptoms such as obesity, elevated blood lipids, and insulin resistance that often predicts type-2 diabetes and cardiovascular disease. The growing prevalence of this disease suggests a changing interaction between genotype and environment that is not yet fully understood. *Drosophila melanogaster* is an important model organism for studying the genetic and environmental factors and their interaction regarding metabolic syndrome. Our experiment involves crosses of recombinant inbred lines of *D. melanogaster* from the Drosophila Synthetic Population Resource, whose offspring are then raised on either high fat or control diets starting at the 1st instar stage. The larvae develop on their respective diets and are then measured for triglyceride concentration in the 3rd instar larvae. We have used this data to map multiple qualitative trait loci (QTLs) associated with genotype by environment interaction underlying triglyceride storage in order to gain a better genetic understanding of these effects. The overall concentration of triglycerides does not differ in larvae raised on a high fat diet compared with those on a normal diet but there is a significant amount of variation in levels between the crosses due to genetic variation. In fact, more than 15% of the phenotypic variation can be attributed to genotype by diet interaction effects. The results of this experiment will help us gain insight into the complex genetic interactions associated

with obesity so it can be applied to prevention of this costly disease.

998C

A Mitochondrial DNA Mutation Drosophila. zhe chen, hong xu. NHLBI, NIH, Bethesda, MD.

A dramatic accumulation of mitochondrial DNA mutations in somatic tissues appears to contribute to age-related disorders in humans, and numerous mutations on human mtDNA have been linked to maternally inherited diseases. The mitochondrial genome has not been very amenable to functional genetic studies in metazoans. Applying a selection scheme based on mitochodnirally targeted restriction enzymes, we isolated a Drosophila mutant with homoplasmic mitochondria DNA mutation in cytochrome c oxidase (COX) subunit I locus: mt:CoI^{T300I}. Phenotypes associated with mt:COI^{T300I} Drosophila mimic most of the symptoms of mitochondrial encephalomyopathies, including shortened life span, age-dependent muscle and neuron degeneration. mt:COI^{T300I} flies are lethal at pupae stage but not the larvae stage at non-permissive temperature. The COX activity was significantly decreased in the young mutant flies, while the ATP level was impaired at old age. Cytochrome a was dissociated from complex IV, which might play an important role in the COX activity decline. A microarray gene expression analysis was carried out to find the molecules potentially related with the pathology of the mutant flies. Furthermore, using this genetic tool, we originally studied mitochondrial disorder in a tissue-specific manner. By comparing different tissues, we found the impairment of nerve system plays an important role in the lethality of the mt:COI^{T300I} flies.

999A

The roles of *cis*- and *trans*-regulation in the evolution of regulatory incompatibilities and sexually dimorphic gene expression. Colin D. Meiklejohn[1], Joseph D. Coolon[2,3], Daniel L. Hartl[4], Patricia J. Wittkopp[2,3]. 1) Biology Department, Indiana University, Bloomington, IN; 2) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 3) Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI; 4) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Evolutionary changes in gene expression underlie many aspects of phenotypic diversity within and among species. Understanding the genetic basis for evolved changes in gene expression is therefore an important component of a comprehensive understanding of the genetic basis of phenotypic evolution. Using introgression hybrids, we examined the genetic basis for divergence in genome-wide patterns of gene expression between *D. simulans* and *D. mauritiana*. We find that 90% of gene expression changes are attributable to divergence at *trans*-regulatory loci and that *cis*-regulatory and *trans*-regulatory divergence differ significantly in patterns of genetic architecture and evolution. The effects of *cis*-regulatory divergence are approximately additive in heterozygotes, quantitatively different between males and females, and well predicted by expression differences between the two parental species. In contrast, the effects of *trans*-regulatory divergence are associated with largely dominant alleles, have similar effects in the two sexes, and generate expression levels in hybrids outside the range of expression in both parental species. Although the effects of introgressed *trans*-regulatory alleles are similar in males and females, expression levels of the genes they regulate are sexually dimorphic between the parental strains, suggesting that pure-species genotypes carry unlinked modifier alleles that increase sexual dimorphism in expression. Our results suggest that independent effects of *cis*-regulatory substitutions in males and females may favor their role in the evolution of sexually dimorphic phenotypes, and that *trans*-regulatory divergence is an important source of regulatory incompatibilities.

1000B

A long term demasculinization of X-linked intergenic noncoding RNAs in *Drosophila melanogaste*r. Maria Vibranovski[1,2], Ge Gao[3], Li Zhang[3], Zheng Li[3], Ming Liu[4], Yong Zhang[2,5], Xinmin Li[6], Wenxia Zhang[4], Qichang Fan[4], Nicholas VanKuren[2,7], Manyuan Long[2], Liping Wei[3]. 1) Genetics and Evolutionary Biology, University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA; 3) Center for Bioinformatics, National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing, P.R. China; 4) Center of Developmental Biology and Genetics, College of Life Sciences, Peking University, Beijing, P.R. China; 5) Key Laboratory of the Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China; 6) Genome Facility, University of Chicago, Chicago, IL, USA; 7) Committee on Genetics, Genomics, and Systems Biology, University of Chicago, Chicago, IL, USA.

Recent studies revealed key roles of noncoding RNAs in sex-related pathways, but little is known about the evolutionary forces acting on these noncoding RNAs. By profiling the transcriptome of *D. melanogaster* with whole-genome tiling arrays, we found that one-quarter of male-biased transcribed fragments are intergenic noncoding RNAs (incRNAs), suggesting a potentially important role for incRNAs in sex-related biological processes. Statistical analysis revealed a paucity of male-biased incRNAs and coding genes on the X chromosome, suggesting that similar evolutionary forces could be affecting the entire genome. Expression profiling across germline and somatic tissues further suggested that both male meiotic sex chromosome inactivation (MSCI) and sexual antagonism contribute to the chromosomal distribution of male-biased incRNAs. Comparative sequence analysis showed that evolutionary time of male-biased incRNAs has significant effect on their chromosomal locations. In addition to identifying abundant sex-biased incRNAs in fly genome, our work unveils a global picture of the complex interplay between non-coding RNAs and sexual chromosome evolution.

1001C

Genomic and phenotypic influences by misfolded proteins. Jun Zhou, Timothy Sackton, Elena Lozovsky, Daniel Hartl. Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Owing to typically complicated systems and a lack of tools, protein evolution is still poorly understood. Mutations destabilize the folding of proteins, and can presumably impose cytotoxicity in cells. How organisms are affected by and respond to misfolded proteins appears extremely interesting. Here, we constructed lines of transgenic fruit flies including a line expressing a gratuitous, exogenous YFP protein (WT), a second line expressing a misfolded YFP protein (MF) and a third line that only contains an empty transgenic vector (EMP). Gene expression profiling revealed that hundreds of genes were transcriptionally influenced by the introduction of the misfolded proteins. Many genes involved in protein folding (e.g. heat shock proteins) were significantly up-regulated in MF flies relative to EMP. Some of the heat shock genes also expressed at higher levels in WT compared to EMP. Genes functioning in protein digestion and proteolysis were over-represented in the contrast of MF and WT flies. These results suggested that the misfolded, and perhaps even normally expressed wild type exogenous, proteins could provoke cellular responses combating potential fitness burden. Very interestingly, in contrast to WT and EMP, MF flies exhibited significantly shorter life span in females. Yet the longevity differences were not as striking in males. Moreover, preliminary findings suggested that MF flies might have overall lower fecundity. Finally, a number of immune genes appeared enriched in the differentially expressed gene groups. This work will further our understanding on the genomic and phenotypic consequences of misfolded proteins such as the fitness cost to the organisms and cellular responses to overcome the threat. More extensive studies are needed to elucidate the evolutionary relevance of protein misfolding, independent of protein functions.

1002A

Higher polyandry is associated with lower prevalence of *sex-ratio* drive in natural populations of *Drosophila neotestacea*. Cheryl Pinzone, Kelly Dyer. Department of Genetics, University of Georgia, Athens, GA.

Selfish genetic elements can spread very rapidly through populations, even if they are harmful to their hosts. A sex-ratio (SR) X-chromosome causes Y-bearing sperm to die, and as a result SR is passed on to 100% of the offspring, all of which are female. In the presence of SR, the sex ratio of a population may become severely female biased, and theoretically could go extinct due to a lack of males. However in many natural populations, SR alleles are often observed to be segregating at stable polymorphism frequencies. One potential explanation is that SR males may have lesser fertility than normal males, and are therefore disadvantaged when females mate multiply due to both sperm competition risk and intensity. **Here we investigate SR male fertility and female mating rate in natural populations of*Drosophila neotestacea*.** We show that SR males produce fewer offspring than non-SR males, especially after multiple mating. We collected five wild populations of *D. neotestacea* that vary in SR prevalence, and found significant variation in female mating rate among populations. We found that higher female mating rates were associated with lower SR prevalence, which suggests that SR is at a disadvantage where females mate often. Furthermore, we performed reciprocal matings between males and females from populations with naturally high and low female mating rates, and found that males had a large effect on female mating rate. **Additionally, we report the first observation of a local extinction event likely due to SR drive.** In sum, our results suggest that polyandry may play an important role in regulating the frequency of SR in natural populations, and may prevent populations from extinction.

1003B

DNA methyltransferase (Dnmt2) is Conserved in Drosophilidae. Vera Gaiesky, Gilberto Vieira, Maríndia Deprá, Marícia D'Ávila. Genética, UFRGS, Porto Alegre, RS, Brazil.

Cytosine-5 methyltransferases of the Dnmt2 family are evolutionary conserved. To all drosophilids studied, it has been described only one enzyme, of the type Dnmt2. Studies have been performed aiming to characterize the DNA methyltransferase 2 (Dnmt2), responsible for DNA methylation in *Drosophila*, but the most data are available only for *Drosophila melanogaster*. The knowledge about conservation of DNA methylation in *Drosophila* related species and its evolutionary consequences is scarce. On this landscape, our study aimed to assess the degree of conservation of *Dnmt2* gene in different species of*Drosophila* in order to infer the evolutionary significance of DNA methylation into the genus. Therefore, samples of genomic DNA of 60 species of the family Drosophilidae were evaluated by PCR technique using primers for *Dnmt2* gene described previously to *D. melanogaster.* The fragments obtained were purified and submitted to automatic sequencing. The chromatograms analysis and alignments were made by the Staden Package and Mega 5.05 software's, and to access the percentage of identity and sequences conservation we used the GeneDoc. We obtained amplicons from 18 species of the *Drosophila*genus in addition to insects sequences whose genomes are available in Flybase database; a total of 30 species belonging to the groups: *guarani, guaramunu, tripunctata, calloptera, immigrans, mesophragmatica, flavopilosa, repleta, melanogaster* and *willistoni*. The analysis of evolutionary divergence estimates of the 13 groups of *Drosophila* presented a minimum value of 14.1% between*guaramunu* and *calloptera* groups, and a maximum value of 35.5% between *willistoni* and *mesophragmatica* groups. The *Dnmt2* amino acid alignment showed large conservation in catalytic portions of Dnmt2 enzyme but there are considerable differences at the recognizing region of target sequence to be methylated. Phylogenetic analyses of nucleotide sequences revealed that the three main clades of the Drosophilidae species *(virilis-repleta* radiation, *quinaria-tripunctata* radiation and *Sophophora* subgenus) were recovered.

1004C

Phylogenetic considerations on the *Drosophila willistoni* species group: morphological versus molecular approaches. Rebeca Zanini[1,2], Maríndia Deprá[1,2], Vera Valente-Gaiesky[1,2]. 1) Programa de Pós-Graduação em Biologia Animal, UFRGS, Porto Alegre, RS, Brazil; 2) Laboratório de Drosophila, Departamento de Genética, UFRGS, Porto Alegre, RS, Brazil.

The *Drosophila willistoni* species group currently is composed by 23 species grouped in three subgroups: *willistoni*, *alagitans* and *bocainensis*. The *willistoni* subgroup encompasses six cryptic species, including *D. paulistorum* species complex, which is formed by six semi-species. The species and semi-species of this subgroup are probably in different evolutionary stages, which lead to a difficult stablishment of the phylogenetic relationships in this subgroup. In the past years some studies tried to solve this issue using both nuclear and mitochondrial genes. However, the phylogenetic relationships recovered were not congruent when the different datasets were considered. In the present study we attempt to compare morphological and molecular phylogenetic reconstructions, in order to improve the evolutionary relationships of the willistoni species subgroup. For morphologial approach we used several characters of the adults and immature stages. For molecular data, were used the mitochondrial genes*Cytb* and *COII* and the nuclear genes *Adh* and *Ddc*. Phylogenetic trees were constructed by Maximum Likelihood and Bayesian inference using appropriated models in *Phyml* and *Mr. Bayes*, respectively; the morphological tree was generated in *TNT* under Maximum Parsimony. Despite being cryptic species, we found several characters of the male genitalia and of the eggshell useful when differentiating this species. Mitochondrial data and nuclear data produced trees with some differences in topology. Semi-species of *D. paulistorum* complex were recovered as a monophyletic clade in nuclear and morphological dataset and as a polyphyletic clade in mitochondrial dataset. *D. tropicalis* and *D. willistoni* were grouped together in nuclear, morphological and combined dataset; in mitochondrial dataset *D. tropicalis* was showed as more basal related to *D. willistoni*. Morphological and nuclear data are in accordance in the position of several taxa in phylogenetic trees.

1005A

Thermal Plasticity of Body and Organ Size in *Drosophila melanogaster*. Shampa Ghosh Modak, Johnathon W. Constan, Alexander W. Shingleton. Dept. of Zoology, Michigan State University, East Lansing, MI.

Phenotypic plasticity, the ability to vary phenotypic expression with environment, is essential to allow organisms to cope with short-term environmental changes. The genetic basis of phenotypic plasticity remains poorly understood. Body and organ size in *Drosophila* show differing levels of plasticity in response to the developmental temperature. We are interested in understanding the genetic basis of thermal plasticity of body and organ size in *Drosophila melanogaster*, and the extent of genetic variation for thermal plasticity in natural populations. We reared 100 wild-derived inbred lines of the Drosophila Genetic Reference Panel (DGRP) in the laboratory at 17°C, 25°C and 28°C and measured the thermal plasticity of thorax, wing and femur size. Average wing size underwent 30% reduction from 17°C to 25°C, whereas thorax and femur length both showed a 6% reduction in the same thermal range. From 25°C to 28°C, however, wing and thorax size showed small but significant reduction of 3-4%, but the femur did not undergo significant change. We found considerable genetic variation not only in body and organ size, but also in the degree of thermal plasticity across lines, evident from the crossing of thermal reaction norms of the DGRP lines. Our phenotypic data of DGRP lines therefore show that not only do adult flies display different body proportions at different temperatures but that there is considerable amount of genetic variation for the relationship between body proportion and temperature. A GWAS study to identify SNPs associated with natural variation in thermal plasticity of wing, thorax and femur size is under way, which will allow us to identify the genetic loci responsible for this variation.

1006B

Characterizing the Mechanisms that Regulate Gonad Morphogenesis in the *Drosophila* Embryo. Jennifer C. Jemc[1], Mark Van Doren[2]. 1) Dept. of Biology, Loyola University Chicago, Chicago, IL; 2) Dept. of Biology, Johns Hopkins University, Baltimore, MD.

During organogenesis, different cell types must migrate and interact to form a functional organ with the proper architecture. The *Drosophila* gonad is an excellent model for this process. During embryonic development, the gonad is formed from two populations of cells: the somatic gonadal precursors (SGPs) and germ cells (GCs). While GCs are specified at the posterior pole of the embryo , SGPs are specified in three bilateral clusters in parasegments (PS) 10-12 of the mesoderm. A fourth cluster of SGPs are specified in PS 13, but only survive to join the gonad in males. GCs migrate toward mesoderm and intermingle with SGPs. The three clusters of SGPs undergo a process called SGP cluster fusion, and the SGPs and GCs coalesce to form a contiguous tissue. At this time, the GCs are surrounded by extensions from the SGPs in a process called ensheathment. Ensheathment is maintained as the gonad undergoes compaction to form a sphere. Previous studies identified a number of mutants that regulate GC migration, and more recent work has resulted in the identification of a number of genes that are required for SGP cluster fusion, GC ensheathment and gonad compaction. Currently, we are exploring the mechanisms by which these genes function to regulate SGP cluster fusion and ensheathment during gonad morphogenesis. One class of genes that we have found to regulate gonad formation are laminins. Previous work identified LanB1 as regulator of the process of ensheathment. Our studies have found that LanB1 specifically cooperates with LanA to mediate ensheathment. We observe localization of laminins within the gonad, suggesting that laminins promote the ability of the SGPs ensheath GCs. Currently, we are working to identify the receptors to which laminins bind to promote ensheathment, and to characterize the downstream molecular mechanisms by which they function.

1007C

Spatiotemporal control of Cindr at ring canals during incomplete cytokinesis in the Drosophila male germline. Åsmund H Eikenes[1,2], Harald Stenmark[1,2], Kaisa Haglund[1,2]. 1) Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway; 2) Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Norway.

In species ranging from insects to mammals, male and female germ cell cyst formation by incomplete cytokinesis involves the stabilization of cleavage furrows and the formation of stable intercellular bridges called ring canals. Accurate regulation of incomplete cytokinesis is required for both female and male fertility in *Drosophila melanogaster*. The molecular control of complete versus incomplete cytokinesis is however largely unknown. Here we show that the scaffold protein Cindr is a novel component of mitotic and meiotic ring canals during *Drosophila* spermatogenesis. We show that Cindr co-localizes with Anillin at both mitotic and meiotic ring canals. Interestingly, upon completion of the mitotic germ cell divisions, Cindr and F-actin dissociate from mitotic ring canals and translocate to the fusome, whereas other components, including Anillin and Pavarotti, remain. We provide evidence that the loss of Cindr from ring canals is coordinated by signals mediating the transition from germ cell mitosis to differentiation. Strikingly, Cindr loss from mitotic ring canals coincides with the endpoint of the mitotic germ cell divisions in both *Drosophila* females and males, thus marking a novel step of gametogenesis. During meiosis, Cindr is required for efficient execution of ring canal formation, because Cindr depletion gives rise to an increased number of binuclear cells. We also show that Cindr is recruited to the contractile ring by Anillin during meiosis, thus functioning downstream of this master cytokinesis regulator. Taken together, our analyses reveal a key step of incomplete cytokinesis during mitotic germ cell divisions and contribute to understanding the spatiotemporal control of incomplete cytokinesis *in vivo*.

1008A

The actin binding protein profilin controls soma-germline interaction and differentiation upon exit from the stem cell niche in the Drosophila testes. Michael J Fairchild, Guy Tanentzapf. University of British Columbia, Vancouver, BC, Canada.

The Drosophila testes house a stem cell niche containing both somatic and germline stem cells that co-regulate one another's proliferative capacity. Upon exiting the stem cell niche, somatic daughter cells encapsulate germline daughter cells forming the mature spermatogenic cysts. This close association and communication between the somatic and germline daughter cells throughout spermatogenesis ensures proper differentiation of the germline into mature sperm. In order to better understand how the soma contributes to regulation of germline stem cell maintenance and differentiation we undertook a forward genetic screen in the somatic cells of the testes using tissue specific RNAi knockdowns. Using sterility as a phenotypic assay we identified upwards of 100 genes required specifically in the soma for fertility. Among these, we found chickadee, the Drosophila homologue of the actin binding protein profilin, to be strongly required in the soma, and was analyzed in detail. We found that profilin was enriched in the somatic cells of the testes, both in the somatic stem cells and their early daughter cells during germline encapsulation. As profilin is essential for cell viability we investigated somatic cell survival and differentiation and found that profilin deficient somatic cells not only survived and differentiated, but also over proliferated. While profilin deficient somatic cells were often found in contact with the germline we found that many germline cells were not encapsulated, consequently forming large germline tumors with spermatogonial characteristics. We found that this may be due to profilin deficient somatic cells having defects in EGFR-MapK signaling which controls germline encapsulation. Our results show that profilin is required in the somatic cells of the testes for effective soma-germline communication, ensuring effective spermatogenesis.

1009B

p53 is sequestered to nuclear bodies in spermatogonia. Gary R Hime[1], Adrian C Monk[1], Helen E Abud[2]. 1) Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, Australia; 2) Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia.

Drosophila p53 is the sole member of the larger p53 superfamily. The vertebrate p53 family consists of three paralogues: p53 - often referred to as a "genomic gatekeeper" due to its role in responding to cellular stresses by activating expression of genes that trigger cell cycle arrest, p63 and p73 - that form complexes with p53 and each other and also have roles in the regulation of cell differentiation. All of the vertebrate family members have been associated with nuclear PML bodies which function as protein anchoring sites in the nuclear matrix associated with DNA replication, transcription and epigenetic silencing. We found *Drosophila* p53 to be expressed at high levels in pre-meiotic male germ cells where it was also localised to nuclear bodies. This localisation is dependent upon p53 complexes being able to bind DNA. *p53* loss of function mutants are viable, fertile and do not affect germline stem cell (GSC) maintenance or differentiation however GSCs are sensitive to overexpression of p53 but maturing spermatogonia are not. The ability of p53 to induce apoptosis in the male germline thus depends upon the stage of germ cell maturation.

1010C

The Highly Conserved LAMMER/CLK2 Protein Kinases Prevent Germ Cell Overproliferation in Drosophila. Shaowei Zhao[1,2], Di Chen[1,2], Qing Geng[1,2], Zhaohui Wang[1]. 1) State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, P.R. China; 2) Graduate School, Chinese Academy of Sciences, Beijing.

Germ cells undergo proper mitotic amplification before entering meiosis. The mitosis/meiosis switch drives the germ cells to leave the potential stem cell pool and become terminally differentiated. This important process is tightly controlled in the

spermatogenesis of all animals. However, a unifying mechanism has yet to be unraveled. Drosophila spermatogenesis is an ideal system to dissect the regulatory program of the mitosis/meiosis switch. The timely accumulation of the pro-differentiation factor Bam has been shown to be central in this process. In a Drosophila genetic screen, we discovered that the mutations in Doa, a gene encoding a member of the highly conserved LAMMER/Cdc2-like kinase (CLK) family, cell-autonomously induced the germ cell overproliferation due to the failed transition from mitosis to meiosis. Additional Bam expression in Doa mutant germline promoted the differentiation from the mitotic to the meiotic state. Remarkably, the human or murine CLK2 could prevent the germline overproliferation and even restore the fertility of Doa mutant flies. Such rescuing activity of Doa or its human homolog requires a conserved residue in their predicted kinase catalytic domain. We propose that LAMMER/Cdc2-like kinase, represented by Doa and its mammalian homolog CLK2, is a critical and conserved component in the regulatory program of the mitosis-to-meiosis switch.

1011A

A wealth of novel and conserved bioactive molecules identified in the venom gland profile of a Drosophila parasitic wasp, Leptopilina heterotoma. Mary Ellen Heavner[1,2], Gwenaelle Gueguen[1], Roma Rajwani[1], Chiyedza Small[1,2,3], Shubha Govind[1,2]. 1) Biology, City College, CUNY, NY; 2) Graduate Center, CUNY, NY; 3) Medgar Evers College, CUNY, NY.

The host range of the generalist endoparasitoid wasp Leptopilina heterotoma includes the highly-studied model organism, D. melanogaster. This host is one among approximately a dozen other host Drosophila species. The accessory long gland-reservoir complex of the wasp L. heterotoma produces venom and virus-like particles. VLPs are thought to be responsible for causing immune deficiency in larval stages of the fly hosts. Venom components also delay larval development. To understand how VLPs are produced in the venom gland and to characterize the bioactive VLP/venom components that might modulate host immunity and development, we have initiated a transcriptome analysis of the long-gland reservoir apparatus. We will present results of pilot sequencing and analysis of 827 unigenes. This analysis showed that approximately 25% of the sequences are novel or classified as hypothetical proteins. A majority of the remaining expression is related to cell cycle, signaling, and cell physiology. This analysis demonstrates striking conservation among the stinging Apocrita species such as the honeybee, ants, and the well-characterized ectoparasitoid jewel wasp, Nasonia vitripennis. We will present enzyme and KEGG pathway profiling of molecules predicted in the L. heterotoma venom and discuss their potential function as virulence factors.

1012B

A sensillum-selective screen identifies a gene required for olfactory response to ammonia. Karen Menuz, Nikki Woodward, Joori Park, John Carlson. Yale University, New Haven, CT.

In Drosophila, olfaction relies upon olfactory receptor neurons (ORNs) housed in three morphological classes of sensory hairs: basiconic, trichoid, and coeloconic sensilla. Many coeloconic ORNs in insects primarily respond to amines and acids, two classes of odors that are not generally detected by other ORNs and are behaviorally relevant for many insects. For example, a subset of coeloconic ORNs in many insect species detect ammonia, a key volatile involved in the chemoattraction of mosquitos to humans. However, the contribution of coeloconic ORNs to olfactory coding and behavior remains poorly understood compared to the function of basiconic and trichoid ORNs. To identify genes that are highly enriched in coeloconic sensilla and that may contribute to their unique function, we undertook a high-throughput RNA sequencing screen of antennae from wild-type flies and atonal mutants, which selectively lack coeloconic sensilla. We identified 326 candidate genes whose expression is eliminated or greatly reduced in atonal antennae. One candidate gene is predicted to be an ammonia transporter. Using in situ hybridization, we found that this ammonia transporter is selectively expressed in support cells in a subset of coeloconic sensilla. Electrophysiological analysis of a mutant defective in this transporter indicated that it is critical for ammonia responses in coeloconic ORNs. This phenotype validates the power of our screen, and suggests that support cells can substantially contribute to ORN coding. This study will guide further research into the molecular underpinnings of olfactory coding in the antenna.

1013C

New insights into the influences of SHAGGY (SGG) on the circadian clock. Robin Fischer, Nicolai Peschel. Neurobilogy and Genetics, University of Würzburg, Würzburg, Bavaria, Germany.

Circadian rhythmicity in Drosophila melanogaster is caused by two negative feedback loops in discrete clock neurons. One of the loops is described by the cycling of two proteins, PERIOD (PER) and TIMELESS (TIM). Thereby many kinases are involved in fine tuning of the core machinery and in adjusting to environmental cues, like daylight. Glycogen synthase kinase 3 (GSK3), the human ortholog of SGG, is one putative regulator, although yet usually described as an important pivot in cellular processes influencing protein synthesis, cell pattern organization, proliferation, differentiation and motility. Despite all these multifunctional features, an additional connection addressing the circadian clock could be shown. We started leant on earlier publications which showed an interaction of SGG and TIM (Martinek et al., 2001), as well as CRYPTOCHROME (CRY) (Stoleru et al., 2007), the light detector molecule of the clock. Latter work showed that an overexpression of SGG leads to rhythmicity under constant light conditions, a situation where normally a total arrhythmic behavior prevails. Here we examined the circadian rhythmicity in flies with increased or reduced SGG levels by using deficiency- or RNAi-lines for SGG, as well as flies overexpressing the endogenous sgg-gene or different constructs, comprising SGG isoforms. So far, we could not confirm the results of Stoleru et al., but observed an influence of SGG on PER as well as significant changes in the locomotor activity of the

affected flies up to total arrhythmicity under constant darkness.

1014A

Role of *dati* in the neuronal circuitry of female acceptance of male courtship in *Drosophila melanogaster*. Rachel Giesey, Joseph Schinaman, Claudia Mizutani, Rui Sousa-Neves. Department of Biology, Case Western Reserve University, Cleveland, OH.

Female acceptance or rejection of males during courtship of Drosophila melanogaster is an excellent model system to study how complex innate behaviors are controlled by the nervous system. To investigate this process further, we exploited a 4th chromosome mutation dati, which causes females to reject males' courtship at a much higher rate than their wild type counterparts. dati encodes a transcription factor expressed in a large subset of cells in the adult brain. To narrow down the regions within the brain that require dati expression for female acceptance, we used RNA interference to knock down dati expression in all neurons, all glial cells, and in five specific neuronal sub-populations. Through this screening, we found out that female acceptance is disrupted when dati is exclusively removed from cholinergic neurons. We are currently testing the role of dati in the development and regulation of cholinergic neurons. Our studies also complement an alternative approach to map brain regions involved in female acceptance using somatic clones mutant for dati that are induced at random regions.

1015B

Tay negatively regulates ERK activity in imaginal discs and motoneurons. Cristina Molnar, Jose F. de Celis. Centro de Biología Molecular , Madrid, Madrid, Spain.

The Epidermal Growth Factor Receptor (EGFR) signaling pathway is required during central nervous system development for the specification of cell fates in neuroblasts and neuronal lineages, and also regulates the consolidation and maintenance of sleep during the adult life. The key transducer of this pathway is the extracellular signal-regulated kinase ERK, which activity promotes the destabilization of the transcription repressor Capicua in the nucleus. It is not known whether other additional regulators modulate EGFR signaling in the developing CNS. The gene tay-bridge (tay) encodes a protein with a 30% homology to the human protein AUTS2 (Autism candidate susceptibility gene 2), and its function is required for the correct formation of the protocerebral bridge. Tay mutations display reduced walking speed and reduced sensitivity to alcohol. We find that Tay functions as a negative regulator of the EGFR pathway in imaginal discs, antagonizing EGFR signaling through the modulation of ERK activity. We also analyzed the effects of tay and some of the EGFR pathway components in locomotion. We find that both knock-down of tay expression and increase in EGFR signaling in the motoneurons cause a reduction in walking speed. The expression of Tay is maximal in motoneurons with low levels of activated ERK, suggesting that Tay restrict the activity of EGFR signaling to a particular sub-population of motoneurons to regulate locomotion.

1016C

Polyamines induced behavior in Drosophila melanogaster. Ashiq Hussain, Ilona Grunwald Kadow. Max Planck Institute of Neurobiology, Sensory Neurogenetics Research Group, Am Klopferspitz 18, 82152 Martinsried, Germany.

Carrion smell is strongly repugnant to humans and triggers distinct innate behaviors in many other species. This smell is mainly carried by two small aliphatic diamines, putrescine and cadaverine, which are generated by bacterial decarboxylation of the basic amino acids ornithine and lysine. Depending on the species, these diamines may also serve as attractants or social cues. Interestingly, the level of putrescine also increases during the development and ripening period of several fruits (banana, mango, blueberry, tomato and peach). Here, we describe that in a classical olfactory choice assay, polyamines (spermine, spermidine, diaminopropane, putrescine, cadaverine, diaminohexane, diaminoheptane, diaminooctane and diaminodecane) trigger robust attractive behavior in Drosophila melanogaster with high sensitivity. Similar olfactory behavior was observed in odorant receptor negative flies suggesting that classical odorant receptors are not involved in perception of polyamines. Interestingly, ionotropic receptor negative flies could not detect putrescine and cadaverine evidencing that IRs are involved in perception of these diamines. In contrast to the olfactory choice assay, in oviposition assays, flies avoid to lay eggs on putrescine or cadaverine containing food. These data suggest that flies display opposite context dependent behaviors to these diamines. We now aim at the identification of sensitive and specific Drosophila chemosensory receptors that will provide a molecular basis for studying the underpinning neural circuits. These behaviors and responsible neural mechanisms provide a powerful model for studying how organisms balance opposing behavioral signals involved in choice-like processes.

1017A

Drosophila WDR40A facilitates evoked neurotransmitter release at presynaptic terminals via a novel lipid based mechanism. Lilian Patron[1], Mays Imad[1], Kei Nagatomo[2], Konrad Zinsmaier[1,2]. 1) Department of Neuroscience; 2) Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ.

We conducted a forward genetic screen to identify novel genes regulating neurotransmitter release. Two of the isolated lethal alleles, B332 and B417, mutate the Drosophila gene CG3313, which encodes an ortholog of the mammalian WD repeat-containing protein 40A (WDR40A). The fly protein is ubiquitously expressed in the cytoplasm and the nucleus of many cells, including neurons and their axons and synaptic terminals. dWDR40A mutant neuromuscular junctions (NMJs) show a significant decrease in the number and size of glutamatergic type I boutons and exhibit abnormal octopaminergic type II axonal innervation. In addition, dWDR40A mutants show a severe impairment of evoked neurotransmitter release to 30% of control flies, which can be restored by presynaptic expression of normal dWDR40A. Since the structural synaptic defects

cannot explain the more severe functional defects, we propose that dWDR40A has 2 mechanistically independent synaptic roles. dWDR40A likely facilitates normal neurotransmitter release by regulating the activity (or levels) of the presynaptic lipid flippase dATP8B. At the presynaptic membrane of larval NMJs, dATP8B mediates the inward translocation of the anionic lipid phosphatidylserine (PS), a critical requirement for normal evoked neurotransmitter release (unpublished). Heterozygous double null mutations of dWDR40A and dATP8B severely reduce evoked release, suggesting that both proteins act in a common signaling pathway. Consistently, dWDR40A mutants show increased PS and dATP8B levels in the presynaptic membrane. Furthermore, overexpression of dATP8B in dWDR40A mutants, but not in wild type animals, causes embryonic lethality. Hence, we propose that dWDR40A controls dATP8B and PS levels at presynaptic terminals, thereby mediating a novel lipid based mechanism regulating presynaptic function.

1018B

Dscam Exon4 Isoforms Show Stochastic and Class-Specific Expression in Da Neurons. Wenke Zhou, Yang Hong. Department of Cell Biology, University of Pittsburgh Medical school, Pittsburgh, PA.

Down syndrome cell adhesion molecule (Dscam) plays critical roles in neuronal recognition and circuit formation. An extraordinary feature of dscam in Drosophila is that it encodes a potential 38,000 isoforms through alternative splicing of several exons. The isoform-specific adhesion between Dscam proteins suggests that each isoform may confer unique identity on a given neuron during circuit assembly. However, it remains unknown as to how individual Dscam isoforms are expressed in vivo at single neuron level. Using our recently developed genomic engineering method, we investigated the expression profiles of alternatively-spliced dscam exon 4, which contains twelve alternative splicing forms, in the dendritic arborization (da) neurons in Drosophila larva. The da neurons, which consist of 4 different classes, can be precisely and reproducibly identified at single neuron level, allowing us to profile isoform expression in single neurons. We generated 12 dscam knock-in alleles that each only expresses one of the 12 splicing isoforms of the exon 4. We then quantitatively measured the expression levels and patterns of these isoforms in single da neurons. Our results showed that each exon 4 isoform showed distinct expression levels in da neurons, while different class of da neurons were also biased to different isoforms. However, in each individual da neurons expression patterns of different exon 4 isoforms appear to be stochastic. Our data support the hypothesis that a stochastic but biased repertoire of Dscam isoforms, rather than a specific single isoform, confers neuronal identity in development and circuit formation.

1019C

Lk6 regulates the translation of glutamate receptors at the Drosophila NMJ. Nizar Hussein, Brittany Tounsel, Faith Liebl. Biological Sciences, Southern Illinois University, Edwardsville, IL.

Glutamate receptors (GluRs) are important for learning and memory. Disruptions in the molecular pathways required for the production or localization of GluRs impair synaptic plasticity. We have found that mutations in *lk6*, the Drosophila ortholog of the MAP kinase-interacting kinases *mnk1* and *mnk2*, significantly reduce postsynaptic glutamate receptor cluster sizes. Both overexpression and knockdown of the*lk6* lead to significant decreases in GluR cluster sizes. Lk6 is directly activated by ERK signaling and is suggested to be involved in protein synthesis through its phosphorylation of eIF4E, which initiates translation by binding to the 5' cap of mRNA. Similar to *lk6*, knockdown of *eIF4e* in both motor neurons and postsynaptic muscle resulted in loss of GluRs at the synapse. Further, overexpression of a nonphosphorylatable allele of *eIF4e*, $eIF4E^{S251A}$, in both motor neurons and muscle also produced a loss of GluRs at the synapse. In addition to Lk6, target of rapamycin (TOR) has also been shown to phosphorylate eIF4E. To assess the relative contributions of Lk6 and TOR to eIF4E phosphorylation and regulation of GluRs, we examined the effects of the TOR inhibitor, rapamycin, on synaptic GluR levels. Collectively, our results support the hypothesis that Lk6 regulates GluR translation.

1020A

The autophagy genes, atg1 and atg8a, positively regulate glutamate cluster formation at the Drosophila NMJ. Samuel Peters, Faith Liebl, Derek Beatty. Department of Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL.

Synaptic communication depends on the spatially correct formation of presynaptic terminals and the localization of postsynaptic receptors. The development and assembly of glutamatergic synapses is of particular importance because the majority of excitatory transmission in the mammalian CNS occurs via ionotropic glutamate receptors. We found, through a forward genetic screen, that the autophagy-specific gene 1 (Atg1) is necessary for the formation of glutamate receptor (GluR) clusters at the Drosophila neuromuscular junction. Although autophagy is necessary for cell survival, animals lacking a functional atg1 gene develop normal muscle size and morphology. Atg1 mutants, however, exhibit a reduction in the synaptic levels of the glutamate receptor subunits, GluRIIA, GluRIIB, and GluRIIC. Similarly, mutations in atg8a also reduce synaptic levels of GluRs. Interestingly, loss of Atg13, which forms an initiation complex with Atg1 on the isolation membrane during autophagy, does not affect synaptic levels of GluRIIA. To determine whether the GluR phenotype is a consequence of autophagy, we examined TOR, PKA, and AMPK mutants, all of which affect pathways that activate autophagy. The resulting phenotypes did not mimic the atg mutant phenotypes. Based on this and the phenotype of other atg mutants, we are currently testing the possibility that Atg1 and Atg8a signal independently of autophagy and one another to regulate GluR trafficking.

1021B

A forward genetic screen reveals novel players involved in synaptic bouton growth at the *Drosophila* neuromuscular junction. Chi-Kuang Yao[1,2,3], Shu-Hui Lin[1], Jhan-Jie Peng[1], Tzu-Li Yen[1]. 1) Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan; 2) Neuroscience program in academia sinica, Academia Sinica, Taipei, Taiwan; 3) Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan.

A synapse serves as the fundamental unit that dictates information flow between individual neurons. It is widely believed that long-lasting structural change at synapses of mature neurons in response to activity stimuli as well as environmental inputs holds the cellular basis for learning and memory. Furthermore, aberrant synaptic growth has been indicated as an early event in more and more neurological disorders. In order to systematically unravel the molecular mechanisms that control proper synaptic growth, our lab has carried out a forward genetic screen to identify mutations affecting synaptic bouton architecture at the *Drosophila* NMJ. In our screen, about 300 uncharacterized EMS mutagenized stocks were collected from previous ERG or bristle morphology screens done in Dr. Hugo Bellen's lab, BCM. We first determined their lethal phase, and homozygous larvae of 89 mutant stocks were able to survive at least to early 3rd instar stage. We further dissected NMJs and labeled pre- and postsynaptic membranous compartments with anti-HRP and anti-Dlg antibodies, respectively. Our preliminary results showed that 7 mutants display the reduced number of NMJ boutons. In contrast, 15 mutants display the increased number of NMJ boutons. Interestingly, NMJs derived from 4 out of 15 mutants significantly were composed of excess satellite boutons, which have been documented in mutants showing defects in numbers of growth signaling pathways, such as BMP and Wnt signaling pathways and synaptic vesicle endocytosis. We are currently mapping their corresponding genes. We hope that identification of these new components will give great insights into the molecular control of synapse formation as well as synapse plasticity.

1022C

MicroRNA regulation of *Drosophila* maternal mRNAs. John M. McLaughlin[1,2], Abigail Carr[1], Diana P. Bratu[1,2]. 1) Department of Biological Sciences, Hunter College, CUNY, New York, NY; 2) CUNY Graduate Center, New York, NY.

Embryonic axis patterning in Drosophila is initiated by maternally deposited mRNAs, many of which code for transcription factors, RNA-binding proteins, and cell cycle regulators that function during embryogenesis. Translational control of maternal transcripts, both spatially and temporally, is a prominent feature of oogenesis and essential for early development. Protein regulatory factors, bound to cis-acting sequence elements, control the translation of some maternal transcripts, including oskar and gurken. Small RNA pathways are now also known to regulate gene expression in eukaryotes. MicroRNAs (miRNAs) are small RNA regulators of gene expression, widely conserved throughout eukaryotic genomes and essential for animal development. We hypothesized that the miRNA pathway mediates the translational repression of a subset of maternal mRNAs during oogenesis. Several maternal mRNAs were examined and found to have one or more predicted miRNA binding sites within their 3'UTRs. In S2 cells, translation of 3'UTR-linked luciferase reporters was reduced specifically in the presence of each miRNA, and this repression was abolished when the miR's seed-binding region was mutated. Further studies will be carried out in vivo using maternal transgenes bearing mutations in their miRNA-binding sites to observe developmental defects caused by translational misregulation. We suspect that miRNA regulation of maternal mRNAs may serve as an additional layer of translational control in oocytes.

1023A

Systematic organization of intestinal cells and stem cells along the anterior posterior midgut axis Alexis Marianes and Allan C Spradling, Department of Embryology, Carengie Institution for Science, Howard Hughes Medical Institute, Baltimore, MD 21218. Alexis Marianes. Carnegie Institute, baltimore, MD.

The adult Drosophila midgut is maintained by intestinal stem cells (ISCs), which asymmetrically divide to replenish themselves and produce differentiated cell types of the adult midgut. We analyzed the properties of intestinal cells along the anterior-posterior axis and found that the tissue contains more specialized regions than the medial zones specialized for iron and copper absorption. By analyzing nutrient staining, cell morphology, and gene expression, we identified at least 10 distinct midgut subregions. Each such zone was isolated using lines from the Janelia GAL4 collection, and characterized by RNAseq to identify their candidate digestive pathways. The existence of significant physiological diversity along the length of the midgut raised the question of whether ISCs can support all midgut cells or if they are region specific. Using lineage tracing system in conjunction with regionally restricted gal4 lines we show that intestinal stem cells differ regionally in behavior and in some cases that their progeny do not cross subregional boundaries.

1024B

Identification of Spalt target genes and analysis of their regulatory regions. Mercedes Martin, Jose F. de Celis, Maria F. Organista. Centro de Biologia Molecular, Cantoblanco, Madrid, Spain.

The Drosophila genes spalt major (sal-m) and spalt-related (sal-r) encode Zn-finger transcription factors, and their expression is regulated by the Decapentaplegic (Dpp) signaling pathway in the wing imaginal disc. We have characterized the requirements of the Sal genes during wing disc development, identifying which aspects of the Dpp functions depend on Sal activity. We found that cell survival and proliferation in the central region of the wing are regulated by Dpp signaling through Sal activity, but that vein differentiation and growth in the lateral regions of the disc are regulated by Dpp independently of Sal. The Spalt proteins are transcription factors that most likely regulate gene expression by repression. However, the identity of their target genes in the wing is still unknown. We have compared the expression profiles of sal and salr mutant wing discs

with control discs, with the aim of identifying candidate Sal/Salr targets. We studied by in situ hybridization the expression pattern of the genes whose mRNA levels varied significantly, and selected 40 genes which expression change dramatically in sal mutant disc compared to wild type discs. For these genes, we will present a preliminary analysis of the structure of their regulatory regions. The identification of direct Sal and Salr target genes and the analysis of their functions is a critical step towards understanding the control of growth and patterning of the Drosophila wing imaginal disc by Dpp and its downstream genes Sal and Salr.

1025C

OR22a, A Canonical Odorant Receptor, Promotes Longevity in *D. melanogaster*. Ceyda Bilgir[1], Xiowen Chu[2], 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.

Olfactory signaling has been shown to modulate *D. melanogaster* lifespan. The increased lifespan phenotype of diet-restricted flies can be partially rescued by exposing animals to odorants from live-yeast paste; anosmic flies that lack the atypical odorant receptor OR83b, which is normally expressed broadly in roughly 80% of all olfactory receptor neurons (ORNs), live longer and have increased stress resistance. Canonical odorant receptors (ORs) are selectively expressed in small sets of olfactory neurons distributed throughout the olfactory organs. Most fly ORNs contain OR83b and one or two conventional ORs, which confer ligand specificity and potential functional specificity to the ORN. Whether these standard olfactory receptors (or their associated neurons) are capable of modulating lifespan is unknown. Here we describe a role for Odorant Receptor 22a (OR22a) in regulating lifespan and altering aging physiology in the fly. Mutant flies that lack the *Or22a-Or22b* gene cluster exhibit significantly reduced lifespan. Physiological parameters are also altered in these mutant animals: they do not accumulate wild-type levels of fat, and their stress resistance is low. Targeted RNAi knock-down experiments suggest that the mutant phenotypes are caused loss of OR22a and not OR22b. This is consistent with the decreased lifespan we observed following inhibition of OR22a-expressing neurons. Moreover, the longevity phenotype is rescued by expressing an OR22a transgene using the UAS/GAL4 system. Our data indicate that OR22a function promotes normal, healthy lifespan. Overall we present yet another evidence that sensory systems rival the insulin-signaling, TOR, and translation-related pathways in the vast number of effective manipulations that induce potent and reproducible effects on organism lifespan.

1026A

TGF-β/Activin Signaling, The Downstream Target of dFOXO, Regulates Longevity Through Muscle Autophagy in Drosophila. Hua Bai, Ping Kang, Ana Hernandez, Marc Tatar. Ecology & Evolutionary Biol, Brown University, Providence, RI.

Reduced insulin/IGF-1 signaling increases the life span of nematodes, flies and rodents. The underlying molecular mechanisms of this robust effect are unknown. To understand how insulin/IGF-1 mediates lifespan in Drososphila we performed chromatin immunoprecipitation-sequencing (ChIP-seq) analysis with the insulin/IGF-1 regulated transcription factor dFOXO. Dawdle, a TGF-β/Activin ligand, is directly repressed by dFOXO when insulin/IGF-1 extends lifespan. Consequently, reduced TGF-β/Activin signaling improved muscle performance and protein homeostasis as a function of age, while inactivation of TGF-β/Activin signaling in muscle prolonged lifespan. TGF-β/Activin signaling, via smox response elements, inhibits the transcription of Atg8a/LC3, a factor controlling the rate of autophagy that is known to modulate Drosophila lifespan. These data reveal how insulin signaling can regulate aging through control of TGF-β/Activin signaling that in turn controls autophagy, representing a potentially conserved molecular basis for longevity assurance.

1027B

Genome-wide analysis of Heat Shock Factor-mediated activation in *Drosophila melanogaster*. Fabiana Duarte, Michael Guertin, John Lis. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

The transcriptional Heat Shock (HS) Response in *Drosophila melanogaster* is regulated by the transcription activator HS Factor (HSF). Upon stress, HSF trimerizes and is recruited rapidly to chromatin, where it binds to specific regulatory sequences called HSF Sequence Elements (HSEs). We previously used ChIP-seq to characterize the genome-wide distribution of HSF in *Drosophila* S2 cells before and after HS. By comparing the data to ModENCODE ChIP-chip datasets generated during non-HS conditions, we demonstrated that the chromatin landscape prior to HS is a primary determinant of whether HSF can bind an HSE after induction. This study also demonstrated that HSF recruitment to a promoter does not necessarily result in gene activation. To better understand HSF-mediated activation and identify factors that affect the activation potential of target genes, we used a new technique developed by our lab called Precision Run On Sequencing (PRO-seq) to investigate first-order changes in gene expression that occur in S2 cells after HS. This method provides a genome-wide snapshot of transcriptionally engaged RNA polymerases with base pair resolution and was used to identify and quantify the differentially expressed genes during a HS time course. We observed a rapid and pervasive response to HS induction, with more than 2000 genes being differentially expressed after 20 minutes. We compared the changes in nascent transcription of activated genes with the HSF ChIP-seq and ModENCODE datasets to determine the relationship between HSF and other chromatin factors' binding intensities in the promoter with the activation status of target genes. We observed that the orientation of insulator proteins relative to the TSS and HSF binding site is an important determinant of the activation potential of HSF. We also identified upregulated genes that do not show inducible HSF binding to the promoter in order to determine other factors that may be involved in this response. This study provides a general understanding of HS induced transcriptional activation and how chromatin landscape affects this process.

1028C

Probable genetic sensors of Drosophila melanogaster which detect gravity waves. David A. Lavan[1,6], Javier Sánchez[2], Julio Valdivia-Silva[3], Raúl Herranz[4], Juan Agapito[5], Diego Orihuela[6], Gabriela Sanabria[6], Yuriko Ortega[6], Olga Bracamonte[6], Jesús Cruces[7], Juan Arredondo[7], Daniel Diaz[1], J.J.W.A van Loon[8], Roberto Marco[7]. 1) National Institute Research of Telecommunications, Lima - Peru; 2) Preventive Department, School of Medicine, Universidad Autónoma de Madrid - Spain; 3) Sciences Division, NASA Ames Research Center; Moffett Field, California, USA; 4) Biological Research Center CSIC, Madrid - Spain; 5) Peruvian Nuclear Energy Institute, Lima - Peru; 6) Cytogenetics Laboratory, Universidad Nacional Mayor de San Marcos, Lima - Peru; 7) Department of Biochemistry, School of Medicine, Universidad Autónoma de Madrid - Spain; 8) Dutch Experiment Support Center, DESC at OCB-ACTA, Netherlands.

In this study, we used two essential physic-matemathical principles in order to describe the genes analyzed as "sensors": first, the possible sensor must to be based in the spatial-temporal linearity, and second, the sensor will be influenced by gravitational forces generated into a region subjected to Zero gravity. Therefore, our group of genes, which have the same factor of regulation but no a common biological function, were searched using a computational algorithm which compared the genetic expression profiles of pupae of Drosophila melanogaster exposed to simulated and real microgravity. Interestingly, the algorithm found a small group of genes with fit sensor characteristics. Temporal expression analyses of mRNA during embryonic phase showed that this group of genes are mainly expressed between first 8 and 18 hours of fly's development. In addition, studies of hybridization of mRNA in situ showed three or more genes are involved in myosin actine fylaments. Studies of correlation showed that the simulated μg is a excellent method to mimic the real gravitational gradient inside the ISS. Overall, our results are consistent with the presence of genes that present characteristics as molecular sensors of gravitational waves. Importantly, these genes could be related to embryogenesis and have interesting implications in the biological evolution.

1029A

Post-transcriptional Regulation of *oskar* mRNA in Drosophila melanogaster. Livia V. Johnson[1,2], Steven K. Formel[1], Diana P. Bratu[1,2]. 1) Department of Biological Sciences, Hunter College, CUNY, New York, NY; 2) CUNY Graduate Center, New York, NY.

During Drosophila melanogaster oogenesis, messenger RNAs (mRNAs) localize to specialized regions in the oocyte, thus giving rise to the body axis of the future fly. They use the microtubule cytoskeleton to be actively transported after their exit from the nurse cells nuclei. During this transport, the mRNA remains translationally silent until properly localized. One such mRNA is oskar, which encodes a posterior pole morphogen. A key protein that plays an important role in the translational repression process of oskar mRNA is Cup. Premature Oskar protein expression has been demonstrated in cup mutant ovaries, however the dynamics of temporal and spatial recruitment of Cup to the oskar mRNP complex have been elusive. We hypothesize that Cup protein is recruited to the oskar mRNP complex as early as nuclear processing in the nurse cells, and it remains with the mRNP complex throughout transport to the posterior of the oocyte. In order to visualize oskar mRNA in various cup mutant backgrounds in vivo and in real time, we use fluorescent probes, named molecular beacons, that specifically bind to oskar mRNA and identify its location. For higher resolution studies, we utilize fluorescent DNA FISH probes, named Stellaris probes, to achieve single molecule resolution in fixed egg chambers. Via these approaches, we will gain a better understanding of the role Cup plays in oskar mRNA transport and translational repression.

1030B

The role of dHey in tissue homeostasis. Amir M. Orian[1], Eliya Lotan-Bitman[1], Olga Boiko[1], Naama Flint[1], Christos Delidakis[2], Manfred Gessler[3]. 1) Rappaport Res Inst, Faculty Med, Technion, Haifa, Israel; 2) Institute of Molecular Biology and Biotechnology (IMBB) Heraklion, Crete Greece; 3) Theodor-Boveri-Institute, Physiological Chemistry, Biocenter, University of Würzburg, Germany.

Hey proteins are HES-related transcriptional repressors that regulate diverse biological processes including cardiogenesis and angiogenesis. dHey is the sole ortholog in *Drosophila*, and is key for sex-determination and neurogenesis during embryogenesis and larval development. However, the genomic program/s regulated by dHey, as well as its role in adult tissue homeostasis are yet unknown. As a first step towards elucidating the genomic program regulated by dHey we used chromatin-profiling technique DamID, in KC cells and identified 2278 bound dHey genomic loci. GO analysis revealed that these loci are enriched for DNA binding proteins, as well as genes involved in the Notch signaling pathway. Furthermore, comparison of Notch-regulated genes and Su(H) direct targets with the genomic loci bound by dHey identified a subset of genomic loci co-bound by dHey in the context of Notch. Yet, our mapping and bioinformatics analyses pointed to targets and processes regulated by dHey independent of Notch. Subsequently, we analyzed genetically and gnomically the role of dHey in tissue homeostasis focusing on the adult gut as a model system. Using surface-marking and affinity purification we mapped the genomic program of gut cell differentiation and identified cell-specific dHey transcriptional programs. In addition we characterized the genetic and genomic impact of conditional inactivation of dHey in specific gut cells populations.

1031C

Evaluating the role of transcription into chromatin domain activation of iab-6 in the BX-C. Francois Karch[1], Sandrine Galetti[1], Annick Mutero[1], Robert Maeda[1], Fabienne Cléard[1], Welcome Bender[2]. 1) Dept Genetic and Evolution, Univ Geneva,

Geneva, Switzerland; 2) Harvard Medical School, Dept of Biological Chemistry and Molecular Pharmacology02115Boston MA, USA.

A large (> 60 kb) regulatory region downstream of Abd-B is divided into 4 regulatory domains designated iab-5, iab-6, iab-7 and iab-8, which direct Abd-B expression in abdominal segments 5, 6, 7, and 8 respectively. Each regulatory domain is modular in nature and comprises various cis-regulatory modules (CRM) such as 1) initiator, 2) cell-type-specific elements, 3) Polycomb-Response-Elements (PRE) and 4) boundaries. Using site directed mutagenesis, we have shown that the activity of the whole iab-6 regulatory domain depends on the presence of the initiator that function as <domain control region>. How the initiator control the activity of the other CRMs within a domain remains unknown. We are presently testing if transcription could be involved. Intergenic transcription across the regulatory domains has been known for 25 years. The anterior limit of expression of these transcripts is colinerar with the location of the probes along the chromosome. Expression of the early transcripts is transient between 2 and 4 hours of development and coincides with the initiation phase of BX-C regulation. We present here a detailed characterization of the early ncRNA emanating from iab-6. We find that the iab-6 domain is the subject of divergent transcription originating from the initiator and moving towards the extremities of the domain. By double FISH with an eve probe, we determined that the anterior border of transcriptions corresponds to PS11, the parasegment whose identity is determined by iab-6. The iab-6 domain is also transcribed in PS12, a feature reminiscent of the Lewis model in which once a regulatory domain is activated in a given parasegemnt, it remains active in more posterior parasegments. In our working model we believe that these early transcripts serve to open up the chromatin structure of the iab-6 domain, thereby enabling the multiple CRMs. We report our attempts to block these early transcripts by targeting transcriptional terminators.

1032A

A Rapid Strategy for Characterizing miRNA Functions in Specific Signaling Pathways. Kevin Kim[1], Arunachalam Vinayagam[1], Norbert Perrimon[1,2]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute, Harvard Medical School, Boston, MA.

MicroRNAs (miRNAs) are a class of small noncoding regulatory RNAs that regulate gene expression by binding to sequences within the 3' untranslated region (3'UTR) of an expressed mRNA. miRNAs are causing tremendous excitement in all fields of research as numerous studies have already demonstrated that many miRNAs regulate differentiation and proliferation to apoptosis in diverse organisms. Although current computational predictions of miRNA-3'UTR interactions provide important guidance for experimental analysis of miRNAs, many algorithms available predict target sets with only limited overlap and cumulatively identify several hundred potential target mRNAs per miRNA. To overcome this bottleneck in miRNA research, we generated a library of Drosophila miRNAs to screen core components in the Hedgehog pathway. From the screen, we identified multiple miRNAs that can regulate one or more of the Hedgehog pathway genes. We have characterized one of these miRNAs, miR-14, and demonstrate in vivo the role it plays in fine-tuning gene expression within the Hedgehog pathway.

1033B

Probing for IRES Function in the gurken 5' UTR. Jacob Merle, Janelle Gabriel, Scott Ferguson. Biology, SUNY Fredonia, Fredonia, NY.

The Drosophila *gurken* (*grk*) gene is required to establish the anterior/posterior and dorsal/ventral axes of the egg. *grk* translation is tightly regulated and many signaling pathways influence this process. When insulin levels are high such as when food is readily available, *grk* is translated using a common mechanism that requires recognition of the 7-methylguanosine cap at the 5' end of the RNA. An alternative mechanism of translation can occur through a secondary structure in the 5' UTR of the mRNA called an Internal Ribosomal Entry Site (IRES). The utility of the IRES is that it allows for translation of important mRNA's even in the absence of canonical cap-dependent translation factors. These conditions arise in nature when insulin levels or nutrient availability is low. Cap-dependent translation of *grk* is specifically blocked in *spindle*-class mutants due to inhibition of Vasa activity. It was discovered in our lab that Insulin/Insulin-like Signaling (IIS) mutations or inhibition of TOR by rapamycin result in increased *grk*translation in *spindle*-class mutants thereby overcoming this cap-dependent block. Based on these data, we hypothesize that the *grk* 5' UTR contains an IRES. This will be explored *in vivo* through a comparison of transgenic reporter lines containing different luciferase reporter constructs. Here we will present data demonstrating the activity of reporter constructs that have been generated by fusion with the 5' UTR of *grk*. These constructs will be used to test the hypothesis that the *grk* 5' UTR contains an IRES and examine the effect of nutrient limitation, inhibition of TOR, or IIS mutations on *grk* translation.

1034C

***gurken* mRNA is alternatively polyadenylated during oogenesis.** Amanda Norvell, Letitia Thompson, Jason Wong. Dept Biol, Col of New Jersey, Ewing, NJ.

The TGF-alpha like protein Gurken (Grk) is required for proper patterning of the egg and future embryo. During oogenesis expression of Grk is tightly regulated, both spatially and temporally. Appropriate distribution of Grk protein is accomplished by coupled *grk* mRNA localization and translational regulation. The hnRNP protein Squid (Sqd) plays a critical role in both of these processes, yet the molecular basis for its translational repression of *grk* is not fully understood. Evidence suggests that *grk* mRNA, like other translationally regulated transcripts, may be modulated by alterations in the 3' poly(A) tail. While investigating whether *grk* mRNA is subjected to cytoplasmic polyadenylation, we found that there are two distinct *grk* transcripts in the ovary. These RNAs differ by approximately 15 nucleotides within the 3' UTR and appear to be

the result of alternative polyadenylation. Current efforts are aimed at characterizing the expression of these transcripts; both a temporal analysis throughout oogenesis and in ovaries isolated from a variety of female sterile mutants.

1035A

Large-scale RNAi screen identifies genes involved in germline stem cell regulation. Dong Yan[1], Ralph Neumuller[1], Michael Buckner[1], Kathleen Ayers[2], Yanhui Hu[1], Donghui Yang-Zhou[1], Richard Binari[1], Ian Flockhart[1], Arunachalam Vinayagam[1], Sakara Randklev[1], Lizabeth Perkins[1], Stephanie Mohr[1], Lynn Cooley[2], Norbert Perrimon[1]. 1) Department of Genetics and Howard Hughes Medical Institute, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA; 2) Department of Genetics, Department of Cell Biology, School of Medicine and Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520.

Stem cells play essential roles during animal development and homeostasis. The key question in stem cell biology is to understand how stem cell self-renewal and differentiation are precisely controlled. Here we systematically screened the TRiP collection of transgenic RNAi lines for genes involved in female germline stem cell (GSC) regulation. We have screened 4570 lines including both long double-stranded RNA lines and short hairpin RNA lines. We identified 350 genes that affect the fertility of the females and amongst those identified 112 genes that are required for stem cell maintenance or differentiation. Many of those genes can be grouped into gene networks including mRNA splicing, mitochondria, phosphoinositol, ubiquitination/sumoylation, kinetochore, insulin signaling, etc. Cross correlation with regulators of Drosophila neural stem cell self-renewal allowed us to identify GSC specific as well as commonly required regulators of self-renewal. Our study provides a systematic map of germline stem cell self-renewal that will further help to dissect the genetic networks underlying binary cell fate decisions in stem cell lineages.

1036B

Neural stem cell regulation in the developing visual system. Katrina S Gold[1], Boris Egger[2], Andrea H Brand[1]. 1) Wellcome Trust/CRUK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, United Kingdom; 2) Department of Biology, Zoology, Chemin du Musée 10, CH-1700 Fribourg, Switzerland.

Stem cells can self-renew by symmetric cell division, favouring expansion of the stem cell pool, or by asymmetric cell division, favouring cellular diversification. During the development of the *Drosophila*optic lobe, a symmetrically dividing pool of neuroepithelial cells transform into asymmetrically dividing neuroblasts, which in turn give rise to the neurons of the visual system in the brain. This transition is reminiscent of the switch from symmetric to asymmetric stem cell division found in the mammalian cortex. Since the symmetrically and asymmetrically dividing cell populations are clonally related, the optic lobe provides an excellent model system for studying the molecular mechanisms underlying the transition in division mode.

We used transcriptome profiling to identify candidate genes that regulate this switch. Neuroepithelial cells and neuroblasts were genetically labeled and isolated from living larval brains. cDNA libraries from the two cell types were compared directly on microarrays to generate transcriptional profiles. This analysis has led to the identification of a number of candidate genes and signalling pathways, including the Notch pathway, the proneural bHLH protein Lethal of scute and the Six-type homeodomain transcription factor Optix.

Optix is expressed in a subset of neuroepithelial cells throughout larval development. It has two closely related vertebrate orthologues: Six3 and Six6. These genes regulate neural stem cell proliferation and patterning in the developing eye and brain, and human mutations have been linked to severe brain malformation. This suggests that Optix has an evolutionarily conserved role in regulating the formation of the nervous system. We are currently investigating the role of Optix in regulating neural stem cell fates in the optic lobe.

1037C

Survival of Apoptosis by Stem Cells in Drosophila testis niche. Salman Hasan, Phylis Hétié, Erika Matunis. Cell Biology, Johns Hopkins Medical Institute, Baltimore, MD.

Stem cells are generally thought to resist damage better than their differentiating progeny, but underlying mechanisms are not well-understood. We have successfully developed an assay for inducing and detecting DNA damage in adult Drosophila testis. High doses of irradiation (100 Gy) cause all stem cells and their progeny from the tissue to be lost within 4 days, leaving only quiescent hub cells intact. In contrast, reducing this dose to 50 Gy induces death in differentiating germ cell and cyst cell progeny but does not eliminate stem cells. Thus, we hypothesize that cells within the niche receive signals that confer survival advantages. Work by Betz et al. (PNAS, 2008) has shown that the pro-survival factor Drosophila inhibitor of apoptosis 1 (DIAP1 also known as thread) gene is a STAT92E target in the wing imaginal disc. Additionally, the authors showed that cells expressing high levels of DIAP1 have a survival advantage under stress as opposed to neighboring cells that express low levels. Consistent with this, we find that DIAP1 is activated by STAT92E in both germline and cyst stem cells in the testis. Our work also shows that *diap1* is required for the maintenance of CySCs in the adult testis as the *diap1* null CySCs are lost quickly from the niche. We seek to further characterize the role of *diap1* in the testis niche, and to investigate whether the levels of DIAP1 in stem cells correlate with the ability of these cells to resist irradiation-induced damage and death.

1038A

Visualization of Sqd-*grk* Interactions in Live Drosophila Oocytes Using Tri-molecular Fluorescence Complementation (TriFC). Nancy J. Levensailor, Steven J. Gangloff, Alicia R. Watson, Dane M. Buenten, Scott B. Ferguson. Biology, SUNY Fredonia,

Fredonia, NY.

Gurken (Grk) is a developmental morphogen that is subject to strict translational control during Drosophila oogenesis. *grk* mRNA is kept translationally silent until it achieves an appropriate localization in the oocyte where it specifies the anterior / posterior and dorsal / ventral axes. Sqd is a protein that binds to *grk* mRNA and precludes translation during its transport from the nurse cells to the oocyte. We have visualized a specific interaction between *grk* mRNA and Sqd protein in real-time by developing a novel technique, Trimolecular Fluorescence Complementation (TriFC). As the name implies, TriFC is composed of three interacting molecules; first a split fluorescent protein is fused to the MS2 Coat Protein (MCP). MCP interacts with a stemloop engineered into an RNA of interest, thereby decorating the RNA with split fluorescent protein that is not fluorescent on its own. Finally a candidate bait protein is fused to the complementary half of the fluorescent protein. If the candidate protein interacts with the RNA of interest, the two halves of the fluorescent protein fold together to form a functional fluorophore. We have demonstrated proof of the TriFC principle between Sqd and *grk* using the Venus fluorescent protein. We see fluorescence in the nurse cells, ring canals, and a transient anterior cortical ring that later relocalizes to the dorsal anterior corner. These observations are consistent with existing models of grk regulation. We are currently generating a Red Fluorescent Protein (RFP) version of the TriFC reagents to permit visualization of the interaction later in oogenesis where yolk autofluorescence interferes with the signal from Venus. This powerful technique should be broadly applicable to any protein-RNA combination and can lend both spatial and temporal resolution to studies of these interactions.

1039B

DISCO: Drosophila Inventory System for Complete Organization. David P. Argue, Ying Peng, Stephen C. Ekker, Yi Guo. Mayo Clinic, Rochester, MN.

Data organization is critical to the success of any research lab. At a minimum, *Drosophila* research typically involves managing an inventory of fly vials and trays, tracking flipping of these trays, and recording genetics details of the organisms within each vial. A software based solution to manage all these details successfully is almost required for any decent sized research laboratory, but existing software solutions do not incorporate the required features to maintain an organized inventory with minimal effort. We present the "DISCO" *Drosophila* management software. This software is a combination web application and iPad app developed with input from experienced *Drosophila* researchers. The inventory component of the system utilizes linear barcodes to track individual vials and trays of vials. Standard barcodes are generated by the system that can be read using off-the-shelf barcode readers. The iPad camera doubles as a barcode reader, so that purchase of a standalone barcode reader is not required. The software incorporates features for advanced searching for vials, recording notes for each vial, running tray flip reports and automated backup of all data. Complete vial histories are also instantly available. The iPad app offers the same features as the browser-based system, but in a mobile form factor. DISCO serves as a complete *Drosophila* management solution.

1040C

Evaluation of SILAC (Stable Isotope Labeling with Amino Acids in Cell Culture) strategies for in vivo quantitative proteomic analyses in Drosophila. Guang-Chao Chen[1,3], Ying-Che Chang[1,3], Suh-Yuen Liang[2], Hong-Wen Tang[3], Tsung-Hsien Pu[2], Tzu-Ching Meng[1,3], Kay-Hooi Khoo[1,3]. 1) Inst Biological Chemistry, Academia Sinica, Taipei, Taiwan; 2) NRPB Core Facilities for Protein Structural analysis, Academia Sinica, Taipei, Taiwan; 3) Institute of Biochemical Sciences; College of Life Science; National Taiwan University; Taipei, Taiwan.

Mass spectrometry combined with SILAC technique has been demonstrated as a powerful tool for quantitative proteomics. Recently SILAC technique was also applied to multi-cellular organisms such as mice, fruit flies, and nematode. In this work, we have further evaluated the SILAC fly strategies with an aim to determine its quantification accuracy, versatility with both heavy lysine/arginine labeling, and to reduce its consumable cost. Only the deuterium-labeled lysine alone engendered high mortality rate and deemed not suitable. In vivo conversion of heavy lysine and/or heavy arginine to several non-essential amino acids was observed, leading to distorted isotope pattern and significantly underestimated H/L ratio output by MaxQuant. We further showed that peptide ratios calculated from the summed intensities of all isotope peaks are less affected by the heavy amino acid conversion and therefore less sequence dependent and to be more reliable. Applying either the single Lys8 or double Lys6/Arg10 in vivo labeling strategy to SILAC flies, we quantitatively mapped the proteomic changes during the onset of metamorphosis and upon amino acid deprivation.

1041A

Global analysis of phase locking in gene expression during drosophila melanogaster development. Shouguo Gao[1,2], Xujing Wang[1,2]. 1) Deptment of Physics, University of Alabama at Birmingham, Birmingham, AL; 2) The comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, AL.

Gene expression profiling during development offers data source to investigate frequency modulation in transcription regulation. For this type of time series data, correlation metrics usually ignore dependence of the order of the data points, thus not only loses sensitivity toward detecting interactions but could also lead to erroneous predictions. We investigate the potential of phase locking analysis in the transcription network modeling of gene expression data during development. Using the Drosophila melanogaster RNA-seq data (27 time points) and REDfly database as positive control and random gene pair as negative control, we utilize Area Under Curve (AUC) of Receiver Operating Characteristic curve to quantitatively assess the phase locking performance. The results show that significant phase locking exists between transcription factors and their

targets (AUC=0.59), between the co-regulated genes (AUC=0.54). We also found that the phase locking increases with the number of shared transcriptional factors (AUC=0.54, 0.63 and 0.68 for 1, 2 and 3 transcriptional factors). Compared with simple correlation the phase locking metric can identify interacting gene pairs more efficiently. In addition, it can automatically address issues of arbitrary time lags. Many regulatory relationships in REDfly missed by correlation metrics are grasped by phase locking. Our result demonstrates the phase locking analysis of gene expression during development can assist transcription network modeling, which provide an additional dimension to reconstruct regulatory networks.

1042B

Small Brains, Big Ideas: Seeding Ideas for the future of Science in Latin America. Yuly Fuentes-Medel[1], Jenifer Pirri[2], Jimena Sierralta[3], John Ewer[4]. 1) MIT Sloan School of Management,MIT, Cambridge, MA; 2) Department of Neurobiology, UMass Medical School,Worcester,MA; 3) Facultad de Medicina, Universidad de Chile, Chile; 4) Centro Interdisciplinario de Neurociencia, Universidad de Valparaiso, Chile.

•Expanding opportunities for Latin American students •Creating a world-class community of scientists •Enhancing new scientific collaborations •Showcase amazing science using invertebrate biology The biannual “Small Brains, Big Ideas” course first took place in Santiago, Chile, October 2010 and again in 2012. So far it has successfully trained 55 Latin-American students on recent advances and modern techniques in neurosciences, primarily focusing on the use of Drosophila melanogaster and Caenorhabditis elegans for biomedical research. While widespread use of these powerful model organisms for research is common in the United States and Europe, their adoption in Latin America has been relatively slow. Part of the difficulty has been the lack of local expertise and limited exposure to the utility of these systems. The purpose of Small Brains, Big Ideas is to overcome this knowledge barrier by providing hands-on experience with invertebrate models and opportunities to interact with world-class scientists who use these systems for their own research. How? By seeding the initiative with important biological questions, counting with outstanding teaching faculty and students who want to make an impact in science and science teaching, and financial support. The course covered areas ranging from molecular neuroscience to behavior genetics, through lectures and laboratory exercises. Small Brains, Big Ideas allowed students to gain firsthand experience with approaches in these model systems and provided the opportunity for students to interact and network with leaders in biomedical research. We plan to extend the impact of the course by creating a permanent platform to provide knowledge-based support and networking capabilities to Latin-American students. A web site has been created; visit us at www.smallbrains.org.