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Late Abstracts

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893B

X Chromosome Screen for Novel Regulators of Oocyte Polarity. Dániel Lakatos¹, Andrea Leibfried¹, Shinya Yamamoto², Hugo J. Bellen², Anne Ephrussi¹. 1) Developmental Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany; 2) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX. USA.

Most cells are polarized, their proteins and even cellular structures showing an asymmetric spatial distribution. The distinct localization of proteins can be achieved by the regulated transport of the encoding mRNA followed by translation activation. In the developing oocyte, maternally provided mRNAs specify the body axes of the future embryo and thus of the animal. *oskar* mRNA is localized to the posterior of the oocyte where it is translated and required for the formation of abdominal structures and the germline. The X chromosome has not been extensively screened so far for lethal mutations affecting these processes due to the lack of viability of hemizygous males. Two recently generated collections of duplications covering the X chromosome now allow easy and straightforward mapping of lethal mutations. We performed a screen on the X chromosome to find novel regulators of oocyte polarity. We screened a large collection of EMS-induced lethal mutants generated in the Bellen laboratory. To this end, we generated germ line clones, using the dominant female sterile technique with an *in vivo* reporter of *oskar* mRNA localization, GFP:Staufen. Among the nearly 2000 lines tested, 47% have no phenotype, 45% arrest oogenesis at an early stage, and 8% show a phenotype with respect to GFP:Staufen localization. We are currently mapping 54 lines that display highly penetrant and reproducible GFP:Staufen and *oskar* mRNA mislocalization. Mapping of the mutations is carried out in two successive steps, making use of duplications. Our first read-out is the rescue of male lethality. In the next step we are performing Sanger sequencing of the candidate genes. Finally, we test homozygous mutant females rescued by a small duplication covering the mapped lethal mutation for loss of the mutant phenotype and thus correct *oskar* mRNA localization. We will present a detailed description of the screen and its results obtained so far.

894C

Analyzing role of different SNXs in the apical-basal polarity in epithelial cells. Bo Zhou¹, Peng Zhang², Xinhua Lin^{1,2}. 1) Dev Biol, Cincinnati Children's Hosp, Cincinnati, OH; 2) Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Retromer is an evolutionarily conserved membrane-associated protein complex. Retromer is composed of two sub-complexes: Vacuolar protein sorting35 (Vps35), Vps26, Vps29 form a cargo selection subunit; Sorting Nexin (SNX) form a membrane targeting/remodeling subunit. Retromer regulates retrograde transportation of transmembrane cargos from endosomes to trans-Golgi network. Trafficking of different retromer cargos relies on the same cargo selection subunit, but on different SNXs. SNX1/2 and SNX5/6 are required for trafficking of retromer cargos, CI-MPR and CED-1. However, the trafficking of another retromer cargo, Wntless, relies solely on SNX3. Recent work has identified one apical polarity regulator, Crumbs, as one of retromer cargos. We have generated deletion mutants of SNX1, SNX3 and SNX6 in *Drosophila* and analyzed which SNX is required for recycling Crumbs and for maintaining the apical-basal polarity.

895A

Contact dependent uptake of Dpp over long distance by *Drosophila* tracheal cytonemes. Sougata Roy, Thomas Kornberg. Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA.

Cytonemes are types of filopodia first identified in the *Drosophila* wing imaginal disc that were proposed to be involved in long distance signaling during development. The present work reports Dpp playing a role in appropriate morphogenesis of larval trachea associated with wing discs. The work identifies Dpp signal specific cytonemes from trachea and shows Dpp receptor Tkv sorting in signal specific cytonemes. Tkv distributes in motile punctal forms in these cytonemes. Vesicular puncta containing both co-localized ligand and receptor were found in cytonemes that touch ectopic ligand-expressing cells, providing direct evidence that cytonemes transporting signaling ligands in receptor dependent manner. Non-specific receptors like FGFR in cytonemes on the other hand do not uptake Dpp from ectopic Dpp source. GFP reconstitution technology in tracheal cytonemes indicated that cytonemes establish molecular contact with the source cells to receive signals. Thus, cytonemes are diverse in composition and function, can ferry specific signaling proteins from far away source at the point of direct contact. These findings first time show a cell-cell contact mediated exchange and transport of morphogens mediated by cytonemes. Cytoneme-based movement of signaling proteins as a mechanism for cell-cell communication ensures controlled amounts of signaling protein targeting to a specific recipient cell.

896B

***Drosophila* king tubby (ktub) mediates light-induced rhodopsin endocytosis and retinal degeneration.** Shu-Fen Chen, Yu-Chen Tsai, Seng-Sheen Fan. Life Science, Tunghai University, Taichung, Taiwan.

The tubby (tub) and tubby-like protein (tulp) genes belong to a small family of genes which their functions remain unclear. The tub and tulp genes are found in multicellular organisms including both plants and animals. The C-terminal of Tub and Tulps proteins are highly conserved. Mutation of members of this protein family causes disease phenotypes including retinal degeneration and obesity. In this study, we find that *Drosophila* king tubby (ktub) participates in rhodopsin (Rh1) endocytosis in response to light stimulation. Immunocytochemical analysis shows that Ktub expresses at the rhabdomere domain in the dark condition. When flies received light stimulation, the Ktub translocates from rhabdomere to the cytoplasm and the nucleus of the photoreceptor cells. Studies further show that wild type photoreceptor forms Rh1-immunopositive large vesicles (RLVs) shortly after light stimulation. In light-induced ktub mutants, the majority of Rh1 remains at the rhabdomere; only few RLVs in the cytoplasm of photoreceptor cells. To further investigate the role of ktub in Rh1 endocytosis, we examine its ability to block norpA mediated Rh1 endocytosis. Mutation of norpA allele causes massive Rh1 endocytosis in light condition. However, majority of Rh1 remains at the rhabdomere in ktub and norpA double mutant in response to light induction. In addition, we also find that ktub and norpA double mutant rescues the light-induced norpA retinal degeneration. Using deletion constructs, we further demonstrate that Tub domain is important for Rh1 endocytosis. Together, these results delimitate the novel function of Ktub in Rh1 endocytosis and retinal degeneration.

897C

Role of SPARC in *Drosophila melanogaster* Fat Body Development and Basal Lamina Stability. Cristina Baratta, Maurice Ringuette. University of Toronto, Toronto, Ontario.

SPARC (Secreted Protein, Acidic, Rich in Cysteine) is an evolutionarily conserved calcium- and collagen-binding extracellular glycoprotein associated with tissue development, remodeling, and wound repair. Previous studies in our lab have demonstrated that Sparc is expressed

by hemocytes and the fat body during *Drosophila* embryogenesis and the resultant protein is concentrated in the basal laminae. We now report that high-level expression of SPARC by the fat body continues throughout larval development. Loss of SPARC results in a reduction of fat body tissue during larval development with rounding and blebbing of remaining fat cells. Knockdown of SPARC with RNAi in the fat body results in similar changes to the shape of fat cells as well as formation of necrotic masses in the larva. In addition, RNAi knockdown results in developmental arrest and larval lethality at the 2nd and 3rd instar larval stages with very few larvae reaching pupal stages. Analysis by scanning electron microscopy indicates that knockdown of SPARC disturbs the surface of the fat body such that the divisions between adipocytes are unclear and obscured by excessive pitting of the surface compared to wild-type fat body cells. Preliminary analysis by transmission electron microscopy suggests knockdown of SPARC causes an accumulation of cellular material in the intercellular spaces between fat body cells and their associated basal lamina. While SPARC is a secreted protein that is distributed throughout the basal lamina surrounding fat body cells, mosaic analysis suggests the above indicated change in cell morphology is cell-autonomous. Furthermore, the other major components of the basal lamina (Collagen IV, Laminin, Nidogen, and Perlecan) appear to concentrate at cell borders upon SPARC knockdown in a cell-autonomous manner. Lastly, cell-autonomous expression of a dominant negative Ecdysone Receptor in the fat body causes a similar accumulation of basal lamina components in fat body cells. These results suggest SPARC plays a critical role in fat body maintenance and stability of its basal lamina.

898A

DE-Cadherin Has an Essential Role in Border Cell Migration. Danfeng Cai^{1,2}, Jessica Sawyer¹, Denise Montell¹. 1) The Department of Biological Chemistry, Johns Hopkins University, Baltimore, MD; 2) Johns Hopkins School of Medicine, Baltimore, MD.

The differential adhesion hypothesis formulated by Malcolm Steinberg 50 years ago states that: when cells of different origin are randomly mixed, cells with most adhesive strength will sort to the center and less adhesive cells surround them. A number of in vitro experiments have been designed to test and evolve the hypothesis. The *Drosophila* ovary serves as a unique in vivo model to test the DAH. At the beginning of stage 9, a group of four to six anterior-located border cells surrounds a pair of cells called polar cells which will specifically increase the expression of an adhesion molecule DE-Cadherin, and start migrating towards the posterior end. We hypothesize that border cell migration is also a cell sorting process determined by differential adhesion of the cells: the higher level of DE-Cadherin in the border cells and polar cells is sufficient to cause them to sort into the nurse cells which have lower level of DE-Cadherin expression. Using Gal4/UAS system and inducible RNAi, and with the combined analysis of fixed tissue and live-cell imaging, we have demonstrated that changing the relative levels of DE-Cadherin in the different cell types, specifically, up-regulating its amount in the DE-Cadherin-scarce nurse cells, and down-regulating its amount in the DE-Cadherin-abundant border cells and polar cells, all cause migration defects. Moreover, decreasing the level of DE-Cadherin in polar cells, the cells that have highest level of DE-Cadherin, disrupts the central localization of the polar cells in the cluster, and causes the border cell cluster to disintegrate. The results indicated that DE-Cadherin plays an essential role in border cell migration and cluster organization. In our system, differential adhesion hypothesis is able to account for the cell sorting behavior of border cells and polar cells into the nurse cells.

899B

Regulation of the Hedgehog Gradient: Transduction of Different levels of Hedgehog Signaling. Pascal Therond, Nadia Ranieri, Laurent Ruel, Armel Gallet, Sophie Raisin. CNRS iBV, Univ Nice Sophia-Antipolis, Nice.

Proteins of the Hedgehog family (Hh) are involved in many developmental processes, including cell proliferation, embryonic development and morphogenesis in metazoans. In several cases, including the *Drosophila* wing, Hh is required for tissue organization during development and acts as a morphogen, specifying cell fate in a concentration-dependent manner. Hh proteins are the only known molecules to be naturally covalently modified with a cholesterol moiety. Perhaps due to this atypical feature, Hh reception and transduction remain poorly understood. In *Drosophila*, Hh binding to its receptor, Patched, leads to Smoothened phosphorylation and stabilization at the plasma membrane. Smoothened interacts with a cytoplasmic complex composed of the kinesin-like protein Costal2, the serine/threonine kinase Fused and the zinc-finger transcription factor Cubitus interruptus (Ci). Smo C-terminal dimerization promotes high-magnitude signaling but it remains unclear how Smoothened and the cytoplasmic complex responds to and transduce different levels of Hh signaling. I will show here that the subcellular distribution of Costal2 is regulated differentially by several Fused-dependent phosphorylations within the Hh gradient field. I will also provide evidence that, within this gradient, Smoothened adopts different conformations, such as monomeric or dimeric forms. These forms control different levels of Hh signaling through the differential phosphorylation of Costal2. I will present a model suggesting that the switch of Smoothened from the monomeric to the dimeric conformation dictates the level of Fused kinase activity, resulting in the relocation of Costal2 from the cytoplasm to the plasma membrane and facilitating high-level Hh signaling through the control of Ci nuclear translocation. Altogether, this study reveals an unanticipated complexity in the regulation of the kinesin Costal2 and provides the first evidence that Fused and Costal2 activities sense differential level of Hh signaling.

900C

Screening for genetic modifiers of AP-3, a protein complex involved in intracellular protein trafficking and implicated in Hermansky-Pudlak syndrome. Imilce A. Rodriguez-Fernandez, Veronica T. Cheli, Diego J. Hoyle, Esteban C. Dell'Angelica. Department of Human Genetics, UCLA, Los Angeles, CA.

The Adaptor Protein-3 (AP-3) is a multi-subunit molecular machinery important for endosomal protein trafficking and lysosome-related organelle biogenesis. Defects in human AP-3 result in a form of albinism called Hermansky-Pudlak syndrome. Analogous to the human disorder, mutations in the *garnet* (*g*) gene encoding one component of AP-3 in *Drosophila melanogaster* cause eye pigmentation defects. We performed a large-scale screening to identify genetic modifiers of the function of AP-3 in the fly eye. The hypomorphic *g²* mutant line was crossed with more than 200 lines of the Bloomington Deficiency Kit (DK) and more than 1,250 Potential Misexpression Insertions (PMI) lines. The DK screening and follow-up validation uncovered four distinct deletions in chromosomes 2 and 3 that in heterozygous form modified the *g²* phenotype (as determined by red pigment content) by at least 1.5-fold. The PMI screening resulted in the identification of 12 genes that when misexpressed modified the *g²* eye color phenotype at least 1.5-fold. One of these genes is *fat facets* (*faf*), which encodes a deubiquitinating enzyme previously implicated in eye development. Strong misexpression of *faf* caused abnormal eye morphology, which was dramatically exacerbated by the *g²* allele. Another gene identified through the screening encodes a Rab GTPase not previously characterized in flies but implicated in protein trafficking within the late secretory and endocytic pathways of other organisms. The identification of modifiers of AP-3 function in flies may help understand the physiological roles of this complex in metazoans.

901A

Understanding the spatio-temporal dynamics of guidance signaling during border cell migration in *Drosophila*. Rishita Chagede, Pernille Rørth. IMCB, Singapore, Singapore.

Cell signaling is a fundamental process required to sense the surroundings and respond to cues, which would direct cell fate during developmental processes such as differentiation, migration, and also help cells to respond to other environmental changes and thereby maintain homeostasis. Cells signal in response to various ligands by activating the specific receptors presented on the cell membranes. The signaling process is usually short lived and highly regulated, to bring about specific responses to the signal. During development and immune response, cells migrate towards or away from the source of a ligand. Cell migration is a dynamic process that can be observed in real time providing an opportunity to study the spatio-temporal dynamics of guidance signaling. During *Drosophila* oogenesis, a group of 6-8 cells, border cells, delaminate from the follicular epithelium and perform a guided migration through the germline tissue to reach the oocyte. The oocyte secretes chemoattractants such as PVF1 that guide the migration via activation of the receptor tyrosine kinases (RTKs), PVR and EGFR expressed by the border cells. I have developed a biosensor probe, RASA, to observe the endogenous guidance signaling in real time during migration. Activated Ras is used as a read out of the activation of both the RTKs. As expected no signal is observed when Ras is knocked down using RNAi. When RTKs are knocked down using RNAi, no signal is observed, showing that, in the border cells activation of Ras is stringently dependent on RTK activation. This probe allows us to observe endogenous signaling and, in wild type, we observe that during migration, the signal is polarized to the front of the leading cell with higher levels. The signal is also outwardly polarized, but to a lesser extent, on the rare cell of the migrating clusters. The front polarization of the cells is drastically reduced in the PVF1 mutant or when the dominant negative RTKs are expressed. We are currently studying the characteristics of the guidance signal in space and in time and its relationship to guided movement.

902B

Roles of microtubules in border cell migration. Nachen Yang, Adam Cliffe, Pernille Rørth. Institute of Molecular and Cell Biology, Singapore.

Unlike actin that is required for almost all eukaryotic cell migrations, roles of the other cytoskeleton component, the microtubules, are less clear. Much of our basic knowledge in understanding how cells migrate has largely come from studies in tissue culture assays, raising the lack of *in vivo* relevance a concern. Border cells are a group of somatic follicle cells that perform stereotypic migration through interacting with nurse cell substrates during *Drosophila* oogenesis, serving as a convenient model to study collective migration *in vivo*. Through imaging of both stable and dynamic microtubules, we found differential microtubule organization and dynamics within the cluster: microtubules are highly organized in the polar cells and form a MTOC-like structure that orientated towards the front prior to migration. The outer border cells, in contrast, have some cortical microtubules, but are less organized; they grow preferentially towards the center of the cluster. We study the general effects of microtubules in the system upon disruption by nocodazol. Both cell motility and apparent cluster directionality are reduced. The specific microtubule depolymerization factor stathmin has a subtle role in migration, and is found to be largely required in the nurse cells. To find additional regulators, we conduct a RNAi screen against genes encoding known or potential microtubule regulators. Among the 80 genes screened, the dynein interactors Lis-1 and NudE, together with dynein are found to be required both in the polar cells (in agreement with previous published results) and outer border cells, primarily for the initiation of migration. Compromising their activities severely disrupts the organization of the border cell cluster, as visualized by the abnormal distribution of adhesion molecules. In summary, we found microtubules do play roles in both migratory border cells as well as their interacting cells. Specifically, the Lis-1-NudE-dynein complex is required, possibly through regulating the reorganization of the follicular epithelium, that ensures a proper organized migratory cluster.

903C

Akt is Negatively Regulated by Hippo Signaling for Growth Inhibition in *Drosophila*. Yaoting Deng¹, Xin Ye², Zhi-Chun Lai³. 1) Biochemistry and Molecular Biology, Penn State University, University Park, PA; 2) Department of Genetics, Penn State University, University Park, PA; 3) Department of Biology, Penn State University, University Park, PA.

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

904A

Genome-wide RNAi screen to identify glia specific function in adult *Drosophila*. Aniket Ghosh¹, Aaron Voigt², Jörg B Schulz², Mikael Simons¹. 1) Cellular Neuroscience, Max Planck Institute for Experimental Medicine, Goettingen, Germany; 2) Department of Neurology, University Medical Center, RWTH Aachen, Aachen, Germany.

All complex nervous systems mainly consist of two cell types, neurons and glia. Glia cells are important for the maintenance of architecture of nervous system as they insulate neurons and provide trophic support. Although glial population contributes significantly in nervous system, a little is known about glial morphology and functions. Therefore, we used a genome-wide RNAi screening in adult *Drosophila* to identify new glial functions and the genes involved in it. *Drosophila* was used for two reasons. First, it is the only possible way to have conditional gene inactivation *in vivo* in high-throughput. Second, basic glial functions are likely to be conserved across the species. RNAi screening was performed in adult *Drosophila* using pan-glial promoter repo-GAL4 carrying tubulin-GAL80(ts) to restrict shRNA expression only in adult stage. A library of ~ 8000 RNAi lines having human homolog was used in the screening. Primary screen identified ~ 600 candidate genes that are required for the long-term survival of adult *Drosophila*. Several categories of candidates viz. cell adhesion, transmembrane proteins, kinases and phosphatases are likely to have glia specific functions. By characterizing the specific biological functions of several of these interesting candidates, we will dissect the molecular details of neuron-glia communication. Given a striking homology of *Drosophila* and vertebrate glial factors, which includes several of our candidates as well, this study will also serve as a valuable tool for the better understanding of the vertebrate nervous system.

905B

Ca²⁺ buffering by calphotin is essential for prevention of light induced retinal degeneration. Shirley Weiss, Elkana Kohn, Daniela Dadon, Ben Katz, Maximilian Peters, Baruch Minke. Department of Medical Neurobiology, Hadassah Medical School, The Hebrew University, Jerusalem, Israel.

Prevention of Ca²⁺ overload is crucial for cell survival. Fly photoreceptors are polarized cells containing an extended interface between their cell body and the microvilli-containing light-signaling compartment. Upon intense illumination, microvillar calcium concentration reaches millimolar levels that would be toxic if Ca²⁺ diffusion between these two compartments were not tightly regulated. Yet, it is unclear how such effective regulation is obtained. Here we show that this regulation is achieved via separation of the two compartments by calphotin, an intrinsically unstructured protein, that forms a massive non-mobile Ca²⁺ buffer along the base of the microvilli. We generated and analyzed transgenic *Drosophila* strains in which calphotin expression levels were reduced in a graded manner, using a combination of genetics, electrophysiology, Ca²⁺ imaging and ultrastructural analysis. Calphotin elimination caused severe light-dependent photoreceptor degeneration, which was Ca²⁺-dependent. The degeneration was rescued by the prevention of Ca²⁺ overload via overexpression of a Na⁺-Ca²⁺ exchanger. Calphotin dependent Ca²⁺ buffering was demonstrated by its expression in HEK cells, while reduced levels of native calphotin resulted in abnormal kinetics of Ca²⁺-elevation in photoreceptor cells. Thus, Ca²⁺ buffering at the microvilli-cell body interface by calphotin constitutes an effective strategy for protection of cells from Ca²⁺ overload and degeneration.

906C

Differential response of *Drosophila* cell lines to extracellular adenosine. Michal Zurovec¹, Jana Fleischmannova², Lucie Kucerova^{1,2}, Vaclav Broz^{1,2}. 1) Dept. Genetics, Biology Centre, Inst Entomology, Ceske Budejovic, Czech Republic; 2) Faculty of Sciences, University of South Bohemia, Ceske Budejovic, Czech Republic.

Adenosine (Ado) is a crucial metabolite that affects a wide range of physiological processes. Key proteins regulating adenosine signaling, transport and metabolism are conserved among vertebrates and invertebrates. It is well known that Ado influences proliferation and viability of several vertebrate and invertebrate cells. Here we show that Ado negatively influences viability, changes morphology and mitochondrial polarity of the *Drosophila* imaginal disc cell line (Cl.8+) via a mechanism exclusively dependent on cellular Ado uptake. High transport of Ado is followed by phosphorylation and ATP production as a part of Ado salvation, which at higher concentrations may interfere with cellular homeostasis. In contrast, hematopoietic cell line Mbn2, Bg2-c2 neuroblasts and embryonic cell line S2 seem to be more Ado-tolerant and grow well in high Ado concentration. These cells preferentially utilize Ado deamination as a part of the purine catabolic pathway. In addition, Bg2-c2 and Mbn2 cells seem to decrease the level of adenosine transport and adenosine phosphorylation in high extracellular adenosine concentrations. Our results show that different types of *Drosophila* cell lines use different pathways for Ado conversion and suggest that such differences may be an important part of complex mechanisms maintaining energy homeostasis in the body.

907A

Regulation of adult *Drosophila* cuticle pigmentation by Insulin signaling. Jennifer A. Kennell, Iryna Shakhmantsir. Biology, Vassar College, Poughkeepsie, NY.

We present results suggesting a novel role for Insulin signaling in directly regulating pigmentation in the developing adult cuticle of *Drosophila*. Misexpression of Insulin signaling pathway inhibitors using the *pnr-Gal4* driver resulted in decreased pigmentation along the dorsal midline in the expected region of *pnr* expression. Conversely activation of the pathway caused increased pigmentation along the dorsal midline. This effect is not sex specific as we see changes in pigmentation in both males and females. In addition, the effect appears to be cell autonomous as we do not see changes in pigmentation outside the expression domain of the *pnr-Gal4* driver. We are currently investigating the mechanism by which Insulin signaling contributes to the production and deposition of pigment in the developing cuticle.

908B

***In vivo* and *in vitro* functional characterization of RhoGEF3, a new guanine nucleotide exchange factor (GEF) of *Drosophila melanogaster*.** Alejandro Zúñiga, Leandro Farías, Verónica Cambiazo. INTA - Universidad de Chile & Center for Genome Regulation (CGR). Santiago, Chile.

The actin cytoskeleton is essential for the mechanical processes driving morphogenesis. Actin dynamics is controlled by RhoGTPases, which in turn are temporal and spatially activated in a specific manner by guanine nucleotide exchange factors proteins (GEF). Twenty two genes that encode putative GEF proteins have been annotated in the *Drosophila* genome, however only reduced number of them have been involved in a morphogenetic process during development. Here we report the molecular and functional characterization of a novel GEF protein, RhoGEF3. In this study we used: 1) *In vitro* assays of RhoGEF3 activity in S2R+ culture cells, 2) *In vivo* analysis of RhoGEF3 loss- and gain-of- function in developing embryos. Temporal and spatial expression of RhoGEF3 was assessed by *in situ* hybridizations and immunohistochemistry using an antiserum against the protein RhoGEF3. Our results indicated that RhoGEF3 promotes the remodeling of the actin cytoskeleton in S2R+ cells in a Rac1 dependent manner. The *rhogef3* transcript and RhoGEF3 protein were detected in tubular tissues, mainly in salivary glands (SG) and tracheal system (TS) during embryogenesis. In the SG, RhoGEF3 was detected in the apical region of the cells that form the secretory portion of the gland. Both gain- and loss-of-function of *rhogef3* resulted in severe defects in SG lumen expansion and in the formation of tracheal tubes at later stages of embryogenesis. We concluded that throughout its capacity to modulate the remodeling of actin cytoskeleton, RhoGEF3 is involved in the regulation of SG lumen formation and in tracheal cell migration and/or cell adhesion processes. Funded by: Fondecyt-1090211, Fondecyt Postdoctoral-3110147.

909C

Roles of N-glycosylation and lipidation in Wg secretion and signaling. Xiaofang Tang¹, Yihui Wu², Tatyana Belenkaya¹, Qin Zhu Huang³, Lorraine Ray¹, Jia Qu³, Xinhua Lin^{1,2}. 1) Developmental Biol, Cincinnati Children's Hosp, Cincinnati, OH; 2) State Key Laboratory of Biomembrane and Membrane Biotechnology, and Key Laboratory of Stem Cell and Developmental Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China; 3) Wenzhou Medical College, Wenzhou, Zhejiang, 325000, China.

Wnt members act as morphogens essential for embryonic patterning and adult homeostasis. Wnt proteins are known to be both glycosylated and lipid modified at conserved sites, which are, for *Drosophila* Wnt member Wingless (Wg), N103 and N414 for glycosylation and C93 and S239 for lipidation. While most of the functional studies employed tagged Wnt molecules in tissue culture systems, we seek to examine the roles of lipidation and glycosylation on native Wg proteins both *in vivo* and in cultured cells. We show that Wg mutant devoid of

all the N-glycosylations exhibits no major defects in either secretion or signaling, indicating that N-glycosylation is dispensable for Wg activities. We demonstrate that lipid modifications at Serine 239 (S239) rather than that at Cysteine 93 (C93) plays a more important role in regulating Wg signaling possibly through affecting interaction with the receptor Frizzled 2 (dFz2). Importantly, while single Wg C93 or Wg S239 mutants can be secreted, removal of both acyl groups at C93 and S239 renders Wg incapable of reaching the plasma membrane for secretion. Together, our data demonstrate the *in vivo* roles of N-glycosylation and lipid modification in Wg secretion and signaling. Our results also clarified some controversial issues in previous functional studies about the roles of lipidation in Wg signaling and secretion. Further studies are being done to analyze the functional relationship between N-glycosylation and porcupine-mediated lipid modification by using mutants lacking both types of modifications.

910A

Snx3 influences Wnt/Wingless secretion through regulating retromer-dependent recycling of Wntless. Yihui Wu¹, Peng Zhang¹, Tanya Belenkaya², Xinhua Lin^{1,2}. 1) State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R.China; 2) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, 45229, USA.

Wnt proteins play essential roles in many biological processes during development and diseases. Recent studies have identified Wntless (Wls) and Retromer complex as key components involved in Wnt/Wingless(Wg) secretion. As sorting nexin (Snx) molecules are essential for anchoring Retromer on membrane, we hypothesize that specific Snx(s) is required for retromer-mediated Wg secretion. For this purpose, we generated *Drosophila* mutants for all of the eight *snx* members, and found that *Dsnx3* was involved in Wg secretion. In wing discs, Wg secretion and its signaling activity are defective in *Dsnx3* mutant clones. In *Dsnx3* dsRNA treated *Drosophila* S2 cells, Wg levels in culture medium are also remarkably reduced. More importantly, Wls levels are strikingly reduced in *Dsnx3* mutant cells, and over-expression of Wls can rescue the Wg secretion defect in *Dsnx3* mutant cells. Moreover, *Dsnx3* can interact with *Drosophila* Vps35 in S2 cells, and co-localize with human Vps35 in early endosomes. Taken together, these data indicate that *Dsnx3* influences Wg secretion by controlling retromer-dependent Wls recycling.

911B

Sulfated is a negative feedback regulator of wingless in *Drosophila*. Jia You¹, Tatyana Belenkaya¹, Xinhua Lin^{1,2}. 1) Dev Biol, Cincinnati Child Hosp Med Ctr, Cincinnati, Oh; 2) State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Drosophila Wingless (Wg) acts as a morphogen to control pattern formation in a concentration dependent manner. Previous studies demonstrated important roles of heparan sulfate proteoglycans (HSPGs) in controlling Wg signaling and distribution. Here, we examined the role of Sulfated (Sulf1), a *Drosophila* homolog of vertebrate heparan sulfate 6-O endosulfatase, in Wg signaling and distribution. We show that *sulf1* is specifically up-regulated by Wg signaling in the wing disc. We found that expression of Wg target gene *senseless* (*sens*) was elevated in the *sulf1* mutant wing discs. Sulf1 also negatively regulate extracellular levels of Wg. Genetic interaction experiments indicate that Wg antagonist Notum may work synergistically with Sulf1 to restrict Wg signaling, and Dally, a member of *Drosophila* HSPGs, is a potential target of Sulf1. Our results demonstrate that *sulf1* is a novel Wg target gene and by a feedback mechanism, it negatively regulated Wg signaling and distribution *in vivo*.

912C

Cyclin B3 appears a better target for inhibition of mitotic exit in reducing mitotic slippage. Beatriz P Alvarado, Blake Riggs. San Francisco State University, San Francisco, CA.

The primary approach taken for the treatment of different types of cancer is using chemotherapeutic agents; such as vinca alkaloids and taxanes are anti-mitotic drugs that function by activating the Spindle Assembly Checkpoint (SAC) and arresting the cell in mitosis by blocking microtubule dynamics. Unfortunately these types of chemotherapeutic agents are broad acting and carry with them a high degree of neurotoxicity. Other small molecules that target mitotic specific proteins have been tested for effects on cancer progression but are less effective than the vinca alkaloids and taxanes. This is believed to be due to allowing the cancerous cells to slip out of mitosis before apoptosis can occur. Because of this mitotic slippage, these treatments aren't as effective against more aggressive forms of cancer. It is now believed that blocking mitotic exit downstream of the SAC is a better strategy for reduction of mitotic slippage. We aim to show that arresting cells downstream of the SAC by inhibition of the Anaphase Promoting Complex (APC), mitotic slippage will be decreased in *Drosophila* S2 cells. We have examined the change in mitotic index and apoptosis in *Drosophila* S2 cells, some of which have been treated with common chemotherapeutic agents such as Taxol, Monastrol, Colchicine and Binucleine. The data was collected through FACS analysis by using a TUNEL assay and looking at condensed DNA in cells. This data has been compared to the mitotic index and apoptosis rates in cells that have cyclin B3 knocked down by RNAi-mediated inhibition. The results from the cyclin B3 knockdown are comparable to that of *cdc27* knockdown as well as the chemotherapeutic agents used. It appears to be a good target for the reduction of mitotic slippage. This data will provide the basis for an RNAi screen on proteins involved in late mitotic events that reduce mitotic slippage.

913A

Regulation of DNA copy number during development. Jared T. Nordman¹, Helena Kashevsky¹, Terry L. Orr-Weaver^{1,2}. 1) Whitehead Institute, Cambridge, MA; 2) Dept. of Biology, MIT, Cambridge, MA.

It has become increasingly clear that the DNA replication program is profoundly affected during development and differentiation. But, the mechanisms responsible for the programmed changes in the DNA replication program, as well as the biological consequences of these changes still remain unclear. *Drosophila* polytene chromosomes provide a unique opportunity to understand how developmental control of DNA replication can lead to changes in gene copy number. Polytene chromosomes contain multiple copies of the genome, but DNA copy number is not uniform throughout the genome, a process referred to as differential replication. Previously, we have used array based comparative genomic hybridization (aCGH) to monitor genome-wide copy number changes in larval salivary gland, larval midgut and larval fat body tissues. These studies revealed tissue-specific changes in differential replication. We have now used aCGH to monitor differential replication in the adult malpighian tubules, adult midgut and adult fat body tissues. These data sets represent the most exhaustive survey of differential replication to date. We have generated a six tissue comparative genomic analysis of differential replication, revealing informative biological insights while providing an invaluable tool kit to dissect this process mechanistically.

914B

Investigating the mechanism of homolog recognition in *Drosophila melanogaster*. Tharanga N. Senaratne, Eric F. Joyce, C.-Ting Wu. Genetics, Harvard Medical School, Boston, MA.

Homologous chromosomes are intimately paired in virtually all cell types throughout development in *Drosophila melanogaster*, which leads to interesting questions about chromosome dynamics, homolog recognition, and nuclear organization. Recently, we conducted a fluorescence in situ hybridization (FISH)-based genome-wide screen that identified candidate proteins involved in the pairing of heterochromatic regions of homologous chromosomes in *Drosophila* cell culture. Hits from the screen have highlighted several important processes in the regulation of pairing including cell cycle regulation, chromosome structure, and microtubule organization. To identify which of these processes are required for homolog recognition, we propose examining the pairing of chromosomal rearrangements, such as small translocations and balancer chromosomes, which should be particularly sensitive to the loss of factors required to detect homology. In addition, none of the known cohesin subunits, which facilitate cohesion between sisters, were identified in the screen, despite the fact that a large proportion of culture cells are in G2 and thus possess two chromatids per chromosome. Cohesin knockdowns were expected to cause sister chromatid separation, and possibly homolog unpairing, and thus increase the number of FISH signals per nucleus. We confirmed efficacy of RNAi against cohesins by examining cells in mitosis, where we observed sister chromatid separation. The maintenance of pairing in interphase cells in the absence of cohesins intriguingly suggests that sister chromatids can be held together with no or with very little cohesins. We hypothesize that homolog pairing mechanisms can compensate for the loss of cohesins, and present further data supporting this model. Funding: Ruth L. Kirschstein National Research Service Award to E.F.J. (F32CA157188) and an NIH/NIGMS grant (RO1GM61936), SPARC Award from the Broad Institute, and Cox Program Award from Harvard Medical School to C.-t.W.

915C

The role of apoptosis and JNK signaling in *dpp*-mediated ventral head development. Sung Yeon Park, Brian Stultz, Deborah Hursh. CBER/FDA, Bethesda, MD.

We are studying the role of decapentaplegic (*dpp*) in the formation of the adult head capsule. *dpp^{hc}* mutations eliminate peripodial-specific *dpp* expression in the eye/antennal disc, and affect structures of the ventral head such as vibrissae, gena, rostral membranes, and maxillary palps. In these *dpp^{hc}* mutations, we observe increased apoptotic cell death during the third larval instar in both the peripodial epithelium (PE) and disc proper (DP) tissue layers of the eye/antennal disc. To determine how this apoptotic cell death is related to the mutant phenotype, we examined apoptotic pathway mutations to determine their requirements for the *dpp^{hc}*-induced pattern defects. The anti-apoptotic *diap* allele dominantly enhances recessive *dpp^{hc}* mutations, while mutations in pro-apoptotic genes can rescue the vibrissae defect of the *dpp^{hc}* mutant. Furthermore, using the *Gal 4-UAS* binary system, we observed that the vibrissae phenotype in strong mutant backgrounds can be suppressed by the provision of anti-apoptotic gene products driven by the ubiquitous *69B-Gal4*, but not by *dpp^{hc}-Gal4*, whose expression is limited to the PE. We also observe activation of the JNK pathway in *dpp^{hc}* mutations, both in the PE, in the region where *dpp* expression is lost, and in regions of apoptotic cells in the disc proper. While JNK activity in the disc proper is correlated with regions of apoptosis, we found that the association of JNK expression and cell death in the PE is less direct. This suggests that the JNK pathway may function differently in the PE, and this pathway may play different roles in the two tissue layers of the eye/antennal disc. Interestingly, induction of the JNK pathway in the PE was detected only in backgrounds in which loss of maxillary palps was observed. We can conclude that the loss of the PE-Dpp causes two partially separable defects: 1) a defect in the sensory vibrissae associated with apoptosis in the DP 2) a defect in the maxillary palps arising from JNK induction in PE, but not obviously associated with apoptosis.

916A

Caspase activity in gut mediates systemic response against tissue damage. Asuka Takeishi¹, Erina Kuranaga², Ayako Tonoki³, Hirota Kanuka⁴, Masayuki Miura¹. 1) Department of Genetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan and CREST, JST, Tokyo, Japan; 2) Laboratory for Histogenetic Dynamics, RIKEN Center for Developmental Biology, Kobe, Japan; 3) Department of Neuroscience, The Scripps Research Institute, Jupiter, Florida, USA; 4) Department of Tropical Medicine, The Jikei University School of Medicine, Tokyo, Japan.

Damaged tissue triggers various defense responses involving innate immunity to maintain the homeostasis of the whole body. In mammal, it is known that inflammatory caspases, such as caspase-1, play central roles as a component of inflammasome to induce innate immune response against infection and endogenous danger signals secreted from damaged tissue. Although inflammatory caspase has not been reported in invertebrate, previous reports revealed apoptotic caspase contributes to the host defense response against infection in *C. elegans*. We thus used *Drosophila* mutant of apoptotic caspase activator, *dark/dpf-1/HAC-1*, to elucidate the mechanism to overcome tissue damage *in vivo*. Epidermal damage was caused by pricking the flies' abdomen, and we found mutant of *dark* is sensitive to this epidermal injury. Epidermal injury induced caspase activation within 30 min in gut, the tissue distant from injured site. *dark* overexpression in gut fully rescued the injury-induced lethal phenotype of *dark* mutant. Moreover, wild type showed lethality by the injection of the hemolymph of pricked flies that caspase activity is inhibited in gut, suggesting the toxic factor was induced in the hemolymph after injury. These results suggest caspase activity is required in gut to regulate the systemic defense response in order to overcome tissue injury *in vivo*.

917B

Endocytosis, vacuolation, non-lysosomal endosome trafficking, and massive calcium release are major activities of *Drosophila* prepupal salivary glands prior to their ecdysone-triggered programmed histolysis. Robert Farkas^{1,2}, Lucia Mentelova^{1,2}, Zuzana Datkova^{1,2}, Daniel Vlcek², Milan Beno¹, Silvia Mahmood^{1,3}, Peter Danis^{1,2}, Ludmila Pecanova^{1,2}, Dusan Cmarko⁴, Lubos Kovacic⁴, Ivan Raska⁴, Bruce Chase⁵, Bernard Mechler^{4,6}. 1) Inst Experimental Endocrinology, Slovak Academy Sciences, Vlarska 3, 83306 Bratislava, Slovakia; 2) Department of Genetics, Faculty of Science, Comenius University, 842 15 Bratislava, Slovakia; 3) Department of Biochemistry, Jessenius Faculty of Medicine, Comenius University, Mala Hora 4, 03645 Martin, Slovakia; 4) Institute of Cellular Biology and Pathology, 1st Faculty of Medicine, Charles University, Albertov 4, 12801 Prague, Czech Republic; 5) Department of Biology, University of Nebraska, Omaha, NE 68182-0040, USA; 6) Department of Developmental Genetics, Deutsches Krebsforschungszentrum-ZMBH Allianz, INF 581, D-69120 Heidelberg, Germany.

It is widely accepted that at the onset of *Drosophila* metamorphosis the steroid hormone ecdysone activates a cell death program (PCD) that leads larval salivary glands (SGs) to rapidly disintegrate about 14-16 hr after puparium formation. However, very little attention has

been paid to fate of *Drosophila* SGs in the period between puparium formation and execution of PCD. By using specific inhibitors, mutations or transgenes for *shi*, *Rab5*, *Rab11*, *vha55*, *vha68-2*, *vha36-1*, *syx1A*, *syx4*, and *Vps35* we show that between pupariation and execution phase of PCD the prepupal glands are very active in endocytosis, acidic vacuole formation which are key structures for iron transfer and release for highly demanded anabolic metabolism, followed by late endosomal trafficking, vacuole disappearance and membrane consolidation, and calcium oxalate (CaOx) extrusion resembling renal excretory activity. In spite of these processes including lost of majority of detectable proteins, SGs remain steadily active in de novo RNA and protein synthesis documenting their high viability till last moments prior to execution of PCD. (Supported by GACR P302/11/1640, EEA & NFM Norwegian Fund # SK-0086/3655/2009/ORINFM, MSM 0021620806 and LC535 grants).

918C

***lin-52* prevents apoptosis in *Drosophila* imaginal discs.** Cuiyun Geng¹, Peter Lewis², Michael Botchan³, Joseph Lipsick¹. 1) Department of Pathology and Genetics, Stanford University, Stanford, CA; 2) Laboratory of Chromatin Biology and Epigenetics, The Rockefeller University, New York, NY; 3) Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA.

Myb-MuvB/dREAM is an evolutionally conserved multiprotein complex that regulates gene expression and site-specific DNA replication in *Drosophila*. This complex contains nine subunits, including the RBF tumor suppressor proteins, *E2f2*, *Dp*, the p55/CAF1 histone chaperone, the Myb oncoprotein, three Myb-interacting proteins (*Mip130*, *Mip120*, *Mip40*), and *Lin-52*. *Lin-52* is a small pioneer protein with a highly conserved domain of unknown function. To dissect the role of the *lin-52* gene during *Drosophila* development, we employed a combination of genetics and cell biology. Here we show that depletion of *lin-52* results in increased cell death in larval imaginal discs, which is accompanied by activation of the Jun N-terminal Kinase (JNK) pathway. Blocking the JNK pathway suppressed the apoptotic phenotype in *lin-52* mutant wing disc, implying that the cell death observed in *lin-52* mutant cells is mediated by the JNK pathway. In addition, apoptotic phenotype caused by *lin-52* reduction can be suppressed by *P35*, a baculoviral protein that can block the activity of effector caspases. This result implies that the apoptosis observed in *lin-52* mutant cells is executed by a caspase pathway. Our results suggest that *lin-52* plays an important role in regulating programmed cell death in *Drosophila* imaginal discs.

919A

Roles of the *Drosophila* RZZ complex in membrane traffic and cytokinesis. Maurizio Gatti¹, Maria Grazia Giansanti¹, Michael Goldberg², Alan Wainman^{1,3}. 1) Dept Biology & Biotechnology, Univ Rome La Sapienza, Rome, Italy; 2) Department of Molecular Biology and Genetics, Biotechnology Building, Cornell University, Ithaca, NY 14853, USA; 3) Present address: Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK.

The *Zw10* protein, in the context of the conserved Rod-Zwilch-Zw10 (RZZ) complex, is a kinetochore component required for proper activity of the spindle assembly checkpoint in both *Drosophila* and mammals. In mammalian and yeast cells, the *Zw10* homologues, together with the conserved *RINT1/Tip20p* and *NAG/Sec39p* proteins, form a second complex involved in vesicle transport between Golgi and ER. However, it is currently unknown whether *Zw10* and the *NAG* family member *Rod* are also involved in *Drosophila* membrane traffic. Here we show that *Zw10* is enriched at both the Golgi stacks and the ER of *Drosophila* spermatocytes, and at the Golgi-derived acroblast of spermatids. *Rod* is concentrated at the Golgi and the acroblast but not at the ER, while *Zwilch* does not accumulate in any membrane compartment. Mutations in *zw10* cause defects in Golgi morphology, reduce the number of Golgi stacks, and disrupt acroblast assembly. Mutations in *rod* also affect Golgi morphology and acroblast formation, while *zwilch* mutant do not exhibit gross defects in these membranous structures. Loss of *Zw10* results in frequent failures of spermatocyte cytokinesis, whereas *Rod* or *Zwilch* are not required for this process. *zw10* mutant spermatocytes assemble regular central spindles and actomyosin rings, but furrow ingression halts prematurely due to defective plasma membrane addition. Collectively, our results implicate *Drosophila* *Zw10* in the ER-Golgi traffic, and indicate that this traffic is essential for plasma membrane formation during spermatocyte cytokinesis. Our findings further suggest that *Rod* plays a Golgi-related function that is not required for cytokinesis.

920B

Mutations from a screen for interactors of the *Axs*^{KO} mutation cause divergent meiotic phenotypes. Elisabeth Bauerly¹, Stacie Hughes¹, R. Scott Hawley^{1,2}. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Dominant mutations in the *Aberrant X segregation* (*Axs*) gene cause missegregation of nonexchange chromosomes as well as barrel-shaped spindles and chromosome misalignment during prometaphase I in *Drosophila* oocytes. A mutation that eliminates the *Axs* gene (*Axs*^{KO}) causes no discernable phenotype, possibly due to redundancy with another pathway. To identify genes acting redundantly with *Axs* we initiated a forward genetic EMS screen for mutations that cause phenotypes that differ in severity depending on whether the *Axs*^{KO} mutation is present. While only 368 mutant lines were examined, two lines showing such a genetic interaction and a meiotic phenotype were identified. One mutation, *L31*, is homozygous lethal in an otherwise wild-type genetic background. Homozygosity for the *Axs*^{KO} mutation suppresses this lethality allowing for the isolation of *L31* homozygous flies. While the surviving *Axs*^{KO} ; *L31* males are fertile, females are sterile. Eggs from *Axs*^{KO} ; *L31* mothers fail to hatch and typically contain few, if any, proper mitotic spindles and have disorganized chromosomes. Some oocytes from *Axs*^{KO} ; *L31* mothers appear to have an anaphase I-like DNA configuration. We will present the results of deficiency mapping of this mutation, as well further cytological characterization. This mutation may provide new insights into the regulation of female meiosis I by the *Axs* protein.

921C

Interkinetic nuclear migration in the developing *Drosophila* wing. Yu-ichiro Nakajima, Emily J. Meyer, Matthew C. Gibson. Stowers Institute for Medical Research, Kansas City, MO.

Animal development requires mechanisms to coordinate the process of rapid cell proliferation with the maintenance and morphogenesis of specialized epithelial architectures. Although the regulatory mechanisms of cell division have been vigorously studied with unicellular organisms and cultured cells, precisely how mitosis is spatially and temporally coordinated with cell polarization and epithelial morphogenesis remains poorly understood. Here we use the *Drosophila* wing imaginal disc as a model to dissect mitotic events in the epithelial context *in vivo*. During wing disc growth, mitotic nuclei translocate to the apical epithelial surface at the onset of cell division in a process termed interkinetic nuclear migration (IKNM). By demonstrating a mechanistic link between prophase entry, cortical Actomyosin

contractility, and apically-directed movements of nuclei, we show that IKNM is a consequence of the conserved process of mitotic cell rounding. To further investigate mitotic events in the wing epithelium, we analyzed the cell-cycle dependent positions and movements of nuclei with cell-cycle markers. Our result support relatively stochastic nuclear movements, except during M-phase, when more precise regulation is required. Lastly, using a novel live imaging approach, we demonstrate the dynamic nature of epithelial cell divisions, *in vivo*.

922A

Role of chromatin modifications in *Drosophila* imaginal disc regeneration. Shalaka Chitale, Andrea Skinner, Rachel K Smith-Bolton. Department of Cell and Developmental Biology, University of Illinois, Urbana-Champaign, Urbana, IL.

Imaginal discs have long been used to study regeneration in flies. Initially regeneration studies in imaginal disc were done by manually damaging the disc, a process that is time consuming and not practical for large scale regeneration screens. Our lab has developed an inducible genetic ablation technique that can selectively ablate cells in the wing disc at the desired developmental stage. Using this tool, we can more easily screen for regeneration genes and evaluate their role in this process. Regeneration involves a number of changes in gene expression profiles of the regenerating tissue, both for formation of the blastema and for subsequent differentiation and repatterning of the proliferating tissue. Discs heterozygous for *trithorax* or *brahma* alleles show impaired regeneration, indicating that chromatin modification and epigenetic regulation play a role in the regeneration process. In order to understand the role of epigenetic regulation in regeneration, we are screening mutants in all chromatin modifiers for their effect on regeneration. In addition, we are clarifying the relationships between chromatin modifying enzymes and other signalling pathways that regulate regeneration.

923B

Characterizing the Role of Trithorax in Regeneration. Andrea Skinner, Shalaka Chitale, Rachel K. Smith-Bolton. Cell & Developmental Biology, University of Illinois Urbana-Champaign, Urbana, IL.

Regeneration is a phenomenon that occurs across many species. *Drosophila melanogaster* is able to regenerate lost imaginal disc tissue prior to pupariation. We performed a genetic screen in which tissue was ablated from the wing imaginal discs of third instar larvae. The animals were then screened after eclosion for wing size as a measure of regeneration. From this screen, we found animals heterozygous for *trithorax* mutants have reduced wing regeneration, eclosing with smaller and less than fully regenerated wings. Trithorax Group (TRX-G) and Polycomb Group (Pc-G) proteins regulate gene expression by modifying chromatin. Trithorax (Trx) is associated with active gene expression via histone 3 lysine 4 (H3K4) methylation. In order to understand how Trx regulates regeneration, we are currently characterizing what is occurring in *trx* mutants after tissue damage. We are also screening for potential Trx targets during regeneration using quantitative RT-PCR.

924C

RNAi Screen for Mitotic Spindle Matrix Components among Nuclear Envelope Proteins. Arthur Chase, Blake Riggs. Biology, San Francisco State University, San Francisco, CA.

A key component of cell division is the mitotic spindle, which aligns and segregates duplicated chromosomes to the newly formed daughter cells at the end of mitosis. The mitotic spindle consists of polymerized microtubules and associated proteins, which assist in forming the fusiform structure. Defects in spindle assembly can lead to unequal chromosome segregation, which is a hallmark of many types of cancers. Current biophysics models propose that the spindle forms its shape only through tubulin polymerization forces. While this model is informative, it doesn't fully explain the rapid reorganization of the microtubule network for proper spindle formation. The spindle's well-organized assembly could be explained by a spindle matrix. This putative matrix would provide a scaffolding to support the growing spindle by physically guiding its formation or it could house spindle growth factors. Several proteins have already been proposed as matrix components. However, the spindle matrix model is not completely understood. I propose that during nuclear envelope breakdown in mitosis, specific nuclear proteins are reorganized to help form the spindle matrix. I will use a targeted RNA interference screen in *Drosophila melanogaster* S2 cells to identify nuclear proteins involved in the spindle matrix. I immunostained the nuclear intermediate filament lamin B and found that it did not form into a matrix during mitosis. I knocked down the nuclear envelope proteins Nup214 and Mbo and found no difference in mitotic index compared to controls. Completing the roster of spindle matrix proteins will help in our understanding of spindle assembly and may provide future targets for cancer therapeutics.

925A

Imaging regenerating imaginal discs. Peter C DeJongh, Brenten Popiel, Rachel K Smith-Bolton. University of Illinois at Urbana-Champaign, Urbana, IL.

Damaged *Drosophila* larval imaginal discs are capable of undergoing wound closure and regenerative proliferation to replace lost tissue. Imaginal disc regeneration has been studied by manually cutting the discs and culturing them in adult hosts, as well as genetically ablating tissue and allowing regeneration to occur *in vivo*. Using these methods we and others have characterized signaling and growth pathways important for regeneration. However, few attempts have been made to culture healing and regenerating imaginal discs *in vitro* for extended periods of time, which would enable observation of the morphological events that occur during wound closure and regrowth. We are developing a method to observe regeneration over the course of several days. Using this method we will characterize wound closure, blastema formation, and changes in the extra-cellular matrix. We will present our culture methods and preliminary observations.

926B

Developmental role of the RTK Stitcher. Fergal O'Farrell^{1,2}, Shenqiu Wang², Christos Samakovlis², Tor Erik Rusten¹. 1) Dept of Biochemistry, Institute of Cancer Research, Norwegian Radium Hospital, Oslo, Norway; 2) Dept of Developmental Biology, Stockholm University, Stockholm, Sweden.

The Orphan Receptor Tyrosine Kinase (RTK) Stitcher is a proto-cadherin with at least one cadherin domain within the extracellular portion of the protein. The cadherin region, together with the cytoplasmic kinase domain indicates that the protein may be orthologous to the oncogenic Ret RTK. Activating mutations of the mammalian Ret oncogene are strongly associated with Multiple Endocrine Neoplasia type 2 (MEN2A and MEN2B). A consequence of increased Ret activity is activation of the TOR growth control pathway, a pathway elevated in many different forms of cancer. The Samakovlis lab previously demonstrated that Stitcher is required for efficient closure of the *Drosophila* embryonic epidermis following mechanically induced wounds. We addressed the developmental role of Stitcher and find that null mutant

alleles are lethal indicating it serves an essential function. Stitcher is widely expressed in epithelial tissues throughout development and we selected the wing for functional analysis. Altering levels of Stitcher in the dorsal domain of the wing, gives rise to an upwardly bent adult wing, indicative of reduced growth on the dorsal surface. This is true for clones of stitcher null alleles, RNAi and dominant negative (using a kinase dead Stitcher variant) approaches. The wing growth defect results from a reduction in TOR signaling resulting in fewer cells. Conversely, Stitcher over expression results in an increase in proliferation and increased TOR pathway activity, an effect similar to oncogenic Ret. We are currently investigating the interplay between the Insulin, TOR and Stitcher pathways during epithelial growth.

927C

Annotation of *Drosophila erecta* Fosmid 41. Gury Alvarez, Edgar Nunez, Suryaveer Dogra, Katie Lopez-Galvez, Georgina Aguilar-Portillo, Grigor Deremsezyan, Luz Ramos, Mahyar Pourdavoud, John Samuel Sukumar palukuri, Jasmin Abdulla, Catherine Coyle-Thompson. Biology, California State University, Northridge, Northridge, CA.

Sequence data of *Drosophila erecta* Fosmid 41 of Chromosome 3L was analyzed with known gene sequence data from *Drosophila melanogaster* using the Basic Local Alignment Search Tool (BLAST) to identify 15 predicted genes. Exon sequences from the known *D. melanogaster* predicted genes were used to query *D. erecta* Fosmid 41 using a specialized BLAST search to align the two sequences for each exon of each predicted gene. A gene model was generated and verified for each predicted gene in 40000bp of *D. erecta* Fosmid 41. The model *D. erecta* transcripts and translated products were compared with the known *D. melanogaster* gene products using a specialized BLAST search to align the two sequences: The tblastx and blastp analyses were done in this manner and conservation of the predicted protein product from the gene sequences were observed for each gene model. We were not able to develop models for two of the predicted genes in the region. This may be due to the genes not being present in the region and the alignment could be due to similarities between exons of *CG32413*, *QC*, and *isoQC*. This work was carried out in the Full Immersion Research Experience (FIRE) course at in the Biology Department at CSU, Northridge as a part of Dr. Catherine Coyle-Thompson's participation as a member of the Genomic Education Partnership (GEP). This analysis and annotation of *Drosophila erecta* fosmid 41 of chromosome 3L as a component of research for the GEP is funded in part by the Genomics Education Partnership, HHMI Grant # 52005780 to Professor Sarah C R Elgin. Funding was also provided through the CSU-LSAMP program National Science Foundation Grant #HRD-0802628 and the Chancellor's Office of the California State University, and the Biology Department at California State University, Northridge.

928A

Annotation of *Drosophila erecta* Fosmid 28 of Chromosome 3L. Theresa R. Bustamante¹, Katie V. Lopez-Galvez², Luz Ramos², Catherine Coyle-Thompson². 1) Engineering Department/ Biology Department, California State University, Northridge, Northridge, CA; 2) Biology Department, California State University, Northridge, Northridge, CA.

Sequence data of *Drosophila erecta* Fosmid 28 of Chromosome 3L from the UCSC Genome Browser Mirror at Washington University in St. Louis, was analyzed with known gene sequence data from *Drosophila melanogaster* using the Basic Local Alignment Search Tool (BLAST) to identify 14 predicted genes in the fosmid. Exon sequences from the known *D. melanogaster* predicted genes were used to query Fosmid 28 from *D. erecta* using a specialized BLAST search to align the two sequences for each exon of each predicted gene. The start and stop codons were identified for each transcript isoform of the genes where possible. Splice sites were determined for each *D. erecta* exon and a gene model generated and verified with the Gene Model Checker tool of the Genomics Education Partnership (GEP) for each predicted gene in 78000bp of *D. erecta* Fosmid 28. The model *D. erecta* transcripts and translated products were compared with the known *D. melanogaster* gene products using a specialized BLAST search to align the two sequences: The tblastx and blastp analyses were done in this manner and conservation of the predicted protein products from the gene sequences were observed for each gene model. This work was carried out in the Full Immersion Research Experience (FIRE) course of the Biology Department at CSU, Northridge as a part of Dr. Catherine Coyle-Thompson's participation as a member of the Genomic Education Partnership (GEP) program. This analysis and annotation of *Drosophila erecta* Fosmid 28 of chromosome 3L as a component of research for the GEP is funded in part by the Genomics Education Partnership, HHMI Grant # 52005780 to Professor Sarah C R Elgin. Funding was also provided through the CSU-LSAMP program National Science Foundation Grant #HRD-0802628 and the Chancellor's Office of the California State University, and the Biology Department at California State University, Northridge.

929B

The Role of PI3K and Insulin Signaling in Mediating Fetal Alcohol Syndrome in *Drosophila melanogaster*. Theresa Logan, Melissa Ruiz, Omar Fateen, Janet Lafler, Rachael French. Biological Sciences, San Jose State University, San Jose, CA.

Fetal Alcohol Syndrome (FAS) is a spectrum disorder affecting individuals exposed to ethanol during gestation and often results in growth and development defects, behavioral changes, and altered adult responses to ethanol; it is also the leading cause of non-genetic mental retardation. Previous studies have shown *Drosophila melanogaster* larvae exposed to ethanol-treated food have many of the same phenotypes. Additionally, it has been found that upregulation of insulin during development mediates these ethanol-induced phenotypes. The purpose of this study is to determine which downstream components of the insulin signaling pathways are mediating these phenotypes, looking in particular at the phosphoinositide 3-kinase (PI3K) pathway, which is activated by insulin. We hypothesize that if the PI3K pathway is involved in mediating ethanol-induced phenotypes, then genetic alterations leading to decreased pathway activity will amplify ethanol-induced phenotype(s), while alterations leading to increased pathway activity will mediate the effects. Loss of function alleles of components of this pathway, along with transgenic constructs (both RNAi and wild-type alleles) were tested for developmental delay and survival in ethanol-treated food. To date, *Akt*, *Pdk* and *foxo* genes have been found to influence survival. Gain of function of *Akt* and loss of function of *foxo* were predicted to mediate and found to increase survival. Loss of function of *Pdk* and *Akt* were predicted to amplify ethanol-induced phenotypes, but found to increase survival. This unexpected result, combined with other data, implicates Pk61C in mediating oxidative stress. We conclude that the PI3K pathway is certainly involved in the insulin mediation of FAS in fruit flies. Future work will include testing more transgenic constructs, including RNAi, wild-type, and dominant negative UAS lines, as well as more loss-of-function alleles within the PI3K pathway. Additionally, sedation and tolerance will be tested in all strains shown to influence survival, and a behavioral assay is in development to test learning and memory.

930C

***Drosophila* as model system to study cross-tolerance between nicotine and ethanol and the effects of chronic nicotine exposure on**

behavior. Norma Velazquez Ulloa, Ulrike Heberlein. Anatomy, UCSF, San Francisco, CA.

Tobacco and alcohol addiction pose a public health worldwide problem. These drugs are often used in combination. In rodents, cross-tolerance -a decrease in the effects of alcohol after consumption of nicotine and/or vice-versa- between these drugs has been reported. However, little is known about the genes and molecular mechanisms that mediate nicotine addiction and the interactions between nicotine and ethanol. *Drosophila melanogaster* is a proven model system to study genes and novel mechanisms of addiction. We have developed a *Drosophila* model for nicotine addiction and nicotine and ethanol cross-tolerance. We measured the effects of exposing adult flies to nicotine on the Capillary Feeder (CAFE) or exposing flies to nicotine during development on the behavior of adult flies. The flies were assayed on: their response to an acute nicotine exposure on a negative geotaxis test; an egg-laying preference test with a choice for a substrate with or without nicotine; the loss-of righting reflex test for sedation due to ethanol exposure. We found that nicotine exposure in the CAFE results in tolerance to acute nicotine exposure and to resistance to the sedative effects of ethanol. Similar results were found when flies were exposed to nicotine during development. In addition to these effects, developmental nicotine exposure decreased adult dry weight and survival, and delayed eclosion time in a dose-dependent manner. Larval exposure is sufficient for decreased survival and eclosion delay. Despite these aversive consequences of nicotine, rearing flies on nicotine food increased egg-laying preference for nicotine over non-nicotine food, which suggests a preference for nicotine despite potential negative consequences. These results show that long-term nicotine exposure has specific effects on behavior, some of which parallel the effects of nicotine in mammals. We show that *Drosophila* can be a suitable model system to study the molecular and neural mechanisms that mediate the effects of nicotine and the interaction between nicotine and ethanol on behavior.

931A

A P_{5B}-type ATPase is required for *Drosophila* polyamine transport. Nicole K Barnette^{1,2}, Victoria Kreinbrink¹, Laurence von Kalm^{1,2}. 1) Department of Biology, University of Central Florida, Orlando, FL; 2) Biomolecular Science Center, University of Central Florida, Orlando, FL.

Polyamines are small ubiquitous cationic molecules that are essential for cell growth and proliferation in addition to other cellular processes including regulation of transcription and chromatin structure, and apoptosis. The intracellular polyamine pool is maintained by a balance of synthesis and import. Polyamine synthesis is well understood; however, how polyamines enter the cell from the extracellular environment is unknown in multicellular eukaryotes. Cancer cells have a high requirement for polyamines due to the need to support rapid growth. In response to drugs that target polyamine synthesis cancer cells up-regulate import via the polyamine transport system (PTS), making the PTS an attractive chemotherapeutic target in combination with drugs that block polyamine synthesis. We have previously demonstrated that *Drosophila* imaginal discs have a PTS with vertebrate-like characteristics. Using both classical mutants and RNAi we have identified CG32000, a P_{5B}-type ATPase, as a component of the polyamine transport system (PTS) in *Drosophila*. The ortholog of CG32000 in *C. elegans*, *catp-5*, is required for uptake of the toxic polyamine analog, norspermidine, suggesting that the CG32000 ATPase is a conserved component of the polyamine transport system in higher eukaryotes.

932B

Genetic mediators of diet-induced cardiac dysfunction. Ryan T. Birse, Kathryn Reardon, Hanna Catan, Rolf Bodmer. The Dell E Webb Center for Neuroscience Aging and Stem Cell Research, Sanford-Burnham Institute, La Jolla, CA.

Compelling evidence indicates that the deleterious effects of excessive dietary accumulation of lipids on metabolism and heart function is strongly linked to the progression of cardiovascular disease (CVD) and type 2 diabetes (T2D). Obesity has grown to epidemic proportions globally, yet, the genetic mechanisms that underlie obesity and diabetic related cardiomyopathies are largely unknown. The frequency of obesity is also alarmingly increasing in children, which can predispose them to develop obesity, CVD and T2D in adulthood. Given the early onset of the obesity epidemic, it is plausible that the metabolic state of the pregnant mother may contribute to the susceptibility of the offspring to obesity. Studies have shown that a nutritional difference in the pregnant mothers correlates with disease type and its postnatal appearance. One critical question concerns the physiological and morphological effects of the dietary-induced obesity on cardiovascular function. *Drosophila* has recently emerged as a powerful tool for studying the genetic mechanisms of metabolism and obesity, and its relationship to heart function. Using the genetic model of *Drosophila*, we find post-natal effects in lipid accumulation and heart structure and function on progeny exposed to a maternal HFD. I have also found an important role of the Target of Rapamycin (TOR) signaling in modulating the adverse effects of a maternal HFD on obesity and cardiac dysfunction. We also find a significant protective effect from the regulation of the downstream TOR lipid effector Brummer lipase on the phenotypes of the progeny from maternal HFD. Finally I tested the genetic modulation of epigenetic factors on the offspring from HFD mothers and found a role for HDACs in regulating lipid storage and heart function. By using the simple model system of *Drosophila* I have shown that a maternal HFD altered the metabolic state not only in the somatic tissue of the mother but also in the oocytes/embryos (modulating TOR-dependent homeostasis), leading to post-natal changes in metabolism and heart function in the offspring.

933C

The Role of the EGFR Pathway in a *Drosophila* Model of Fetal Alcohol Syndrome. Peter Luu¹, David Do², Luke LaJoie³, Brianna Hagen¹. 1) Biology, SJSU, San Jose, CA; 2) Computer Science, SJSU, San Jose, CA; 3) Mathematics, SJSU, San Jose, CA.

Fetal alcohol syndrome (FAS) is a spectrum disorder that results from developmental ethanol exposure, leading to reduced body and brain growth, and reduced cognitive development. Previous studies have shown *Drosophila* larvae exposed to ethanol-treated food have many of the same phenotypes. In particular, flies developing in ethanol display reduced viability, developmental delay, and altered behavioral responses to ethanol, all phenotypes shared in common with mammalian models of FAS. Finally, it is known that these phenotypes can be ameliorated with transgenic expression of *Drosophila* insulin-like proteins (Dilps) (McClure et al., 2011). The epidermal growth factor receptor (EGFR) pathway regulates cell proliferation, differentiation, and gene expression; however, it was not previously known to have a role in mediating the developmental response to ethanol exposure. Because previous research has demonstrated an interaction between the insulin receptor pathway and the EGFR pathway, we hypothesized that some of the insulin-dependent effects of developmental ethanol exposure are mediated by the EGFR pathway. To test this hypothesis, we examined the effects of mutations altering the activity of the EGFR pathway on developmental ethanol phenotypes. We found that mutations in at least three genes, *argos*, *rhomboid* (*rho*), and *Egfr*, alter the survival rate of flies reared in ethanol. Both the intramembrane serine protease Rhomboid and the EGF receptor stimulate the pathway, while the ligand Argos inhibits the pathway. We find that loss of function mutations in *Egfr* and *rho* lead to decreased survival. Conversely, and consistent with our hypothesis, a loss of function mutation in *argos* increases survival. Finally, using the GAL4-UAS transgenic

expression system, we have localized the requirement for rho to the developing nervous system. We therefore conclude that the EGFR pathway mediates ethanol-induced developmental mortality, and, further, that this mortality is due to ethanol's effects on the developing nervous system.

934A

***mauve* encodes the *Drosophila* homolog of the Chediak-Higashi Syndrome protein LYST and is necessary late during phagocytosis.** Mokhlasur Rahman¹, Adam Haberman², Charles Tracy¹, Sanchali Ray¹, Helmut Kramer¹. 1) Neuroscience, UT Southwestern Medical Center, Dallas, TX; 2) Oberlin College, Oberlin, Ohio.

Chediak-Higashi Syndrome (CHS) is an autosomal recessive disease, which is characterized by oversized lysosomes and lysosome-related organelles. Patients with CHS show partial albinism, progressive neurological disorder and often suffer from recurring bacterial infections. In a screen for trafficking mutants, we have identified a new eye color mutation, which displayed oversized lysosome-related organelles (LROs) such as pigment granules, and was allelic to *mauve*. We show that *mauve* encodes the *Drosophila* homolog of the CHS protein LYST. One of the most important clinical symptoms of CHS patients is susceptibility to bacterial infections. This phenotype was replicated in *mauve* flies, which displayed reduced survival after infections. Initial uptake of bacteria by *mauve* hemocytes was not altered but degradation of phagocytosed bacteria was impaired. Using markers for different stages of phagosome maturation we found early stages indistinguishable from wild type, but late stages marked by Hook were enlarged and contained an increased number of bacteria per phagosomes. Given this defect, we also tested for changes in the maturation of starvation-induced autophagosomes in fat bodies. We found: (i) *mauve* flies show reduced lysotracker staining indicating reduced autolysosome formation. (ii) *mauve* was not required for induction of autophagosomes since mCherry-ATG8 positive structures were increased in *mauve* flies compared to wild type. (iii) Electron microscopy of larval fat body suggests that autophagosomes were increased in size, and those large autophagosomes were also positive for Rab7, a marker for mature autophagosomes. These data suggest that *mauve* is necessary to suppress homotypic fusion of late phagosomes and autophagosomes and promote their fusion with lysosomes.

935B

Peroxisome Biogenesis in *Drosophila melanogaster*. Joseph E. Faust, Arvind Manisundaram, Michael Stern, James A. McNew. Biochemistry and Cell Biology, Rice University, Houston, TX.

Peroxisomes are organelles that carry out β -oxidation of fatty acids and other lipid processing reactions. Defects in peroxisome biogenesis cause a spectrum of human diseases known as peroxisome biogenesis disorders (PBDs). The most severe PBD, Zellweger syndrome, is characterized by neuronal dysfunction, craniofacial malformation, and muscle weakness (hypotonia). There is currently no cure for PBDs and it is still unclear how the loss of peroxisomes leads to the disease state. We have begun to model PBDs in the fruit fly, *Drosophila melanogaster*, and hope to gain new insight into PBD etiology. Inhibition of peroxisome biogenesis was achieved by disrupting the *pex3* gene, which is required for the early steps of peroxisome biogenesis as well as insertion of proteins into the peroxisomal membrane. Inhibition of peroxisome biogenesis in *Drosophila* is lethal during metamorphosis, a developmental stage that requires the catabolism of nutrients stored as triacylglycerol (TAG). Prior to metamorphosis, these larvae are hypersensitive to starvation, where metabolism of stored lipids is required. Flies with impaired peroxisome biogenesis in muscles have reduced locomotor function and accumulate TAG in muscles. Hypotonia is a characteristic of PBD patients, but is thought to be a secondary effect of nervous system dysfunction. We propose that peroxisome loss may directly affect muscle physiology by causing a buildup of potentially toxic lipids. The precise mechanism of lipotoxicity is not known, but it could result from increased mitochondrial β -oxidation and elevated reactive oxygen species (ROS) production. Further experiment will determine the role of peroxisomes in muscle physiology and may lead to new therapeutic targets for the treatment of PBDs.

936C

Characterization of anti-cancer compounds targeting the polyamine transport system in imaginal discs. Minpei Wang^{1,2}, Aaron Muth³, Otto Phanstiel^{2,4}, Laurence von Kalm^{1,2}. 1) Department of Biology, University of Central Florida, Orlando, FL; 2) Biomolecular Science Center, University of Central Florida, Orlando, FL; 3) Department of Chemistry, University of Central Florida, Orlando, FL; 4) Department of Medical Education, College of Medicine, University of Central Florida, Orlando, FL.

The native polyamines, putrescine, spermidine and spermine, are ubiquitous organic polycations essential for diverse cellular functions including growth and proliferation, transcription and chromatin structure, mRNA stability, and apoptosis. Activated polyamine biosynthesis is a hallmark feature of malignant cells. Difluoromethylornithine (DFMO) is an established inhibitor of polyamine biosynthesis, however, in response to treatment with DFMO, cancer cells compensate by up-regulating import of polyamines from the extracellular environment. Thus, there is a need to develop compounds that inhibit polyamine import for use in combination therapy with DFMO. We have previously demonstrated that the leg imaginal disc has a polyamine transport system that is similar to mammalian cells. In this study, we characterize three candidate polyamine import inhibitors: Anthracen-9-ylmethyl-4,4,4-tetraamine (**Ant444**), N-(4-Amino-butyl)-N'-(3,5-bis-[[4-(4-amino-butylamino)-butylamino]-methyl]-benzyl)-butane-1,4-diamine (**Trimer44**) and Benzene-1,3,5-tricarboxylic acid tris-[[4-(4-amino-butylamino)-butyl]-amide] (**Triamide44**). We find that Ant444 and Trimer44 are twelve-fold and nine-fold more effective than spermidine, and six-fold and four-fold more effective than spermine respectively in blocking uptake of a toxic polyamine analog that utilizes the polyamine transporter to gain entry into the cell. In addition, we find that Ant444 and Trimer44 efficiently block uptake of native polyamines into DFMO treated imaginal discs. A combination treatment using Ant444 and Trimer44 is more effective than either compound alone raising the possibility of multiple transport mechanisms. In summary, our data provide support for future rational design of compounds targeting polyamine import.

937A

***Drosophila* RNAi screen to identify genetic interactors of VAP-B.** Girish S. Ratnaparkhi¹, Senthilkumar Deivasigamani¹, Hemant Verma¹, Ryu Ueda², Anuradha Ratnaparkhi³. 1) Indian Institute of Science Education & Research, Pune, Maharashtra, INDIA; 2) National Institute of Genetics, Mishima Shizuoka, JAPAN; 3) Agharkar Research Institute, Pune, Maharashtra, INDIA.

Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's disease) is a progressive neurodegenerative disorder characterized by selective death of motor neurons. Most cases of ALS occur sporadically. In a subset of the known 5-10% familial cases, the disease is inherited due to a dominant mutation. One such dominant missense mutation, P56S, was identified in Vesicle Associated Membrane Protein Associated Protein B (VAPB or VAP-33-1; Nishimura et. al, 2004, *Am. J. Hum. Genet.* 75:822). VAPB is one of at least a dozen genetic loci associated with ALS.

VAPB is an integral trans-membrane protein of the endoplasmic reticulum and is implicated in a variety of functions including lipid transport, Ephrin signaling and the unfolded protein response. VAPB (P56S) behaves in a dominant negative manner by sequestering the wild type protein into cytoplasmic inclusions.

We intend to identify all genetic interactors of VAP-B. Knowledge of the VAP-B genetic network will lay the groundwork for future experiments that will help unravel the effect of VAPB mutation(s) on cellular genetic networks. A genome wide RNAi screen in *Drosophila* has been initiated in order to identify genetic modifiers of the macrochaetae phenotype observed in flies expressing dVAPB in the *scabrous* expression domain (Ratnaparkhi et. al., 2008, *PLoS One* 4:e2334). After screening 10% of the fly genome we have identified 72 interactors of which 26 are enhancers of VAPB function while 46 are suppressors. The interactors include known ALS loci.

938B

Role of signaling pathways in Amyloid- β -dependent cell death in *Drosophila* eye. Andrew Steffensmeier², Oorvashi Roy Puli¹, Meghana Tare¹, Madhuri Kango-Singh^{1,2,3}, Amit Singh^{1,2,3}. 1) Department of Biology, University of Dayton, 300 College Park Drive, Dayton, OH, 45469; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton, OH, 45469; 3) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, 300 College Park Drive, Dayton, OH, 45469.

Alzheimer's disease (AD) is an age-related, progressive neurodegenerative disorder. The reason for Alzheimer's neuropathology is the generation of large aggregates of A β 42 that are toxic in nature, and induce oxidative stress, aberrant signaling and many other cellular alterations that trigger neuronal cell death. However, the exact mechanisms leading to cell death are not clearly understood. We employ a *Drosophila* eye model of AD to study how A β 42 causes neurodegeneration. Misexpression of higher levels of A β 42 in the differentiating photoreceptors of the fly retina rapidly induced aberrant cellular phenotypes and cell death. We found that blocking Caspase-dependent cell death initially inhibited cell death but did not lead to a significant rescue in the adult eye. However, blocking the levels of c-Jun NH (2)-terminal Kinase (JNK) signaling pathway significantly rescued the neurodegeneration phenotype of A β 42 misexpression both in the eye disc as well as the adult eye. Here we present our findings on the role of signaling pathways controlling patterning and growth in amyloid plaque mediated cell death observed in the *Drosophila* eye.

939C

The Effect of Up-regulation of SOD1 in a *Drosophila* Model of Spinocerebellar Ataxia 3 (Machado-Joseph Disease). Sallie McLean McSwain, John M Warrick. Biology Dept, University of Richmond, Richmond, VA.

Spinocerebellar ataxia 3, more commonly known as Machado-Joseph Disease (MJD), belongs to a family of dominantly inherited human diseases that induce neurodegeneration. The degeneration is caused by an expansion of a normally occurring (CAG) repeats in the coding part of the gene (*mjd1* or *atx3*). This expansion causes the Ataxin 3 protein to aggregate and become toxic to the cell by an unknown mechanism. Research has suggested that MJD may increase the amount of reactive oxygen species thus decreasing cell longevity and increasing neural death. Research has been done to show that over expression of SOD1, which naturally reduces free radicals, in a *Drosophila* motorneurons using the Gal-4/UAS system increased lifespan of the flies by up to 40%, this suggests that SOD1 and the effects of oxidative stress are important factors in aging and lifespan determination. We hypothesized that by increasing naturally occurring antioxidant gene products such as cytoplasmic SOD1, we could decrease the severity and progression of the disease. Mild, moderate, and strongly expressing UAS alleles of mutant and normal MJD with and without additional SOD1 using an additional UAS-SOD1 were expressed in the fly eye using the *gmrGal4* driver. Flies were aged for one or seven days and a western blot was run to examine the effect of additional SOD1 on the aggregation of MJD proteins. Heads of one and seven day old flies were also fixed and embedded in epon resin blocks, and sectioned using the ultramicrotome. Sections were stained and degeneration was assessed using light microscopy. Results show that flies expressing both MJD and SOD1 have increased levels of neurodegeneration. Currently we are testing to see whether the effect is additive or synergistic. Research by others has implicated superoxide down regulating the autophagy pathway, and autophagy has been suggested to reduce the degeneration caused by MJD by removing aggregates. Therefore, we propose that the increase in SOD1 levels interfered with the autophagy pathway causing the increase in degeneration.

940A

Latitudinal cline for sexual dimorphism: correlated response to mating behavior, body size variations and flight adaptations. Veer Bhan. Department of Biotechnology, UIET, M D University, Rohtak, Haryana, India.

Eight populations of *D. melanogaster* were investigated for four behavioral traits (i.e. ovariole number, fecundity, copulation duration and mating latency) and three size related traits (i.e. thorax length, wing length and wing width). Wing loading was calculated by using wing and thorax dimensions. Wing loading and size dimorphism were found to be positively correlated with each other and both were negatively correlated with the latitude of origin. Copulation duration was also found to be positively correlated with the latitude of origin. Mating latency and copulation duration were negatively correlated with each other. It is assumed that the larger females were adapted along the latitude due to fecundity advantage whereas the small size of males was due to reduced male-male competition and higher wing loading leading to larger mating latency and shorter copulation duration.

941B

Generation of a novel wing colour pattern in *Drosophila* : when engrailed crosses the line. Héloïse D. Dufour^{1,2}, Cédric Finet^{1,2}, Shigeyuki Koshikawa¹, Jane E. Selegue¹, Sean B. Carroll¹. 1) Howard Hughes Medical Institute, University of Wisconsin, Madison; 2) These authors contributed equally to this work.

Engrailed, which encodes a homeodomain transcription factor, plays a crucial role in the establishment of the posterior identity of Arthropod segments. This role has been highly constrained for the last 500 million years. There are notably only a couple of examples (in butterflies wings) where engrailed has been documented to derogate from its posterior expression, but no functional role could be attributed to this oddity. Here, we investigate the generation and evolution of a complex white and black spot pattern in the wing of the fruit fly *Samoia leonensis*. We show that the white spots correlate with engrailed pupal expression, not only in the posterior, but also in the anterior part of the wing. Earlier in development though, in the imaginal disc, the engrailed expression is restrained to its posterior canonical pattern. This suggests that the engrailed role in establishing the posterior identity is maintained, while it is later recruited to repress dark pigmentation. Transgenesis was established in this species to test this hypothesis. Furthermore, by collecting closely related species and reconstructing their phylogenetic relationships, we show that the spotty engrailed pattern likely evolved on a black wing

background, thereby providing a visible phenotype for selection to act on. Those results make an example of how, once their role in body plan establishment is accomplished, crucial developmental genes can acquire discrete new functions.

942C

The gene CG6234 is conserved outside Drosophilidae species as part of the genetic regulatory network which contributes to amnioserosa development. Christian E. Hodar^{1,2}, Veronica Cambiazo^{1,2}. 1) Universidad de Chile - INTA, Santiago, Chile; 2) Center for Genome Regulation.

Most insects possess two extra-embryonic epithelia: the amnion located ventrally and the serosa which envelops the embryo. During dipteran evolution these tissues have undergone morphological changes: in lower Cyclorrhapha the serosa covers the embryo and is dorsally fused to amnion and in higher Cyclorrhapha both are fused into one dorsal epithelium, the amnioserosa. In *Drosophila*, differentiation of amnioserosa and dorsal structures is transcriptionally regulated by a network controlled by Mad and Zen. We identified in Drosophilidae species the gene CG6234, which has a dorsal expression in early embryos that became restricted to amnioserosa during development. In *D. melanogaster*, CG6234 expression is regulated by Mad and Zen and loss-of-function of this gene results in amnioserosa alterations. CG6234 has no orthologs in any other sequenced genomes beyond Drosophilidae, but divergence in axis formation and extra-embryonic morphologies among these insects prevent us from concluding whether CG6234 is present only in Drosophilidae group or it arose with cyclorrhaphan flies as part of the genetic network that controls amnioserosa formation. In this work we attempted to identify orthologs of CG6234, Mad and Zen in *Musca domestica* and *Themira biloba*, both higher cyclorrhaphan flies. Degenerated PCR were used to clone CG6234, Mad and Zen orthologs from *M. domestica* and *T. biloba*. In situ hybridizations (ISH) were used to detect expression of transcripts. Immunofluorescence detection of phosphorylated Mad was used to reveal its active form. We identified in both species ortholog sequences for CG6234, Mad and Zen. ISH revealed that dorsal expression for these genes is conserved, suggesting that the genetic network triggered by Mad and Zen in dorsal domains of *D. melanogaster* are conserved in other cyclorrhaphan flies and CG6234 might be originated prior to *Drosophila* radiation as part of the network involved in amnioserosa formation. Fondecyt 3110129 & 1090211.

943A

Regulatory and exon-intron nucleotide sequence variability of gene *Dras1* in *Drosophila virilis* group. Prohor A. Proshakov¹, Anna I. Checunova¹, Maxim I. Barsukov¹, Larisa N. Gause¹, George N. Bachtovarov², Svetlana Yu. Sorokina¹, Vladimir G. Mitrofanov¹. 1) Dept Genetics, Koltsov Institute of Developmental Biology, RAS, Moscow, Russian Federation; 2) Mechnikov Research Institute of Vaccines and Sera, RAMS.

Ras family proteins are one of the key molecules in regulation and coordination of cell life cycle, which explains the high intensity studies of genetic determination of this family, in particular gene *ras1*. However, *ras* gene DNA sequence was not studied in insects, except for *D. melanogaster* and *B. mori*. We investigated *Dras1* regulatory and exon-intron sequences in closely related and morphologically identical species of *D. virilis* group, which can represent special interest to study the initial steps in species divergence. The PCR technique was used to research nucleotide polymorphism of DNA 8 sibling species of the *D. virilis* species group: *D. virilis*, *D. americana-americana*, *D. americana-texana*, *D. lummei*, *D. dezoana*, *D. kanekoi*, *D. laticola* and *D. montana* from collection Dept. of Genetics, Koltsov Institute of Developmental Biology. Comparative sequence analysis and phylogenetic construction was carried out. Average length of the sequences studied was approximately 2000 b.p. We found group and species-specific insertions, deletions in non-coding areas and single nucleotide polymorphism in conservative fragments, GEF-sites and promoter regions. All nucleotide changes in the studied *Drosophila* species were synonymous. This study was supported by the program of the Presidium of the Russian Academy of Sciences Biodiversity and Gene Pool Dynamics.

944B

Genetic and Functional Divergence of Y Chromosomes Over 550 Generations of Mutation Accumulation. Jun Zhou¹, Thomas Eickbush², Lene Martinsen¹, Bernardo Lemos¹, Daniel Hartl¹. 1) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Biology, University of Rochester, Rochester, NY.

The Y chromosome in *Drosophila* is gene poor and degenerate with little polymorphism in unique sequence, owing to lack of recombination, exclusive transmission through males, and reduced effective population size. It has long been thought to play little role in phenotypic variation. However, the recent discovery of Y-linked regulatory variation (YRV) that affects the level of expression of hundreds of genes across the genome suggests an unexpected functional role of the Y chromosome. Here, we studied a set of 15 mutation-accumulation Y chromosomes originating from a single Y 550 generations ago. The Y-linked rRNA gene copy numbers vary more than three-fold among these Y chromosomes. Several Y-specific microsatellites, which were previously suggested to harbor cryptic YRVs, also range up to three-fold in copy number. The divergent heterochromatic Y chromosomes showed phenotypic variation in the form of drastically different levels of suppression of position-effect variegation (PEV). The degree of suppression appears to be negatively associated with the size of the rDNA locus. Gene expression analyses revealed that hundreds of genes were differentially expressed among these Y introgression lines, presumably modulated by the Y chromosomes. Gene ontology analyses showed that many ribosomal protein genes as well as genes located at the boundary of euchromatin and heterochromatin were overrepresented in lines that had higher levels of PEV suppression. This may suggest that the Y chromosomes affect chromatin spreading leading to varying consequences for PEV suppression through epigenetic regulation. In addition, we present multiple lines of evidence for X-linked rDNA silencing in males. The suppression of X-linked rDNA expression by the Y was also shown in other geographic populations tested, and found to be independent of genetic background. Collectively, these findings suggest that the variation on the Y chromosome has important phenotypic consequences.

945C

Bacterial species associated with *Drosophila simulans* and *Drosophila melanogaster* in the wild and in the laboratory. Fabian Staubach¹, Alan O Bergland¹, Sven Kunzel², John F Baines², Dmitri A Petrov¹. 1) Biology, Stanford University, Stanford, CA; 2) Max Planck Institute for Evolutionary Biology, Ploen, Germany.

Microbes have been identified as important interaction partners of a variety of insects including aphids, ants, bee wolves and many others. These interactions range across the spectrum of possible relationships between host and microbe from mutualistic symbiosis to pathogenicity. We have initiated a study of microbial flora associated with *Drosophila*, hoping to employ the wide arsenal of genetic tools available in *D. melanogaster* to start gaining insight into the nature of bacterial communities living in association with insects. To identify key bacterial species associated with *Drosophila*, we have collected bacterial 16S 454 sequencing data from wild caught and lab-reared flies. Preliminary analysis reveals greater taxonomic diversity in the bacterial flora than previously reported. We furthermore find differences

between microbial communities from wild *D. melanogaster* and *D. simulans* pairs each collected from the same fruit, suggesting a genetic component influencing the bacterial communities.

946A

Genome scans for positive selection in African and non-African populations of *Drosophila melanogaster*. Ryuichi Sugino¹, John Pool¹, Kristian Stevens², Charis Cardeno², Marc Crepeau², Russell Corbett-Detig³, Pablo Duchen⁴, James J. Emerson⁵, David Begun², Charles Langley². 1) Laboratory of Genetics University of Wisconsin-Madison; 2) Department of Evolution and Ecology University of California, Davis; 3) Department of Organismal and Evolutionary Biology Harvard University; 4) Department of Evolutionary Biology Ludwig Maximilians University, Munich; 5) Department of Integrative Biology University of California, Berkeley.

Detecting the genetic targets of natural selection is a fundamental goal in evolutionary genetics. Population genomic data offers the opportunity to identify numerous outlier loci based on genome-wide patterns, and these loci may provide insights into the genetic basis of adaptive evolution. Here we perform three genome scans for selection on more than 100 fully sequenced genomes of *Drosophila melanogaster*, mainly from sub-Saharan (ancestral range) populations. First, we search for selective sweeps within a single population sample from Rwanda. Second, we compare variation in Rwanda to a population from France and look for loci that may have been involved in the adaptation of this species to temperate non-African environments. Finally, we identify loci that show elevated genetic differentiation among African populations that were sampled from a variety of environments. Our work contributes toward a genomic and geographic atlas of recent positive selection in the *D. melanogaster* genome.

947B

Changes in body melanisation and not body size affect mating success in *Drosophila immigrans*. SHAMA SINGH. ZOOLOGY, UNIVERSITY OF DELHI, DELHI.

We investigated effects due to changes in body size and body melanisation on mating success (mated pairs, mating latency and copulation duration) in wild flies and laboratory strains of *Drosophila immigrans*. Accordingly, we tested the hypothesis whether changes in body size or body melanisation are correlated with mating success in *D. immigrans*. A comparison of copulating and non-copulating flies of *D. immigrans* in the field showed contrasting differences due to body melanisation but not with body size. Similar results were found in copulating flies with early vs. late mating propensity. We selected isofemale lines varying either in body melanisation or in body size but not in both the traits. Laboratory data on dark vs. light isofemale lines showed significant effect of body melanisation on mating success. By contrast, small vs. large body size strains did not differ in their mating success. Further, laboratory selected dark strain showed significantly higher number of mated pairs and longer copulation duration as compared with light strain. By contrast, mating latency was longer for light strain and much shorter for dark strain. Thus, changes in mating success are associated with body melanisation and not with body size in *Drosophila immigrans*.

948C

Cytoplasmic Rbfox1/A2BP1 regulates early germline cyst development by repressing translation through a 3'UTR mediated-mechanism. Arnaldo Carreira-Rosario, Michael Buszczak. Department of Molecular Biology, University of Texas-Southwestern Medical Center, Dallas, TX.

Germline stem cells of the *Drosophila* ovary undergo an asymmetric division, giving rise to a stem cell and a cystoblast that then divides four times to produce a 16-cell cyst. Previously, we found that RNA binding Fox1 (Rbfox1)/Ataxin 2 Binding Protein 1 (A2BP1) mutant females exhibit germline cystic tumors and are sterile. The human Rbfox1 gene encodes several isoforms, many of which contain a highly conserved RNA recognition motif (RRM). Some of these isoforms localize to the nucleus and regulate tissue specific alternative splicing while others localize to the cytoplasm. The function of these cytoplasmic forms remains poorly understood. In *Drosophila* early cysts, Rbfox1 is observed in the cytoplasm and RNAi designed to specifically reduce cytoplasmic Rbfox1 phenocopies the Rbfox1 mutant phenotype. We have shown that an intact RRM is needed to rescue the Rbfox1 mutant phenotype. This motif associates with (U)GCAUG sites in a specific manner in vitro. Based on these findings and the cytoplasmic localization of Rbfox1 within early cysts, we hypothesize that Rbfox1 binds to (U)GCAUG elements in the 3'UTRs of target genes and regulates their translation. To test this, we have engineered a sensor construct that contains this element within its 3'UTR. We observed repression of this reporter exclusively in Rbfox1-positive cells. Moreover, this repression is no longer observed in Rbfox1 mutants. From these experiments we conclude that Rbfox1 can repress translation of transcripts containing (U)GCAUG elements within their 3' UTRs. Excitingly, our results suggest a novel function for an extensively studied splicing regulator.

949A

Characterization of a calcyphosine-like protein required for proper border cell migration during oogenesis. Lathiena A. Manning, Michelle Starz-Gaiano. Biological Sciences, University of Maryland Baltimore County, Baltimore, MD.

Cellular migration is a critical mechanism for many developmental processes allowing for dynamic tissue rearrangement. Often cells must travel to a satellite location to proceed with their specified task. The timing of this positional change is commonly regulated by signals received by the cell. Once the signal is accepted the cell may undergo a series of morphological alterations initiating the ability for transit. A compelling example of cellular migration occurs during *Drosophila melanogaster* oogenesis. Amid *Drosophila* oogenesis, a small population of migratory cells, referred to as border cells, detach from the anterior epithelium while remaining in a cluster and migrate posteriorly toward the oocyte. Two pathways have been shown to be essential in this process: Ecdysone, the single *Drosophila* steroid hormone, and the JAK/STAT signaling pathways. Both signals ultimately control the expression of specific genes. Using new imaging strategies, we are investigating how the domain of migratory cells is specified. Other work focuses on the role of a calcyphosine-like protein, found through transcriptional profiling assays to be downstream of both signaling cascades. The calcyphosine-like protein contains EF hand domains, which have an aspect in calcium binding activity. Using immunofluorescence and gene expression analysis, we have examined the function for this novel protein in border cell migration. RNA interference (RNAi) knock down of the gene specifically in the border cells disrupted their proper migration. We are testing the hypothesis that the calcyphosine-like protein disrupts actin dynamics preventing the cellular rearrangements needed for migration.

950B

Chk2 mutation rescues germline stem cell loss induced by DNA damage. Xing Ma^{1,2}, Ting Xie^{1,2}. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Dept of Anatomy & Cell Biology, Kansas City, KS.

All kinds of cell types are exposed to DNA damage reagents, such as reactive oxygen species generated from cellular metabolism and UV light from the sun. Like other cell types in the organism, adult stem cells must constantly contend with possible genomic insults to maintain genome integrity. Unlike other cell types in the body, adult stem cells are responsible for generating and replenishing all cell lineages in a specific tissue. Stem pool elimination by DNA damage induced cell death will lead to tissue degeneration, whereas accumulation of damaged DNA in the stem cell may result in uncontrolled proliferation, differentiation defect, or even tumorigenesis. Here we use the *Drosophila* ovarian germline stem cell (GSC) as a model to investigate how a stem cell responds to DNA damage due to the structural simplicity and the availability of a variety of molecular markers. DNA damage is induced by heatshock-induced expression of the I-CreI endonuclease, which can cleave the rDNA clusters in the X and Y chromosomes of the *Drosophila* genome to generate DNA double strand breaks. Interestingly, we found that DNA damage produces two different phenotypes: GSC loss and the accumulation of undifferentiated germline progenitors, cystoblasts (CBs). The accumulation of CBs is caused by germline cyst dedifferentiation instead of GSC or CB overproliferation. More interestingly, removing one copy of *chk2*, a check point kinase induced by DNA damage to arrest the cell cycle and facilitate DNA repair, is sufficient to rescue the GSC loss phenotype but not CB accumulation, but inactivation of both copies of *chk2* is able to rescue both GSC loss and CB accumulation. Taken together, our data show that DNA damage can drive damaged GSCs to differentiation for maintaining the overall wellness of the stem cell pool, and can also convert differentiated germline cysts back into CBs, which might provide the possibility to replenish the stem cell pool.

951C

Nuclear localization of the *Drosophila* ovo protein. Akram M. Abou-Zied. Dept. of Zoology, Faculty of Science, Suez Canal University, Ismailia, Egypt.

The *Drosophila* ovo is responsible for several aspects of germline sex dimorphism and oogenesis. At least two OVO protein isoforms are known. They form a nested pair differing in amino-terminal start points, but sharing most of their coding sequences. One isoform is a transcription repressor, while the other a transcription activator. The corresponding effector domains are located near the amino terminus of each isoform. At the common carboxy terminus, four C2-H2 zinc fingers (ZF) are located. The three tandemly arrayed fingers are well-conserved through evolution. The fourth finger is less well-conserved and is separated from the upstream triplet by a 10-aa-long "linker" composed largely of lysine and arginine residues. The "linker" resembles canonical unipartite nuclear localization signals (NLS). We have engineered expression vectors joining green fluorescent protein (GFP) to wild-type and deletion derivatives of the OVO protein coding region. When the GFP::OVO ZF is expressed in genetically transformed *Drosophila* larval salivary glands, green fluorescence is nuclear-localized. To hasten the pace of discovery, we used transient transfection of *Drosophila* S2 cultured cells. In this assay, a GFP::OVO fusion protein containing the putative NLS and ZF4 was not directed into nuclei, but rather into punctate cytoplasmic aggregates. In contrast, GFP::OVO fusion proteins containing the entire ZF1-ZF3 array were efficiently nuclear-localized. A series of deletion constructs were then made to determine the minimal region essential for nuclear localization. The data allow the following conclusions: First, OVO protein nuclear localization is due to sequences associated with DNA-binding. Second, ZF2 is sufficient to drive GFP fusion protein into nuclei. Third, the N-terminal region, 80% of the overall length of the protein, lacks NLS activity. Finally, the highly basic region between ZF3 and ZF4, which had been postulated as a potential NLS, is insufficient by itself to achieve nuclear localization.

952A

Independent Evolution of a Reproductive Trait Through Distinct Developmental Mechanisms in *Drosophila*. Delbert A. Green¹, Cassandra Extavour². 1) Molecular and Cellular Biology, Harvard University, Cambridge, MA; 2) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

All insect ovaries are partitioned into egg-producing substructures called ovarioles. In *Drosophila* ovariole number is positively correlated with egg production rate, making it a strong determinant of reproductive capacity. Ovariole number is species-specific, shows high evolutionary lability, and is largely under genetic control. Thus, ovariole number determination is an attractive model to study the developmental genetic mechanisms underlying a rapidly evolving trait with direct impact on fitness. We previously showed that the number of a specific ovarian cell type, terminal filament (TF) cells, determines ovariole number. Here we show that in closely related *Drosophila* lineages, ovariole number is modulated by different developmental mechanisms, which are likely to have different underlying genetic bases. Specifically, in these lineages, reduction in ovariole number occurs primarily through two different developmental mechanisms: 1) reduced number of somatic gonad precursors (SGPs) specified in embryogenesis and 2) different somatic cell morphogenesis and differentiation in larval life. Previous quantitative genetic analyses and ovary development studies suggest the Insulin receptor (*InR*) and *bric-à-brac* (*bab*) genes as candidates for loci of evolutionary change in ovariole number. We present results of our experiments analyzing the effect of reduced function of *InR* and *bab2* on SGP number and ovarian morphogenesis. We discuss these results in the context of the possibility that evolution can produce similar ovariole numbers through distinct developmental mechanisms, which may in turn proceed through different genetic mechanisms.

953B

Smt3 Posttranslational Modifications Mediate Critical Regulatory Events during *Drosophila* Spermiogenesis and Spermatogenesis. Janet E. Rollins^{1,2}, Keumil Hwang², Patricia Morris^{2,3}. 1) Natural Sci, Col Mt Saint Vincent, Riverdale, NY; 2) Center for Biomedical Research, Population Council, New York, N.Y.; 3) The Rockefeller University, New York, N.Y.

Drosophila spermatogenesis is a dramatic, temporally-orchestrated developmental stage-specific process. Sperm production includes marked changes in mitosis and meiosis, chromosomes, transcription, translation, and posttranslational modifications, with striking nuclear remodeling during spermiogenesis. The posttranslational small ubiquitin-like modifier (SUMO) protein has been shown to play diverse roles in many highly conserved cellular processes such as spermatogenesis in various species including man and rodents. The purpose of this study was to define the precise stage-specific timing of fly Smt3 (*Drosophila*SUMO)-mediated events during germ cell development, determine whether Smt3-deficiency affects sperm production, and initially identify Smt3-modified proteins for comparison with those during mammalian spermatogenesis. For bioimaging, unconjugated Smt3 and Smt3-modified proteins were detected by immunofluorescence of testis from wild-type and heterozygous Smt3 stocks. SUMOylated proteins were determined by immunoprecipitation (IP) and Western blot analyses. Male fertility of fly mutants was assessed. In wild-type flies, SUMOylated proteins show

strikingly different patterns in most stages of spermatogenesis. The testes of heterozygotes showed reduced levels of Smt3 and an altered SUMOylated protein profile compared to wild-type. Interestingly, the reduction of Smt3 signals was readily observed in meiotic spermatocytes; no change for mitotic spermatogonia was apparent. Heterozygote males exhibited a reduced fertility and their testes show a marked defect in sperm transfer to the seminal vesicles. Our data are suggestive that precise timing of SUMOylation events in developing fly germ cells is required for normal spermatogenesis and sperm transfer, findings consistent with marked reduction in fertility. Taken together, our results indicate important roles for Smt3 and SUMOylation during and after meiosis in *Drosophila* testis.

954C

The antiviral RNAi pathway interactome in *Drosophila Melanogaster*. Carine Meignin¹, Karim Majzoub¹, Yann Verdier², Joëlle Vinh², Jean-Luc Imler¹. 1) CNRS-UPR9022, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France; 2) Laboratory of Biological Mass Spectrometry and Proteomics (SMBP), CNRS USR3149, ESPCI ParisTech, Paris, France.

In *Drosophila melanogaster*, RNA interference (RNAi) is a major defense mechanism against viral infections. Flies that lack one of the three core components of RNAi (Dicer-2, R2D2 or Argonaute-2) show increased susceptibility compared to wild-type flies when infected. The general RNA silencing mechanism in drosophila comprises three pathways (siRNA, miRNA and piRNA) that share common features: involvement of evolutionary conserved protein families (e.g. Argonautes), strong specificity dictated by base-pairing and negative effect on gene expression. Only few studies attempted to unveil the mechanism by which the siRNA pathway targets the viral genomes and limits the viral load. Moreover, the interacting partners of Dcr-2, R2D2 and AGO2 in a viral infection context remain unknown. For instance, Dcr-2 is involved in Vago induction independently of AGO2 and R2D2; on the other hand Loquacious is required for white Inverted Repeat silencing but not for antiviral silencing. We defined the Dcr-2, R2D2 and AGO2 interactome in non-infected cells or cells challenged with different viruses with biotin-tagged versions of these proteins in stable cell lines. Cells were infected with different viruses, and the purified complexes were subjected to a LC MS/MS analysis in order to identify interacting partners. We identified 134 proteins interacting with one or more core component of the siRNA pathway. Interestingly, about a third of the interacting proteins are specifically recruited to the complexes during viral infection. We confirmed that several identified partners contribute to the control of viral replication in tissue culture cells.

955A

Cholera toxin disrupts exocyst-mediated trafficking to intestinal cell junctions. Annabel E. Guichard¹, Beatriz Cruz-Moreno¹, Nina M. Van Sorge^{2,5}, Guillaume P. Pineton de Chambrun³, Declan Mc. Cole³, Victor Nizet^{2,4}, Bier Ethan¹. 1) Dept Biol, Univ California, San Diego, La Jolla, CA, USA; 2) Dept of Pediatrics, Univ California, San Diego, La Jolla, CA USA; 3) Dept of Medicine, Univ of California, San Diego, La Jolla, CA USA; 4) Skaggs School of Pharmacy & Pharmaceutical Sciences, Univ of California, San Diego, La Jolla, CA USA; 5) Medical Microbiology, University Medical Center Utrecht, Heidelberglaan, The Netherlands.

Ctx, a toxin produced by *vibrio cholerae*, can cause the life-threatening diarrhea associated with cholera. After entering intestinal host cells, Ctx activates endogenous adenylate cyclases to elevate cAMP levels. One well-characterized effect of Ctx on intestinal epithelial cells is to trigger Cl⁻ ion efflux through luminal CFTR channels. Cl⁻ secretion is accompanied by Na⁺ and water efflux via the paracellular route to preserve electroneutrality and osmotic balance. Here, we report that Ctx, also disrupts epithelial cell-cell junctions. We first show that expression of CtxA in *Drosophila* epithelial cells inhibits Notch signaling and disrupts membrane trafficking of the Notch ligand Delta and DE-cadherin to adherens junctions (AJs) through inhibition of the small GTPase Rab11 and its binding partner Sec15, a key component of the exocyst. Similarly, in human intestinal epithelial cell lines, Ctx treatment causes mislocalization of endocytic trafficking components (Rab11 and Sec15), disorganization of AJs (E-Cadherin) and tight junctions (TJ): ZO-1, Claudin-2, uncoupled alignment of AJs and TJs, and reduced Notch signaling. Finally, in ligated murine ileal loops, Ctx strongly reduces Rab11 and Sec15 expression and produces apical gaps between epithelial cells. We discuss how this novel activity of Ctx may contribute to the pathophysiology of cholera.

956B

Toxicity response of *Drosophila melanogaster* larvae to *Pseudomonas fluorescens* Pf-5. Kristin L. Latham¹, Amy Nicholson¹, Jenna Schneider¹, Adam Pettitt¹, Patricia Flatt². 1) Biology, Western Oregon Univ, Monmouth, OR; 2) Chemistry, Western Oregon Univ, Monmouth, OR.

Drosophila melanogaster is a useful model organism for examining host-pathogen interactions and immune system response. While immune response to bacteria has been well-studied in late larvae and adults, little is known about response to bacterial exposure during early larval stages. We have exposed first- and second-instar larvae to *Pseudomonas fluorescens* strain Pf-5 by a non-invasive feeding procedure. Larvae fed *P. fluorescens* show dose-dependent differences in time to metamorphosis and survival. Interestingly, larvae fed Pf-5 cultured in different types of bacterial media show distinct differences in growth rate and phenotype, suggesting that this bacterial strain produces metabolites that vary with culture media, with differences in insecticidal properties. *P. fluorescens* Pf-5 is known to produce potent antibiotic compounds such as 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin, and pyoluteorin; we are currently investigating whether differential metabolites can be isolated from Pf-5 grown in different media. These data suggest that toxic response in *Drosophila* larvae elicited by Pf-5 bacteria is dependent not just on bacterial dose but also on which bacterial metabolites are present.

957C

A novel PGRP-LC splice variant encodes a putative negative regulator of the imd pathway. Claudine Neyen, Bruno Lemaitre. Global Health Institute, EPFL, Lausanne, Switzerland.

Drosophila immunity against Gram negative pathogens relies on Peptidoglycan Recognition Proteins (PGRPs) which encompass secreted amidases, capable of degrading bacterial peptidoglycan (PGN), and membrane-bound receptors, ready to signal upon PGN sensing. PGRP-LC, a transmembrane signalling PGRP, activates the imd pathway upon recognition of PGN, leading to antimicrobial peptide release. The PGRP-LC locus encodes a variety of receptor isoforms based on alternative splicing of PGRP-domain-encoding exons to a common intracellular signalling domain. The ligand specificity of each isoform and the signalling functionalities of the shared cytoplasmic tail have been well studied. Recent RNAseq data identified an additional exon encoding an alternative intracellular domain, which generates a complementary subset of isoforms with a distinct cytoplasmic tail. Here we describe a novel function associated with intracellular domain variants of PGRP-LC (rPGRP-LC). Overexpression of rPGRP-LC reduces the imd response to Gram negative challenge, indicating a regulatory role for rPGRP-LC. While activating PGRP-LC is known to localize to the plasma membrane, rPGRP-LC resides on intracellular vesicles carrying early

endosome markers. Upon infection, a portion of activating PGRP-LC relocated to this compartment. *In silico* analysis of the rPGRP-LC cytoplasmic tail shows a conserved RHIM domain, as well as a RING/FYVE/PHD domain, reminiscent of ubiquitin E3 ligases. We therefore hypothesized that rPGRP-LC might interact with ligand-bound, internalized PGRP-LC at the level of early endosomes and downregulate imd signalling through ubiquitination. Ubiquitin-tagged receptors are generally shuttled into multivesicular bodies (MVBs) by the ESCRT machinery, which shuts off receptor-mediated signalling. Interfering with the endocytic machinery at the level of early endosomes, ESCRT, or MVB-to-lysosome traffic resulted in enhanced imd pathway signalling upon infection, tentatively confirming this mechanism. Studies are currently underway to characterize the putative ubiquitination of PGRP-LC.

958A

Increased immunoreactivity in the aged *Drosophila* Malpighian Tubule. Ana M. Hernandez¹, Florentina Rus², Neal Silverman², Marc Tatar¹. 1) Ecology and Evolutionary biology, Brown University, Providence, RI; 2) Division of Infectious Disease, Department of Medicine, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA.

Senescence of the immune system is a hallmark of aging in many living organisms including humans. Disregulation of the immune response contributes to both the onset and continuation of several diseases in the elderly. The *Drosophila* immune system mimics molecular and functional aspects of the human innate immune system, and so it is an ideal model to study aging-related changes in immunity. *Ex vivo* culture of the *Drosophila* malpighian tubule (MT) provides an isolated system to dissect the different pathways playing a role in immunosenescence. Our studies show that the aging malpighian tubule is hyper-responsive to immune challenges. This response is dependent on the NF- κ -B homolog Relish. However, the aged malpighian tubule is incapable of controlling bacterial growth. Possible underlying mechanisms include alterations in protein translation, stability, or secretion, and studies are underway to elucidate between these possibilities.

959B

Behavioral analysis for different sugars in *Drosophila* larvae with respect to survival, choice and learning. Johanna E. Pfitzenmaier^{1,2}, Astrid Rohwedder^{1,2}, Noel Ramsperger¹, Anthi Apostolopoulou^{1,2}, Annekathrin Widmann^{1,2}, Andreas S. Thum^{1,2}. 1) Department of Biology, University of Konstanz, 78457 Konstanz, Germany; 2) Department of Biology, University of Fribourg, CH-1700 Fribourg, Switzerland.

By comparing the *Drosophila* olfactory and gustatory system, it was suggested that the taste system has a lower dimensionality and might be designed to classify substances into a handful of basic classes, such as 'edible' and 'nonedible' (Gerber and Stocker 2007; Schipanski et al. 2008). However, this picture appears rather incomplete, given the fact that substances like sugars not only differ in taste but also in their nutritional value. Recent studies have shown that adult *Drosophila* can detect and remember the caloric content of sugars regardless of the respective taste (Burke and Waddell 2011; Dus et al. 2011; Fujita and Tanimura 2011). We analyzed whether such a nutrition based system exists, by investigating how different sugars affect larval survival, choice and olfactory learning. In an experimental setup, similar to that of Schipanski and colleagues (2008), we applied metabolisable (fructose, sucrose, glucose, sorbitol, maltodextrin; defined as G1) and non-metabolisable (arabinose, sucralose, xylose; defined as G2) sugars at concentrations of 0.1M, 1M, 2M and 4M. We found that larvae raised on pure agarose as well as on agarose containing G2 sugars were reduced in lifespan compared to larvae that were fed on agarose containing G1 sugars. Interestingly, all sugars induced larval choice behavior, though in a dose-dependent way, as well as larval appetitive olfactory memory, irrespective of taste or nutritional benefit. Therefore, we argue that the reinforcing function of a particular sugar is not based on its nutritional benefit only but is rather a part of a complex multi-stimulus system. Taken together, given the lack of information on the basic organization of the external and internal sensory neurons that detect sugar, our data provide further understanding of how sugar information is processed in the *Drosophila* larva.

960C

***longitudinals lacking* cooperates with *fruitless* in generating sexual differences in neuronal structures and behavior.** Kosei Sato, Gakuta Toba, Masayuki Koganezawa, Daisuke Yamamoto. Tohoku University, Sendai, Miyagi, Japan.

The *Drosophila* *fruitless* (*fru*) gene encodes a set of putative transcription factors that play a central role in male courtship behavior through their organizing actions on the sexually dimorphic neuronal circuitry. However, the mechanism whereby *fru* establishes the sexual fate of neurons has been an enigma. By modifier screening in the compound eye, we identified a loss-of-function allele of *longitudinals lacking* (*lola*) as a suppressor of the *fru*-induced rough-eye phenotype. Phenotypically, *lola* and *fru* mutant alleles synergistically enhanced male-to-male courtship. *lola* also interacted with *fru* in neural development: mAL, a sexually dimorphic interneuron group, was feminized by *fru* mutations and this effect was dominantly enhanced by *lola*. Our MARCM analysis on mAL revealed that different *lola* isoforms have distinct functions in neural sexual differentiation. Immunoprecipitates of *Drosophila* CNS extracts with an anti-Fru antibody contained *lola*, suggesting that Fru forms a complex with *lola* *in vivo*. Fru might acquire its ability to orchestrate sexual development of the entire brain by forming complexes with hundreds of *lola* isoforms each with potentially different target specificity.

961A

The *Drosophila* high-conductance Na⁺-activated K⁺ channel. Gonzalo Budelli, Alice Butler, Lawrence Salkoff. Dept. of Anatomy and Neurobiology, Washington University in St. Louis, Saint Louis, MO.

The sodium dependent potassium (KNa) current is one of the largest components of the delayed outward current in many mammalian neurons under physiological conditions (Budelli *et al.*). Sodium activated potassium currents were also observed in *Drosophila* (Saito *et al.*). One gene underlying this current, *SLO2.2* (Slack), was cloned from rat and functionally expressed in our lab (Yuan *et al.*). Because of its prominence and importance in mammals, we are studying it in *Drosophila* which offers unique genetic and molecular tools. Thus, we have cloned and functionally expressed the *SLO2* gene from *Drosophila* (*dSLO2*, CG42732). Rat *SLO2.2* and *dSLO2* have 65% amino acid identity through the region including membrane spanning domains. Using inside-out patches we determined that the channels are conserved with respect to many properties including their sodium sensitivity (Kd \approx 50mM), single channel conductance ($g \approx$ 80ps in 80mM symmetrical K), chloride dependence, weak voltage dependence and inactivation by intracellular divalent cations. Previous work from our lab and others showed that the mammalian KNa channels are controlled by neuromodulators through G-protein coupled receptors (Santi *et al.*, Nuwer *et al.*). We are investigating whether similar neuromodulation is conserved in the *Drosophila* orthologous channel. The neuromodulation of KNa channels and their weak voltage dependence which permits their opening over a wide range of voltages, make these channels important candidates to activate or repress electrical activity in neurons and to play a significant role in plasticity by modulating cell electrical

excitability. References Budelli et al. Nat Neurosci. 2009 Jun;12(6):745-50. Saito M, Wu CF. J Neurosci. 1991 Jul;11(7):2135-50. Yuan et al. Neuron. 2003 Mar 6;37(5):765-73. Santi et al. J Neurosci. 2006 May 10;26(19):5059-68. Nuwer et al. J Neurosci 2010 Oct 20;30(42):14165-72. Supported by NIH.

962B

An actin rich mechanosensory organelle in *Drosophila* somatosensory neurons. Asako Tsubouchi, Jason Caldwell, W. Daniel Tracey. Anesthesiology, DUKE University, Durham, NC.

To sense mechanical stimuli is important for life. In *Drosophila*, two types of sensory neurons (ciliated and non-ciliated) have been found to play a role in neurosensory mechanotransduction. However it is still largely unknown how these sensory neurons convert external stimuli to neuronal electrical signals. Here, we show that *Drosophila* non-ciliated multidendritic neurons contribute to the larval sense of touch. Based on dendritic complexity, the md-da neurons are classified onto class I to class IV. It is known that class IV neurons are receptors for mechanical stimulation (nociception). Here, we report that class III md-da neurons are required for behavioral responses to gentle touch. Class III neurons are characterized by actin-rich spike like protrusions that we term mechanopodia. We show that overexpression of Rac1 in class III neurons, dramatically increased the number of mechanopodia and also resulted in a hypersensitive behavioral response to touch. In contrast, both expression of dominant negative form of Rac1 caused a decrease in the number of mechanopodia of class III neurons. These larvae showed a touch insensitive phenotype. Our results indicate the number of mechanopodia on class III neurons is correlated with the sensitivity of behavioral responses to touch. In addition, we performed a tissue specific RNAi screen to hunt for genes required in Class III neurons for gentle touch responses. We have identified a set of ion channel genes that knockdown to an insensitive touch phenotype. Some of these ion channels are also required for the formation of mechanopodia. Characterization of genetic mutants for the genes identified with RNAi is currently under way. An interesting possibility is that sensory mechanisms of mechanopodia may be relevant to the mechanisms mechanotransduction of the actin rich stereovilli of hair cells or somatosensory neurons of the skin.

963C

Two odor receptors contribute distinct and complex signals in response to structurally similar odor molecules. Scott A. Kreher, Raquel Robles, Michael Wesolowski. Dominican University, River Forest, IL.

The sense of smell is crucial for animals to find food, avoid predators, and to find potential mates. Understanding the sense of smell is a fundamental question in neuroscience, and it is especially worthwhile to investigate the basis of olfaction in insects, since pest insects use their sense of smell to find crops and blood-feeding insects use their sense of smell to find hosts. The first step in sensing an odor is the interaction of an odor molecule with a specific odor receptor protein. In previous research, we electrophysiologically characterized the entire repertoire of odor receptors in the fruit fly (*Drosophila melanogaster*) larva and characterized the behavior of larvae in response to these odors. We found that only two odor receptors, *Or42a* and *Or42b*, responded electrophysiologically to the odor, ethyl acetate. In a subsequent examination of the behavioral responses of mutants for either *Or42a* or *Or42b* to ethyl acetate, we found that each receptor contributed to the behavioral response in a predictable manner: mutants of the high-affinity receptor *Or42b* were not attracted to low concentrations of ethyl acetate and mutants of the low-affinity receptor *Or42a* were not attracted to high concentrations of ethyl acetate. We extended this analysis to examine how *Or42a* and *Or42b* mutants behaviorally responded to three odor molecules that are structurally similar to ethyl acetate. We found that the odor receptor mutations differentially affected behavioral responses to these three odors, and that the responses were not fully predictable from the electrophysiological data set. In two cases, although the odors elicited electrophysiological responses from both *Or42a* and *Or42b*, only one odor receptor mutant displayed altered behavioral responses, which were typically loss of attraction phenotypes. These data taken together suggest the importance of sensory neuron context in odor receptor function. To further address this question, we are ectopically expressing odor receptors in non-native olfactory sensory neurons to examine how the sensory neuron ensemble codes signals.

964A

Identification of Cell Recognition Molecules controlling Mechanosensory Neuron Wiring in the *Drosophila* CNS. Milan Petrovic, Dan Dascenco, Olivier Urwyler, Dietmar Schmucker. VIB, KU Leuven, Leuven, Belgium.

The striking specificity by which neuronal connections are formed represents one of the most intriguing processes in the field of developmental neurobiology. Cellular recognition mediated by cell-surface proteins plays a pivotal role during the targeting of axons and dendrites and subsequent localization of the prospective synaptic connections. To identify novel cell recognition molecules that mediate synaptic specificity, we are conducting a systematic RNAi-based screen in a subset of adult *Drosophila* mechanosensory neurons. The mechanosensory neurons of *Drosophila* that are associated with distinct sensory bristles on the notum project into the CNS and elaborate a complex but invariant axonal branching pattern, in which the single axonal branches bear most of the synaptic connections. We are focusing on a pair of mechanosensory neurons, the posterior Scutellar central (pSc) and the posterior Dorsal central (pDc) neurons, that project together into the CNS but branch differently and target overlapping as well as distinct target regions. To analyze the functional requirement of the cell recognition molecules, genetically encoded axonal and synaptic markers are selectively expressed in a small subset of mechanosensory neurons to visualize the branching pattern and the sites of synaptic connections with target interneurons in the CNS. In addition we are generating MARCM-based single cell clones to determine the precise axonal and synaptic phenotypes caused by the RNAi mediated gene knockdown. Strategies for the labeling and genetic manipulation of the mechanosensory postsynaptic neurons in the CNS will be presented.

965B

A set of reagents for addressing neuroblast hemilineages in the adult VNC. Robin Harris, Barret Pfeiffer, Gerald Rubin, James Truman. Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA.

One of the major challenges in understanding neural circuits is breaking the nervous system into manageable yet biologically relevant units. A plausible grouping system is neurons that are developmentally related, i.e. neurons that come from the same neuronal stem cell (neuroblast). Neurons that are active in the larva (primaries) are born during an embryonic wave of neurogenesis, and these are highly diverse within and between lineages. However, a much larger set of adult-specific/secondary neurons is produced during larval life. For this set, neurons within a hemilineage are much more homogeneous: they form discrete clusters within the adult CNS, and often share similar morphology and gene expression patterns. It therefore seems reasonable to view secondary hemilineages as meaningful neural classes.

MARCM is a powerful technique for accessing neural lineages, but as a stochastic marker, it cannot target lineages repeatably or in multiple segments simultaneously. To overcome such limitations, we have generated a set of lines targeting genetic access to the 34 secondary hemilineages of the *Drosophila* ventral nerve cord (VNC). We have selected GAL4 lines with expression in immature hemilineages in the wandering third instar larva. We use nSyb-GAL80, which is expressed in all mature neurons, to suppress GAL4 expression in non-target (primary) neurons. We then use a hormone-inducible UAS-flippase to excise a stop cassette from a constitutive, non-GAL80-suppressible driver or reporter construct, allowing us to immortalize the larval expression pattern with temporal specificity. These lines can be used both to explore properties of each hemilineage as a unit, and to subdivide broad LexA or QF expression patterns into clusters of developmentally related cells. As a demonstration, we have used these lines to cleanly target subclasses of fru-expressing neurons in the VNC.

966C

Descriptive Genetic and Genomic Analysis of Post-Embryonic Neurogenesis. Haluk Lacin, Elizabeth Cozart, Yi Zhi, Beth Wilson, James Skeath. Dept. of Genetics, Washington University in St Louis, St Louis, MO.

Pioneering studies that used the *Drosophila* embryonic CNS as a model system have unveiled many of the basic mechanisms that govern neurogenesis in invertebrates and vertebrates. Much less, however, is known about the genetic and molecular control of post-embryonic (PE) neurogenesis. While lineage studies have mapped all post-embryonic lineages found in the nerve cord, lack of systematic efforts to identify molecular markers that unambiguously label individual lineages and the genes that regulate the development of these lineages hinder our understanding of PE neurogenesis. Here, we describe our efforts to build a molecular marker map of post-embryonic lineages and to identify the genes that regulate PE neurogenesis. We have identified 13 transcription factors (TFs) that collectively label 22 out of the 25 neuronal lineages in thoracic segments of the larval nerve cord. These markers allow one to identify each lineage based on molecular criteria alone. We find that most TFs label multiple lineages and that within each lineage a TF typically labels neurons in one of the two hemilineages. For example, Hb9 labels the B hemilineage of three defined PE lineages. The identification of molecular markers that label individual PE lineages provided a simple assay to screen for mutations that regulate neurogenesis. Thus, we undertook a classical forward genetic screen to identify mutations that alter the development of Hb9+ PE lineages. Our screen of over 2000 EMS mutagenized second chromosomes identified over 20 mutations that alter Hb9 expression. Whole genome sequencing-based methods together with classical genetic tests have enabled us to identify the causative lesion in five of the six backgrounds sequenced to date, with ten more lineages presently being sequenced. One of these genes encodes a GPCR-like protein. Loss of function in this gene causes an apparent specific block in PE neurogenesis in all lineages. Our future work will focus on the characterizing these genes and identifying molecular nature of the remaining genes.

967A

Identification of localized mRNAs in *Drosophila* sensory neurons using a novel genetic screen. Mala Misra¹, Janet A. Tambasco¹, Marissa A. Schlueter¹, Ida L. Barlow¹, Nair JayaNandanan^{2,3}, Maria Leptin^{2,3}, Elizabeth R. Gavis¹. 1) Molecular Biology, Princeton University, Princeton, NJ; 2) EMBL, Heidelberg, Germany; 3) Institute of Genetics, University of Cologne, Cologne, Germany.

Neurons are among the most extreme examples of cellular polarization, having three functionally and morphologically distinct subcellular domains: dendrites, axon, and soma. Like many polarized cell types, neurons use mRNA localization and local translation to spatially and temporally regulate protein expression within these domains. Until now, technical limitations precluded a large scale effort to identify the full repertoire of localized mRNAs in neurons and to visualize their localization patterns. To this end, we have designed a novel screen combining Gal4/UAS-driven transgene expression and MS2-based fluorescence labeling of mRNAs. Modified EP transposons encoding upstream activating sequences (UAS) and MS2 RNA stem loops are introduced at random sites in the *Drosophila* genome. Expression of Gal4 induces transcription at these sites, yielding an MS2-tagged transcript. MS2 Coat Protein fused with RFP binds the MS2 stem loops, allowing in vivo tracking of tagged transcripts. In a pilot screen of 112 lines carrying EP-MS2 insertions, 28 mRNAs were localized to the processes of larval class IV dendritic arborization (da) neurons. Preliminary analysis showed that in 60% of lines, EP-MS2 transposons incorporated in or within 1kb of known genes, suggesting that many MS2-tagged mRNAs reflect the behaviors of endogenous transcripts. One positive candidate encoded Coracle, a cytoskeletal binding protein and septate junction component. Knockdown of coracle in class IV da neurons decreased dendritic branching, suggesting a role in dendritic morphogenesis. Based on the success of the pilot screen, we have collaborated with other research groups at the University of Cologne and Oxford University to generate a library of 1500 EP-MS2 lines. We are presently screening this library to identify other localized mRNAs in *Drosophila* sensory neurons.

968B

The T-box transcription factor midline collaborates with the insulin-regulated dFOXO transcription factor to regulate cell-fate specification in the developing eye of *Drosophila melanogaster*. Sudeshna Das¹, Deepak Kumar¹, Yan Zong², Brandon Drescher¹, Sarah Morgan², Sandra Leal¹. 1) Biological Sciences, University Of Southern Mississippi, Hattiesburg, MS; 2) School of polymers and high performance materials, University Of Southern Mississippi, Hattiesburg, MS.

The *Drosophila* midline (mid) gene encodes a highly conserved invertebrate ortholog of the mammalian Tbx20 transcription factor gene family and regulates critical aspects of embryonic central nervous system (CNS) development. Embryos homozygous mutant for mid exhibit severe CNS defects due to the misspecification of neuronal subtypes and axon guidance defects within the ventral nerve cord (VNC). To understand the molecular-genetic mechanisms by which mid regulates neuronal specification and axon guidance, it is essential to decode the complex mid transcriptional networks that mediate these functionally integrated processes. For this reason, we are using the *Drosophila* eye as a practical model system to combine a classical genetic modifier screen with both RNA interference (RNAi) and the UAS-Gal4 expression system for the identification of mid-interacting genes. We then determine whether mid-interacting genes guide the differentiation of neurons within the embryonic CNS. We now report that specifically reducing mid expression in the larval imaginal eye disc using RNAi results in significantly fewer interommatidial bristles within the adult eye. These results suggest that mid functions as a cell-fate determinant during the pupal stage of disc morphogenesis, when specialized accessory cells are recruited to surround an R1-R8 photoreceptor neuron cluster. Results from the genetic modifier screen also show that mid collaborates, either directly or indirectly, with the transcription factor dFOXO to regulate interommatidial formation, placing mid downstream of insulin-stimulated signaling pathways that regulate cell growth, metabolism and survival. We are currently examining whether dFOXO also interacts with mid to regulate distinct aspects of embryonic CNS development including cell fate specification and axon guidance.

969C

A NetLogo model of Notch-Delta interactions in the determination of the neural-epidermal lineages. Elaine R. Reynolds¹, Christopher Sanginiti¹, Jeffrey Pfaffmann². 1) Neuroscience Program, Lafayette Col, Easton, PA; 2) Dept of Computer Science, Lafayette Col, Easton PA.

The ligands *Notch* (*N*) and *Delta* (*DI*) are the key signaling molecules involved in the determination of a neural vs. epidermal lineage in the embryonic mesectoderm. *N* and *DI* are expressed initially in all mesectoderm cells, but through a heterodimeric interaction on adjacent cell surfaces, *N* expression becomes high in some cells, inducing an epidermal fate. This process yields a reproducible geometry and consistent number of neural and epidermal cells. We built a NetLogo agent-based model of the interaction with the goal of exploring the factors involved in the stable determination of fate. The model replicates each step within the cell, beginning with the transcription of *N* and *DI*, their transport to a simulated cell surface, and their lateral diffusion across the membrane. When *N* contacts *DI* on an adjacent cell, it is cleaved and transports back to the nucleus, altering *N* and *DI* transcription rates. In programming the model, parameters are initially set, but the response is stochastically varied. Implementations of the model produce fate determination that is robust and stable. We have set up experiments that explore the roles of initial transcriptional rates, processing of *DI* and protect of *N* laterally at the cell surface, processing of *N* after cleavage, and *N* regulation of its own transcription and that of *DI*. For each experiment, we use the development of a stable and accurate pattern of transcription to measure how each variable modulates fate determination. In general, results indicate that initial transcription rates can be varied and a stable pattern is still obtained. However, *DI* processing, autoregulation of *N* transcription, and the timing of *N* transport to the nucleus do affect the model's ability to obtain a stable and correct pattern of fate determination.

970A

Cell type-specific role of ecdysone signal in the optic lobe cell death and development in *Drosophila*. Yusuke Hara^{1,2}, Yu Togane^{1,2}, Hiromi Akagawa^{1,2}, Tatsuya Sudo¹, Ayano Ishitsuka¹, Masashi Iwamura¹, Rie Ayukawa¹, Keiichiro Hirai¹, Kengo Beppu¹, Takashi Takahashi¹, Kikuo Iwabuchi², Hidenobu Tsujimura¹. 1) Developmental Biology, Tokyo Univ of Agriculture and Technology, Tokyo, Japan; 2) Dept of Biological Production Science, Tokyo Univ of Agriculture and Technology, Tokyo Japan.

The *Drosophila* optic lobe develops during metamorphosis. Enormous cells die in the early half of the metamorphosis in the optic lobe. Here, we show that ecdysone regulates the cell death and the development of the optic lobe in cell type-specific manner. Knockdown of *Ftz-f1* resulted in a reduction in the number of dying cells. *Ftz-f1* is a nuclear receptor expressing at mid-prepupa and provides competence to respond to ecdysone signal after pupation. This suggests that ecdysone after pupation is required for the optic lobe cell death. Then, we examined the expression of EcR, a component of the ecdysone receptor, in the developing optic lobe. EcR has three isoforms, EcR-A, B1 and B2. In neurons, EcR-A and EcR-B1 start to express at prepupal stage and the expression gets strong as the optic lobe development proceeds. In contrast, no expression was observed in the neuroblasts and neuro-epithelium. In glia, expression of the both isoforms was different depending on the types of glia. Knockdown of *EcR* in neurons resulted in the reduction in the number of dying cells, suggesting EcR expressed in neurons promotes the cell death. The knockdown caused developmental disorder of the optic lobe: smaller and mis-arranged neuropiles, and prolonged persistence of neuroblasts. In contrast, knockdown of *EcR* in glia resulted in a great increase of the number of dying cells, suggesting that EcR expressed in glia is required for the optic lobe cell survival. Knockdown in glia also caused an aberrant distribution of glia and the mis-arrangement of the neuropiles.

Together, these results suggest that ecdysone signaling exerts different effects on the different tissues and cell types. The optic lobe cell death and the development may be regulated via combination of these effects.

971B

Role of Piccolo homolog Fife in synapse assembly and function. Sam Galindo¹, Joseph Bruckner², Scott Gratz¹, Jessica Slind¹, Richard Geske¹, Kate O'Connor-Giles^{1,2}. 1) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Laboratory of Cell and Molecular Biology, University of Wisconsin-Madison, Madison, WI.

Neurotransmitter release occurs at specific presynaptic sites known as active zones. Active zones comprise the exocytic machinery and a group of proteins referred to as the cytomatrix of the active zone (CAZ) that are thought to coordinate the organization of presynaptic terminals. Characterization of CAZ proteins is critical to uncovering the mechanisms that regulate synaptic architecture and function. Vertebrate CAZ proteins include Piccolo, Bassoon, RIM1, RIM2, Unc-13 and CAST. Invertebrates share a CAST ortholog (Bruchpilot in *Drosophila*), Unc-13 (*Dunc-13*) and a single RIM protein (*DmRIM*). However, Piccolo and Bassoon have not previously been identified in invertebrates. Through a genetic screen for regulators of neural function we have identified a gene, previously misannotated as three genes, that encodes a protein with homology to Piccolo and RIM proteins. We conducted molecular phylogenetic analyses and found that Piccolo and RIM diverged from a common ancestor and the newly identified protein, which we have named Fife, is more related to Piccolo, while the previously identified *DmRIM* encodes the sole *Drosophila* RIM homolog. Because piccolo has long been believed absent from invertebrate genomes, we extended our analysis to other invertebrate genomes and identified piccolo-related genes in multiple species. Together, our analyses demonstrate that piccolo is conserved between vertebrates and invertebrates and suggest greater conservation of the molecular architecture of synapses than previously believed. Piccolo has been implicated in major depressive and bipolar disorders. However, studies of Piccolo function in mammalian systems have yielded conflicting results, raising questions about the endogenous role of Piccolo that may be more effectively addressed in a simpler model system. We will present the results of our ongoing studies into the in vivo role of Fife in synapse assembly and neural function.

972C

Akt1 regulates GluRIIA localization and subsynaptic reticulum expansion in *Drosophila* neuromuscular junction. Hyun-Gwan Lee, Na Zhao, Scott B. Selleck. Biochemistry and Molecular Biology, The Penn State University, University Park, PA.

The Akt family of serine-threonine kinases integrates a myriad of signals governing cell proliferation, apoptosis, glucose metabolism and cytoskeletal organization (Manning and Cantley, 2007). Akt can also affect neuronal morphology and function, influencing dendrite growth and the expression of ion channels. Moreover, Akt is known to influence components of the TSC/Rheb/TOR signaling pathway, a system where misregulation leads to neurological and behavioral deficits, including tuberous sclerosis complex. To explore the function of Akt in synapse assembly and function we have examined its role in the development of the *Drosophila* neuromuscular junction, a glutamatergic synapse that displays developmental and activity-dependent plasticity. The single *Drosophila* Akt family member, *Akt1* selectively affected the postsynaptic targeting of one glutamate receptor subunit, GluRIIA and was required for the normal expansion of a specialized postsynaptic membrane compartment, the subsynaptic reticulum (Jia et al., 1993). Knockdown of *Akt1* also produced defects in muscle

membrane organization indicative of a deficit in exocytosis previously described for larvae bearing mutations in Gtaxin, a *Drosophila* t-SNARE (Gorczyca et al., 2007). Reduction of *Akt1* function resulted in loss of Gtaxin at the SSR, and expression of a constitutively active *Akt1* produced ectopic membrane structures and increased Gtaxin levels in the muscle cell. Reductions in *Akt1* function in the postsynaptic cell also altered the levels and distribution of Dorsal and Cactus, two downstream targets of Akt that affect glutamate receptor localization at the NMJ (Heckscher et al., 2007). Our findings show that Akt1 governs two critical elements of synapse development, neurotransmitter receptor localization and postsynaptic membrane elaboration.

973A

The T-box transcription factor *midline* defines the posterior limit of dorsal anterior fates in the *Drosophila* follicular epithelium. Fiona Hails, Mariana Fregoso Lomas, Laura Nilson. Biology, McGill, Montreal, Quebec.

The *Drosophila* eggshell is distinct in both shape and structure along the anterior-posterior and dorsal-ventral axes. These asymmetries reflect the patterning of cell fates in the follicular epithelium, a tissue that surrounds the germline and secretes the eggshell at the end of oogenesis. During mid-oogenesis, activation of the Epidermal growth factor receptor (Egfr) by the secreted ligand Gurken which is localised to the dorsal anterior of the underlying oocyte, specifies a stereotyped pattern of dorsal anterior follicle cell fates. Graded Egfr activation can account for the ventral limit of these fates, but how the posterior limit is established is not understood. Previous work has shown that activation of the Egfr pathway can only induce dorsal fates in the anterior follicle cells, suggesting that cells in the posterior are not competent to acquire dorsal anterior fates even in the presence of Egfr signaling. However little is known about how this competence is determined. Recently, we have shown that loss of function of the *midline* gene leads to determination of ectopic dorsal anterior fates in the dorsal posterior domain. We hypothesize that *midline* establishes the posterior limit of dorsal anterior fates in the follicular epithelium by modulating the cellular response to Egfr signaling. To test this idea, we generated double mutant clones of *midline* and components of the Egfr pathway in the follicular epithelium using the FLP-FRT mediated mitotic recombination system. In clones mutant for *midline* and *ras*, a downstream effector of Egfr signaling, endogenous and ectopic dorsal anterior fates are lost, indicating that the ectopic dorsal fates in *midline* mutants are dependent on Egfr signaling. In clones mutant for both *midline* and *sprouty*, a negative regulator of the Egfr pathway, dorsal anterior cell fates expand further into the posterior than in *midline* single mutants. These data suggest that *midline* does not modulate the level of Egfr signaling but instead prevents dorsal anterior fates in the posterior of the tissue by making posterior cells refractory to Egfr signaling.

974B

The *Drosophila* Hox gene Deformed (Dfd/Hoxb4-d4) modulates cell adhesion within the eye-antennal imaginal disc. Marie-Anaïs Tiberghien¹, Magali Suzanne¹, David Cribbs², Corinne Benassayag¹. 1) Université Paul Sabatier, LBCMCP UMR 5088, Toulouse, France; 2) Université Paul Sabatier, CBD UMR 5547, Toulouse, France.

Most of the *Drosophila* adult head, including the eyes, ocelli, antennae and maxillary palps, derives from the composite eye-antennal imaginal disc. The antennal part of this complex rudiment is composed by two distinct cell populations: the antenna (Ant) and the maxillary (Mx) territories which must segregate to give rise to the two corresponding adult olfactory organs. We found that the Mx territory is detectable by expression of the Hox gene Deformed (Dfd/ Hoxb4-d4) and is separated by lineage restriction from cells of the Ant territory. Dfd is required for Mx morphogenesis since loss of Dfd function in the eye-antennal disc induce loss of adult Mx organs. Firstly, we have shown that differential expression of Dfd modulates cell properties leading to a cell-sorting phenotype *in vivo*. Indeed, cellular clones harbouring Dfd misexpression show rapid cell shape remodeling accompanied by clone extrusion from the epithelium and by considerable reorganization as seen by cell polarity markers. Secondly, we have shown that Dfd induces cell-sorting by repressing a component of the adherens junctions: DE-Cadherin (DE-Cad). This adhesion molecule is differentially accumulated in loss or gain of Dfd function clones. Moreover, removing both Dfd and DE-Cad from the eye-antennal disc induces a partial rescue of Dfd-dependent adult Mx defects further indicating that DE-Cad is a Dfd effector during adult Mx morphogenesis. Taking together, these data demonstrate that Dfd regulates cell affinity by a direct or indirect repression of the DE-Cad and this is required for Mx differentiation. Such Dfd dependent regulation of cell adhesion might play a key role in segregating Mx cells (expressing Dfd) from the Ant cells (non expressing Dfd) within the antennal disc during Mx development. This novel function of a Hox gene in cell affinity and segregation offers an enticing model for studying cellular Hox functions during morphogenesis.

975C

A screen for genes regulating repatterning in regenerating wing imaginal discs. Keaton J Schuster, Andrea Skinner, Rachel K Smith-Bolton. Cell & Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL.

Drosophila imaginal discs are known to regenerate after fragmentation and implantation into a female host. However, this experimental method is impractical for large-scale genetic screens. Our lab has developed a method to induce tissue ablation in the wing imaginal disc *in vivo* in a spatially and temporally controlled manner. This ablation protocol allows for a rapid, efficient and thus powerful approach to screen for genes involved in regeneration. Regeneration in imaginal discs involves wound healing, blastema formation and outgrowth, and repatterning of the regenerating disc. Proper repatterning is essential for the regenerate to form the correct, functional structure. Using isogenic deficiencies to screen for regeneration-specific patterning mutants, we have found two independent mutant phenotypes with malformed wings. Regenerated wings heterozygous for one deficiency have an aberrant shape. Regenerated wings heterozygous for a second deficiency have an improperly patterned L5 vein. We are currently characterizing these phenotypes and mapping the responsible genes. In conclusion, this study will give insight on how an appendage is able to correctly re-establish and/or maintain proper patterning during the regenerative response in *Drosophila* imaginal discs.

976A

Transcriptional Control of Xenobiotic Detoxification in *Drosophila*. Jyoti R. Misra, Mike A. Horner, Geanette Lam, Carl S. Thummel. Dept Human Gen, Univ Utah, Salt Lake City, UT.

Xenobiotic compounds such as pollutants, pesticides and other foreign chemicals pose a constant threat to the survival of all organisms. To overcome this, animals mount an elaborate transcriptional response, regulating a battery of genes that encode enzymes involved in detoxification of these compounds. Several transcription factors have been identified in vertebrates that contribute to this regulatory response. In contrast, little is known about this pathway in insects. We show that the *Drosophila* Nrf2 ortholog, CncC, is a central regulator of

xenobiotic detoxification responses. A binding site for CncC and its heterodimer partner Maf is sufficient and necessary for robust transcriptional responses to three xenobiotic compounds, phenobarbital (PB), chlorpromazine, and caffeine. Genetic manipulations that alter the levels of CncC, or its negative regulator Keap1, lead to predictable changes in xenobiotic-inducible gene expression. Transcriptional profiling studies reveal that more than half of the genes regulated by PB are also controlled by CncC. Consistent with these effects on detoxification gene expression, activation of the CncC/Keap1 pathway in *Drosophila* is sufficient to confer resistance to the pesticide malathion. These studies establish a molecular mechanism for the regulation of xenobiotic detoxification in *Drosophila* and have important implications for controlling insect populations and the spread of insect-borne human diseases.

977B

CDK8-Cyclin C negatively regulates SREBP-dependent lipogenesis. Xiao-Jun Xie¹, Qun Wang¹, Lu-Ping Liu², Eun Joo Kim¹, Yani Zheng¹, Jian-Quan Ni², Jun-Yuan Ji¹. 1) Department of Molecular and Cellular Medicine, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843, USA; 2) School of Medicine, Tsinghua University, Beijing 100084, China.

Aberrant fatty acid biosynthesis represents a universal feature of human cancers, and is linked to metabolic diseases such as diabetes, obesity and cardiovascular diseases. Despite substantial efforts, the molecular mechanisms that maintain the lipid homeostasis in multicellular organisms remain poorly understood. We use *Drosophila* as a model to study the in vivo functions of Cyclin-dependent kinase 8 (CDK8) and its regulatory partner Cyclin C (CycC), two highly conserved subunits of Mediator complexes. We found that CDK8-CycC is a novel factor that plays an important role in regulating lipid metabolism. Specifically, we observed that CDK8 and CycC mutants accumulate more fat and the expression of lipogenic genes are significantly increased. Consistent with this, knocking down CDK8 or CycC in fat body leads to increased fat accumulation. To explore the underlying mechanisms, the genome-wide gene expression patterns of these two mutants were profiled. Compared to the control, a large number of genes involved in fatty acid biosynthesis are up-regulated in the mutants. Interestingly, mammalian homologs of many of these genes are the targets of sterol regulatory element binding proteins (SREBPs), a family of master transcription factors that plays a key role in regulating lipid homeostasis. Thus we postulated that CDK8 and CycC could inhibit the expression of lipogenic genes by repressing SREBP-mediated transcription. Consistent with this hypothesis, knocking down both CDK8 (or CycC) and dSREBP in fat body results in a significant reduction of neutral lipid levels compared to knocking down CDK8 (or CycC) alone. Together, these observations suggest that CDK8-CycC plays an important role in inhibiting lipogenesis by repressing SREBP-dependent transcription.

978C

The enlarged crop phenotype observed in *drop-dead* mutants does not correlate with starvation. Christine Sansone, Edward Blumenthal. Biological Sci, Marquette Univ, Milwaukee, WI.

Flies mutant for the gene *drop-dead* (*drd*) were originally isolated because of their shortened lifespan. Further examination of the mutant flies revealed diverse phenotypes including digestive system dysfunction, neurodegeneration, tracheal defects, small body size, and female infertility. Without the phenotypes being clearly connected, the function of the gene and the cause of early lethality cannot be determined. To study the various phenotypes in isolation, we utilized the UAS-Gal4 system to drive tissue specific knockdown of *drd*. Three different Gal4 lines recapitulate the early lethality phenotype when *drd* is knocked down: *breathless*, *DJ717*, and *DJ626*. Our interest is primarily concerned with the digestive system defects observed in these three tissue specific knockdown lines. In *drd* mutants, the crop, a food storage organ where minimal digestion occurs, becomes enlarged. Enlarged crops are only temporarily observed in wild type flies, after they have been starved and then are reintroduced to food. The enlarged crop phenotype was observed when knocking down *drd* in the *breathless* and *DJ717* patterns, but not with *DJ626*. The enlarged crop phenotype suggests *drd* mutants are starving due to an inability to properly process food. Consistent with this hypothesis, *drd* mutants have depleted triglyceride and glycogen levels. Examination of triglyceride and glycogen levels revealed that knockdown in the *DJ717* and *DJ626* patterns caused starvation. However, knockdown in the *breathless* pattern did not cause a significant difference in the triglyceride and glycogen levels. Therefore, by employing the UAS-Gal4 system, we have demonstrated that the enlarged crop and starvation phenotype are independent. Furthermore, we provide evidence that there are at least two different causes of death in *drd* mutant flies.

979A

Expression and characterization of *Drosophila* signal peptide peptidase-like (*sppl*), a gene that encodes an intramembrane protease. David J. Casso^{1,2}, Songmei Liu^{2,3}, Brian Biels², Katja Brückner¹, Thomas B. Kornberg^{2,3}. 1) Department of Cell and Tissue Biology, Univ California, San Francisco, San Francisco, CA; 2) Department of Biochemistry and Biophysics, Univ California, San Francisco, San Francisco, CA; 3) Cardiovascular Research Institute, Univ California, San Francisco, San Francisco, CA.

Intramembrane proteases of the Signal Peptide Peptidase (SPP) family play important roles in developmental, metabolic and signaling pathways. Whereas vertebrates have one SPP and four SPP-like (SPPL) genes, we found that insect genomes encode one Spp and just one Sppl. Characterization of the *Drosophila* *sppl* gene using BLASTP and CLUSTAL W revealed that the predicted Sppl protein is a highly conserved structural homolog of the vertebrate SPPL3 proteases with a predicted nine-transmembrane topology, an active site containing aspartyl residues within a transmembrane region, and a carboxy-terminal PAL domain. Expression analysis identified developmental and subcellular differences between *spp* and *sppl*, suggesting distinct functions: *spp* expression was detectable from early embryo to larval stages and Spp protein localized to the ER, while *sppl* was detectable in the early embryo but not thereafter and Sppl protein localized to both the Golgi and ER. Surprisingly, whereas *spp* is an essential gene required during early larval stages, *sppl* loss of function alleles showed no apparent phenotype either alone or in combination with *spp*. This was unexpected given that genetic knock-down phenotypes in other organisms suggested significant roles for Spp-related proteases.

980B

Transcriptional Twister: characterizing the plasticity of a bipartite dTCF binding motif. Hilary Cara Archbold¹, Kenneth M Cadigan^{1,2}. 1) Cellular and Molecular Biology Program, University of Michigan, Ann Arbor, MI; 2) Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI.

In *Drosophila*, TCF (also known as pangolin) acts as a major transcriptional regulator of Wingless (Wg) signaling in multiple stage and tissue specific events. The ability of TCF to bind the correct regulatory elements is critical for proper spatio-temporal expression of target genes. How TCF mediates such a broad range of outcomes is poorly understood. TCF contains a HMG domain which binds the consensus

sequence SSTTTGWW. Previous research in our lab identified a second sequence GCCGCCR (the “Helper site”) which is indispensable for activation of multiple Wg targets. Our data support a model where TCF binds HMG and Helper site pairs, via two closely spaced domains, the HMG and the C-Clamp. Surprisingly, spacing and orientation of these two motifs varies both within and between Wingless Response Elements (WREs). My research plan is focused on understanding how motif architecture acts to regulate TCF binding and/or activity at these cis-regulatory sequences, and using this information to identify new WREs and target genes. Using a luciferase reporter gene system in *Drosophila* Kc cell culture, we have shown that in both synthetic and known target gene WREs, the optimal spacing of the Helper site is orientation specific, is relative to the HMG domain imposed bend of DNA, and can be located either up or downstream of the HMG site. We are currently investigating the correlation between activation levels and binding affinity using EMSA, and are using a site-specific integration strategy to characterize reporter gene expression level and patterns driven by optimal and suboptimal motifs in transgenic *Drosophila*. In addition, using our preliminary knowledge concerning optimal motif configuration, we have identified several novel candidate WREs which will be tested for Wg dependent activity in cell culture and in transgenic *Drosophila*.

981C

Identification of cis-regulatory elements at the endogenous apterous locus. Dimitri C. Bieli¹, Oguz Kanca¹, Martin Müller¹, Daryl Gohl², Paul Schedl², Markus Affolter¹. 1) Biozentrum, University of Basel, Basel, Switzerland; 2) Princeton University Department of Molecular Biology.

The imaginal discs of *Drosophila melanogaster* are an excellent model system to study organogenesis. In order to form a proper adult body, patterning and growth control of the imaginal discs must be tightly regulated. In the case of the wing disc, the tissue is subdivided into different compartments, anterior (A) and posterior (P) as well as dorsal (D) and ventral (V). Those compartments and especially the boundaries between them play a crucial role in setting up the organizers which pattern the tissue. While the subdivision into A and P compartments is determined already during embryogenesis, the subdivision into D and V compartments proceeds during larval development while the tissue is growing. Thereby, the selector gene *apterous* (*ap*) plays a crucial role as it establishes the D/V compartment boundary. This compartmentalization is a prerequisite to set up the wingless organizer, which subsequently patterns the wing disc along the D/V axis. This study aims to identify the mechanisms of transcriptional regulation of *ap* in the growing wing disc. To identify the cis-regulatory elements involved in this process, we used several genetic approaches. First, deletions with defined breakpoints in the *ap* genomic locus were generated and the resulting phenotypes analyzed. Second, we performed a comparative enhancer study by fusing several fragments from the *ap* locus to a reporter gene and tested their ability to drive expression in the D compartment of the wing disc. Third, an *in situ* rescue system was engineered which can be used to investigate the rescue activity of enhancer sub-fragments at the endogenous *ap* locus. Furthermore, we characterized several classical *ap* alleles at the molecular level. By combining all those approaches, we identified an enhancer region of 2.2 kb which is essential for the expression of *ap* in the D compartment of the wing disc. This fragment will now be used to uncover the regulatory input that confers the proper expression of *ap*.

982A

Two-level developmental gene regulatory model to incorporate cis-regulatory and temporal information. Jacqueline M. Dresch¹, Rupinder Sayal², Chichia Chiu¹, David N. Arnosti². 1) Mathematics, Michigan State University, East Lansing, MI; 2) Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI.

High-throughput genome sequencing and transcriptome analysis have provided researchers with a quantitative basis for detailed modeling of gene expression using a wide variety of mathematical models. Modeling of eukaryotic gene regulation has focused on either time-dependent interactions of gene networks or equilibrium approaches that can explore the cis-regulatory grammar of transcriptional enhancers. These models rely on systems of differential equations or thermodynamic descriptions, which can be used either to understand dynamics of a system, or DNA-level regulatory processes. To combine the strengths of each of these approaches, we have developed a novel model that joins these methods to provide a dynamical description of gene regulatory systems, using detailed DNA-based information, as well as spatial transcription factor concentration data. Our ‘two-layer’ modeling approach uses a thermodynamic model as the synthesis term in our differential equation, the term that represents the rate of mRNA production. The differential equation incorporates all other terms, representing decay and diffusion of mRNA, thus the model does not lose its effectiveness in predicting emerging spatial expression patterns over time. By incorporating data on DNA sequence, we are able to successfully model context-specific features of enhancers, as well as replicate the dynamic expression of a simple *Drosophila* gene regulatory circuit that drives development in the dorsal-ventral axis of the blastoderm embryo, involving Dorsal, Twist, Snail, and rhomboid. Where protein and cis-regulatory information is available, our two-layer model provides a powerful method to recapitulate and predict dynamic aspects of eukaryotic transcriptional systems that will greatly improve understanding of gene regulation at a global level.

983B

Regulation of *Sex combs reduced* within the transverse row bristle primordia of legs in the first thoracic segment of *Drosophila melanogaster*. Emily R. Wyskiel, Christopher L. McCallough. Biological Sciences, University of Illinois at Chicago, Chicago, IL.

The *Drosophila* adult has one pair of legs on each of its three thoracic segments (T1-T3). Although these structures exhibit serial homology, the legs from different segments have distinct morphological features. One such feature is the patterning of the peripheral nervous system in the form of small mechanosensory bristles (mCs). In the T2 leg these mCs are organized into a series of longitudinal rows (L-rows) along the circumference of the tibia and tarsal segments. However, at specific positions along the circumference and proximal/distal axis of the T1 leg, the L-rows are replaced by a group of mCs organized into transverse rows (T-rows). Studies have indicated that the position of T-row bristles on the tibia and basitarsus of T1 legs is established as a result of Hox gene modification of the L-row patterning pathway. In T1 prepupal legs, *Scr* is expressed at elevated levels within the T-row primordia. We have found that *Scr* modifies the mC pattern on T1 legs via repression of *Delta*, a key regulator of leg mC patterning. Our model for T-row patterning suggests that a central step in this process is establishment of spatially defined *Scr* expression within defined domains of the leg primordium in response to the global regulators of leg development. The mechanisms that generate morphological diversity among the legs will therefore require an understanding of the regulation of *Scr* in the T-row primordium. Here we will present our genetic and molecular studies on the regulation of *Scr* by genes known to pattern the leg along its circumference and P/D axis.

984C

Exploring the role of the C-clamp in TCF mediated Wnt/ β -catenin signaling. Aditi Ravindranath, Ken Cadigan. Dept. of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI.

The Wnt/ β -catenin pathway is a highly conserved cell-cell signaling pathway that plays a critical role in development and tissue homeostasis and is misregulated in many human disorders. In *Drosophila*, the TCF/Pangolin (TCF/Pan) protein is the key transcription factor in the Wingless (Wg) pathway. TCF/Pan is thought of as a transcriptional switch: repressing and activating Wg target genes in the absence and presence of Wg/Wnt signaling, respectively. TCF/Pan function is dependent on its ability to recognize cis-regulatory modules known as Wnt response elements (WREs). Several WREs contain binding sites for the well-characterized HMG domain of TCF/Pan, but also contain another DNA motif known as the Helper site, which is bound by a cysteine-rich region, termed the C-clamp, located just C-terminal to the HMG domain in TCF/Pan. WRE reporters containing Helper sites require the C-clamp of TCF/Pan for activation by Wg signaling (Chang et al. *Curr. Biol.* 18: 1877). A major question that remains is how important is the C-clamp for TCF function and hence Wg/Wnt signaling in vivo? Preliminary results in fly embryos indicate that the C-clamp is required for Wg dependent patterning of the embryonic epidermis. Molecular readouts of Wg signaling will also be examined. In addition, a structure-function analysis of the C-clamp of TCF/Pan is being carried out, to better understand which residues are required for TCF/Pan's DNA binding in vitro and its transcriptional activity function in vivo.

985A

Corepressor Preferences and Distinct Chromatin Features induced by Hairy Transcriptional Repressor in the *Drosophila* Embryo. KURTULUS KOK¹, LI LI², DAVID ARNOSTI¹. 1) GENETICS, MSU, EAST LANSING, MI; 2) MOLECULAR AND CELL BIOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY, CA.

Transcriptional repressors appear to fall into two categories based on their ranges of action, short-range and long-range. In short-range repression, a repressor works over distances of less than 100 base pairs to block the function of nearby bound activators but not interfering with more distantly bound activators. In contrast, long-range repressors can work over distances of more than 1kb to make a promoter resistant to the influence of all enhancers. This kind of repression is often referred as silencing since it may cause dominant inactivation of entire chromosomal locus. Their differential actions have been attributed to the distinct cofactors they recruit but recent studies showed that these repressors recruit common corepressors such as Groucho and dCtBP. These corepressors seem to act in a context-dependent manner and are likely to utilize different mechanisms of repression. We have used Hairy as model long-range repressor and Knirps as model short-range repressor determine the different effects on chromatin structure, and activator and basal transcriptional machinery recruitment. Hence, we are interested in how and why particular cofactors are recruited by a repressor to silence genes in different contexts. To address these questions, we are misexpressing mutant versions of Hairy that lack specific motifs required for cofactor interaction. Through these studies, we aim to get distinct phenotypes in repression ability, chromatin modification and compaction status, activator and basal transcriptional machinery recruitment, indicating which of these features are influenced by particular cofactors. Genome-wide characterization of chromatin changes and cofactor preferences mediated by Hairy will help us elucidate the repression mechanisms in *Drosophila*, and more generally, pathways of transcriptional repression in metazoans.

986B

Transcriptome-wide profiling implicates *Drosophila* Muscleblind in mRNA subcellular localization. Neal A.L. Cody^{1,2}, Eric T. Wang^{3,4}, Daniel Treacy³, Thomas T. Wang³, Christopher B. Burge^{3,4}, Eric Lécluyer^{1,2}. 1) RNA Biology, Institute de Recherches Cliniques de Montréal, Montréal, QC; 2) Department of Biochemistry, Université de Montréal, Montréal, QC; 3) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA; 4) Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA.

Myotonic dystrophy type 1 (DM1) is a neuromuscular disease involving the expansion of CUG repeats in the 3'UTR of the DM protein kinase (*DMPK*) mRNA in humans. Expansion containing transcripts are retained in subnuclear foci and exert a toxic RNA gain-of-function effect leading to the sequestration of several RNA binding proteins in the nucleus of affected cells, in particular, members of the Muscleblind-like (MBNL) family of proteins. This mechanism is primarily thought to perturb alternative splicing events regulated by MBNLs, although previous studies have also implicated these factors in mRNA localization. Recent work in the Burge lab at MIT demonstrated that MBNLs regulate subcellular trafficking of a large number of mRNAs in mammals. In a collaborative effort with this group, we assessed whether this property is conserved for the founding member of this protein family, *Drosophila* Muscleblind (Mbl). Biochemical fractionation was used to isolate RNA populations from cytosolic, membrane, and insoluble fractions of control S2R+ cells or cells depleted for Mbl by RNA interference. RNA deep sequencing analysis to compare fraction profiles between Mbl knockdown and control cells indicate that RNAs enriched for Mbl consensus binding sites (UGCU) in their 3'UTRs, exhibit broad displacement from membrane towards insoluble cellular compartments. Similar alterations in RNA localization patterns were observed in mouse C2C12 myoblast cells depleted for MBNL1-2. These results indicate that this family of RNA binding proteins has an evolutionarily conserved role in the control of mRNA localization, which may lead to novel insights in our understanding of the molecular defects leading to DM1.

987C

***bcd* mRNA localizes by random transport and cortical anchoring at stage 9 of oogenesis.** Vítor Trovisco¹, Katsiaryna Belaya¹, Liz Gavis², Daniel St Johnston¹. 1) Gurdon Institute, The University of Cambridge, Cambridge, Cambridgeshire, UK; 2) Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.

Localization of *bicoid* (*bcd*) and *oskar* (*osk*) mRNAs to opposite poles of the *Drosophila* oocyte (anterior and posterior, respectively) defines the anteroposterior body axis. Previous studies have shown that *osk* mRNA is transported to the posterior by Kinesin-1 along a weakly polarized microtubule (MT) network, in which the plus-ends are biased towards the posterior. Here we analyse *bcd* mRNA transport and localization at stage 9 of oogenesis using a similar approach of direct labelling and live imaging. *bcd* mRNA (*bcdMS2**MCP-GFP) is also actively transported in stage 9 oocytes but, unlike *osk*, does not show an obvious directional bias. These results suggest that *bcd* mRNA may be localized by random transport and anterior capture. We tested whether *bcd* mRNA is anchored at the anterior by photoconverting localized *bcdMS2**MCP-Dendra. A considerable amount of mRNA was found to remain at the same anterior location after one hour. Furthermore, *bcd* mRNA remains anteriorly localized after depolymerisation of microtubules. This suggests that *bcd* mRNA is not localized by continual directed transport at stage 9, but by anchoring at the anterior cortex. In agreement with previous studies, we found that *bcd* mRNA is mainly transported by Dynein. The average speed of *bcd* mRNA particles is similar to the speed described for Dynein (~1 μ m/s) and is significantly reduced in *Dhc64C* hypomorphs. However, *bcd* transport seems to rely on an interplay between Dynein and

Kinesin-1, as *bcd* speed is also reduced in *Khc* null mutants. In conclusion, at stage 9 of oogenesis, *bcd* mRNA is randomly transported by Dynein along an unpolarized MT network until it reaches the anterior cortex, where it is anchored.

988A

Ecdysone Regulation of Stem Cell Maintenance in the *Drosophila* Testis Niche. Yijie Li, Qing Ma, Erika Matunis*. Cell Dept, Johns Hopkins Medical Inst, Baltimore, MD.

While local signals are known to regulate stem cells, the roles of systemic signals in stem cell function are largely uncharacterized. In *Drosophila*, the steroid hormone ecdysone is known to be involved in embryogenesis, larval molting and metamorphosis. Relatively little is known about the function of ecdysone in adults, especially in males, however it has been found that ecdysone is implicated in diverse aspects of male reproduction including male spermiogenesis. Previous work in our lab has shown that the Nucleosome Remodeling Factor (NURF) complex is required cell-autonomously for the maintenance of both germline stem cells (GSCs) and cyst stem cells (CySCs) in the testis. Nurf301, a unique component of the NURF complex has recently been shown to interact with the ecdysone pathway during development. This prompted us to investigate the role of ecdysone signaling in the maintenance of stem cells as well as its interaction with NURF complexes in the *Drosophila* testis. We find that the ecdysone signaling pathway is required for GSC and CySC maintenance. Several ecdysone pathway members like ecdysone receptor (ECR), Ultraspiracle (USP) and some downstream targets of ecdysone signal pathways are expressed in the testis niche. By RNA interference and mosaic analysis, we find that ECR, USP and some downstream genes are required cell-autonomously in both GSCs and CySCs. RNAi interference in the cyst cell lineage in the heterozygous *nurf301* background enhances the phenotype, suggesting there may be a genetic interaction between Nurf301 and the ecdysone pathway.

989B

Escort stem cell as germline stem cell differentiation niche in *Drosophila* ovary. Su Wang^{1,2}, Daniel Kirilly¹, Ting Xie^{1,2}. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Dept of Anatomy & Cell Biology, Kansas City, KS.

Generally speaking, stem cells will balance self-renewal and differentiation by both niche signals and intrinsic factors in a variety of systems. In the *Drosophila* ovary, germline stem cells (GSC) locate in the niche at the anterior part of the ovary. GSCs will self-renew and continuously generate differentiated germ cells which will leave GSC niche and interact with another kind of somatic cell, escort cells (ECs). The escort stem cell (ESC) hypothesis has been proposed that ESCs are responsible for the ECs generation. However, it still remains unclear how ECs interact with differentiated germ cells. In this study, we found that lost ECs are repopulated by its neighbors rather than via a stem cell system. On the other hand, ECs will form long cellular processes to encase the differentiated germ cells. The ECs' long cellular processes are disrupted in the tumor germlaria whose germ cells fail to differentiate, which indicates that the germ cell differentiation is required for ECs long cellular processes formation. While to knock-down Rho will result in the failure of ECs long cellular processes formation, which leads to the accumulation of undifferentiated sing germ cells by increasing BMP signaling outside the GC niche. Our research indicates that ECs will interact with differentiated germ cells physically with their long cellular processes and regulate the germ cell differentiation. Therefore, we regard the self-maintained ECs as the differentiation niche of the early differentiated germ cells in the *Drosophila* ovary.

990C

Hippo Pathway effectors Yorkie and Scalloped are required for proper maintenance of hematopoietic progenitors in the larval lymph gland. Gabriel B Ferguson¹, Julian A Martinez-Agosto^{1,2}. 1) Molecular Biology Institute, UCLA, Los Angeles, CA; 2) Department of Human Genetics, UCLA, Los Angeles, CA.

Hematopoiesis in *Drosophila melanogaster* takes place in two stage-specific waves of development, one embryonic and the other during larval stages. Definitive hematopoiesis takes place in the larval lymph gland, an organ that contains a distinct population of slow cycling, niche-dependent stem-like progenitors that give rise to all mature hematopoietic cell types (hemocytes). Maintenance of these progenitors is dependent on highly conserved signaling pathways such as Notch, Wingless/Wnt, and Hedgehog. We have also identified the Hippo pathway as a novel regulator of hematopoiesis. In mammals, Hippo signaling has been shown to help maintain pluripotency in mESCs, regulate organ size, and act as a tumor suppressor pathway as it negatively regulates the oncoprotein YAP-1 via phosphorylation. In *Drosophila* this pathway is highly conserved and acts by inhibiting the homolog of YAP-1, the transactivator protein Yorkie (Yki). Here, we show that Yki and its binding partner, the transcription factor Scalloped (Sd), are developmentally regulated in the lymph gland. Yki is observed throughout the progenitor cell population of the lymph gland while these cells undergo rapid expansion. However, as the progenitors become quiescent in later stages of development, Yki is limited to a small population of intermediate progenitor cells. Conversely, Sd is not robustly expressed until the onset of differentiation and is observed most prevalently among differentiating cells. Furthermore, manipulation of Hippo Pathway Signaling in the lymph gland by RNA interference and overexpression yields significant defects in both maintenance and differentiation of hematopoietic progenitors. These results demonstrate a role for the Hippo pathway within the lymph gland in regulating the proper development and differentiation of hematopoietic stem-like progenitors in *Drosophila*.

991A

Tis11 mediated mRNA degradation regulates Intestinal Stem Cell Quiescence. Lindy A McClelland¹, Heinrich Jasper², Benoît Biteau². 1) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY; 2) Department of Biology, University of Rochester, Rochester, NY.

Maintaining tissue homeostasis is essential to the health of the organism. Multipotent stem cells contribute to tissue homeostasis by giving rise to the functional cells of the tissue as they are needed. This is achieved by coordinating tissue needs with stem cell proliferation rates. Proliferation rates that exceed the demands of the tissue result in dysplasia, while insufficient proliferation rates fail to replenish the functional cells of the tissue. In *Drosophila*, multipotent intestinal stem cells (ISC) respond to many stressors by increasing their proliferation rates. This ensures the proper regeneration of the functional cells damaged by stress. Proliferation rates return to basal levels after the stress has been eliminated, thus preventing overgrowth and maintaining the homeostatic balance. How this is mechanistically achieved remains unclear. Here we show that Tis11, a post-transcriptional regulator of gene expression, plays a role in discontinuing the regenerative program during the recovery from stress. Tis11 targets specific mRNAs for degradation by binding to an adenine-uridine rich element present in the transcript's 3'UTR. By limiting the availability of specific effector proteins, Tis11 is a potentially powerful regulator of the signaling pathways that control ISC proliferation rates. We show that Tis11 is specifically expressed in ISCs and the undifferentiated precursors of the intestinal epithelium. We also show that the overexpression of Tis11 is sufficient to inhibit proliferation. Inhibition of

Tis11 affects the restoration of proliferation rates to basal levels post stress. Our findings establish a role for Tis11 in maintaining tissue homeostasis by terminating the regenerative mode of proliferation. Currently, we are working to identify the molecular targets of Tis11, thus, providing insight into which signaling pathway(s) are involved in coordinating ISC proliferation rates with the needs of the tissue.

992B

Exploring the role of Upd ligands in the adult *Drosophila* midgut. Dani Osman¹, Nicolas Buchon¹, Sveta Chakrabarti¹, Yu-Ting Huang², Yu-Ting Chiu², Mickaël Poidevin³, Yu-Chen Tsai², Bruno Lemaitre¹. 1) GHI, EPFL, Lausanne, Switzerland; 2) Dep. of Life Science, Tunghai University, Taichung, Taiwan; 3) CGM, CNRS, Gif-sur-Yvette, France.

The Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway plays critical roles in development and homeostasis from *Drosophila* to mammals. In particular, it is required in *Drosophila* to promote Intestinal Stem Cells (ISCs) division and differentiation, leading to the regeneration of the adult midgut. While many cytokines can activate the JAK/STAT pathway in mammals, only three ligands called Unpaired (Upd1, Upd2, and Upd3) are known to bind to the receptor Domeless (Dome) in *Drosophila*. Our study aimed to characterize the respective role of the Upd ligands in the midgut. First, we monitored their expression pattern along the midgut cell. While upd1 is mainly expressed in ISCs, upd2 seems to be expressed in enteroblasts and upd3 in enterocytes. Second, using newly generated up specific mutants and an RNAi approach, we performed a loss-of-function analysis and found that all the three ligands impact JAK/STAT transcriptional activity. Interestingly, we showed that Upd1 is required to maintain basal epithelium renewal while Upd2 and mainly Upd3 are involved to induce the regenerative response following an immune challenged condition. Finally, we showed that the three ligands are required to maintain the midgut renewal upon aging. Altogether, these findings demonstrate a cooperative role of the Upd ligands to maintain homeostasis in the adult midgut.

993C

The Role of *Zfh-1* in the Cyst Stem Cells of the Testis Stem Cell Niche. Judith L. Leatherman, Elizabeth Overturf. School of Biological Sciences, University of Northern Colorado, Greeley, CO.

The *Drosophila* testis is a leading model for studying adult stem cells in their niche. This niche is comprised of a group of cells called the hub, around which two stem cell populations cluster, the germline stem cells (GSCs) and the cyst stem cells (CySCs). The transcriptional repressor *zfh-1* is an important regulator of the CySCs, being both necessary and sufficient to direct CySC renewal. To explore the molecular functions of *Zfh-1* that promote self-renewal, we queried the literature on the orthologous ZEB factors in vertebrates. ZEBs are master regulators of epithelial to mesenchymal transition (EMT), via their ability to repress epithelial genes including E-cadherin. We examined E-cadherin expression in testes with a lacZ enhancer trap line. We found E-cadherin-lacZ was high in the hub and differentiating cyst cells, but highly reduced in CySCs where *Zfh-1* accumulates, suggesting that *Zfh-1* may repress E-cadherin. We misexpressed E-cadherin in the CySCs, but found no effect on the CySC population, suggesting that E-cadherin repression is not a self-renewal function of *Zfh-1*. ZEB factors also undergo reciprocal-negative regulation with the miR-200 microRNAs. The miR-200s promote epithelial differentiation via ZEB mRNA repression, and ZEB factors transcriptionally repress miR-200s. We found that the miR-200 ortholog in *Drosophila*, miR-8, is expressed in late cyst cells, consistent with *Zfh-1* repressing miR-8 transcription in CySCs. However, miR-8 misexpression did not reduce *Zfh-1* protein accumulation, and miR-8 mutants did not have an expanded domain of *Zfh-1* protein accumulation. We conclude that miR-8 and *Zfh-1* do not undergo a similar reciprocal-negative regulation in the *Drosophila* testis. We are currently exploring other hypotheses for *zfh-1* function.

994A

Mutagenesis by imprecise excision of the *piggyBac* transposon in *Drosophila melanogaster*. Heuijong Kim, Kiyoun Kim, Jaekwang Kim, Song-Hee Kim, Jeongbin Yim. School of Biological Sciences, Seoul National University, Seoul, Republic of Korea.

Mutagenesis by transposon-mediated imprecise excision is the most extensively used technique for mutagenesis in *Drosophila*. Although *P*-element is the most widely used transposon in *Drosophila* to generate deletion mutants, it is limited by the insertion coldspots in the genome where *P*-elements are rarely found. The *piggyBac* transposon was developed as an alternative mutagenic vector for mutagenesis of non-*P*-element targeted genes in *Drosophila* because the *piggyBac* transposon can more randomly integrate into the genome. Previous studies suggested that the *piggyBac* transposon always excises precisely from the insertion site without initiating a deletion or leaving behind an additional footprint. This unique characteristic of the *piggyBac* transposon facilitates reversible gene-transfer in several studies, such as the generation of induced pluripotent stem (iPS) cells from fibroblasts. However, it also raised a potential limitation of its utility in generating deletion mutants in *Drosophila*. In this study, we report multiple imprecise excisions of the *piggyBac* transposon at the *sepiapterin reductase (SR)* locus in *Drosophila*. Through imprecise excision of the *piggyBac* transposon inserted in the 5'-UTR of the *SR* gene, we generated a hypomorphic mutant allele of the *SR* gene which showed markedly decreased levels of *SR* expression. Our finding suggests that it is possible to generate deletion mutants by *piggyBac* transposon-mediated imprecise excision in *Drosophila*. However, it also suggests a limitation of *piggyBac* transposon-mediated reversible gene transfer for the generation of induced pluripotent stem (iPS) cells.