# Boston Area *Drosophila* Meeting 2019

## June 11, 2019
## Brown University
## Markuvitz Auditorium, Sidney Frank Hall, 185 Meeting St., Room 220

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| 9:30 – 10:30 | **Microtubules stabilize intercellular contractile force transmission during ventral furrow formation**<br>Clint Ko, Martin Lab, Massachusetts Institute of Technology |
| | **microRNA dependent regulatory switch that controls immune responses by regulating stem cell fate in the gut**<br>Pushpa Verma, Van Vacter Lab, Harvard University |
| | **Dissecting the sharp response of a canonical developmental enhancer reveals multiple sources of cooperativity**<br>Jeehae Park, DePace Lab, Harvard Medical School |
| | **[Lightning] KO or OK?: A computational method to uncover regulatory mechanisms of transcription factors during development**<br>Ashley Conard, Larschan Lab, Brown University |
| | **[Lightning] A myotubularin-related phosphatase that regulates autophagic flux**<br>Elizabeth Allen, Baehrecke Lab, University of Massachusetts Medical School |
| 10:30 – 10:45 | **BREAK** |
| 10:50 – 12:00 | ***Drosophila* DEG/ENaCs Expressed in Neurons and Muscle Impact Synaptic Transmission and Behavior**<br>Alexis Hill, Assistant Professor, College of the Holy Cross |
| | **A conserved role for the N-glycosylation pathway in sleep and seizures**<br>Brittany Leger, Walker Lab, Massachusetts General Hospital |
| | **Early integration of multimodal chemosensory stimuli in the *Drosophila* larva**<br>Jess Kanwal, Samuel Lab, Harvard University |
| | **[Lightning] Behavioral features of alcohol reinforcement in *Drosophila***<br>Jamie Catalano, Kaun Lab, Brown University |
| | **[Lightning] Identifying hygrotaxis circuit elements in *Drosophila***<br>Tatevik Sarkissian, Garrity Lab, Brandeis University |
| 12:00 – 1:25 | **LUNCH – Discussion Groups (Signup)**<br>– Apoptosis and Neurodegeneration<br>– Cell Signaling and Development<br>– Neurobiology and Behavior<br>– Transcription and Epigenetics |
| 1:30 – 2:30 | **Vitamin A deprivation as a novel approach to identify neuroprotective molecules**<br>Jens Rister, Principal Investigator, University of Massachusetts – Boston |
| | **Killing time: Decoupling developmental apoptosis and neuroblast proliferation in *Drosophila***<br>Katherine Harding, White Lab, Massachusetts General Hospital |
| | **Generation of neuronal diversity in the *Drosophila* optic lobes through development and evolution**<br>Nikos Konstantinides, Desplan Lab, New York University |
| | **[Lightning] Structure-function analysis of β-arrestin Kurtz reveals a critical role of receptor interactions in downregulation of GPCR signaling *in vivo***<br>Fei Chai, Veraksa Lab, University of Massachusetts – Boston |
| | **[Lightning] Pigment dispersing factor (PDF) signaling: A novel pathway for memory regulation by the circadian clock**<br>Johanna Flyer-Adams, Griffith Lab, Brandeis University |
| 2:30 – 2:45 | **BREAK** |

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| 2:50 – 3:50 | **Glial α-synuclein promotes neurodegeneration characterized by a distinct transcriptional program *in vivo***<br>Abby Olson, Feany Lab, Brigham & Women's Hospital |
| | **Regulation of endosomal Microautophagy in *Drosophila***<br>Ana Mesquita, Jenny Lab, Albert Einstein College of Medicine |
| | **Selective Serotonin Reuptake Inhibitors as Potential Therapeutics for MEGF10 Myopathy**<br>Isabelle Draper, Principal Investigator, Tufts Medical Center |
| | **A new GFP-tagged cell line resource from the DRSC**<br>Stephanie Mohr, Perrimon Lab / DRSC, Harvard Medical School |
| | **[Lightning] FlyBase Updates**<br>Victoria Jenkins, FlyBase, Harvard University |
| 3:50 – 4:00 | **BREAK** |
| 4:05 – 5:15 | **KEYNOTE – Routing and remodeling membranes at the synapse**<br>Avital Rodal, Brandeis University |

**5:15 – Dinner out in Providence**
Most restaurants listed are known for farm-to-table menus. They are largely within walking distance (~15min) or a short Lyft/Uber from campus.

*Organizer Recommendations (General)*

Gracie's – 194 Washington St. – $$$$ – Upscale dining for reasonable prices.
Persimmon – 99 Hope St. – $$$-$$$$ – Upscale tasty small plates. Definitely order dessert.
Hemenway's – 121 S. Main St. – $$$-$$$$ – Great fresh seafood.
New Rivers – 7 Steeple St. – $$$ – Try the cheese & charcuterie plates.
Red Fin Crudo – 71 Washington St. – $$$ – Tapas, great for sharing.
The Grange – 166 Broadway – $$$ – Tasty all vegetarian (and largely vegan) menu.
Los Andes – 903 Chalkstone Ave. – $$$ – Peruvian and Bolivian cuisine. Great for meat-lovers.
Chez Pascal – 960 Hope St. – $$$ – Good for pork lovers.
Garden Grill – 727 East Ave. – $$-$$$ – Vegetarian home-style cooking. Walk-ins only.
KG Kitchen – 771 Hope St. – $$$ – Urban American bistro. Great brews on draft.
Milk Money – 566 South Water St. – $$$ – Southern-themed shared plates.

*Organizer Recommendations (Italian – $$$)*

Massimo – 134 Atwells Ave.
Pan e Vino – 365 Atwells Ave.
Siena – 238 Atwells Ave.
Camille's – 71 Bradford St.
Enoteca Umberto – 256 Atwells Ave.
Andino's – 171 Atwells Ave.
Pastiche – 92 Spruce St. – Great dessert

*At Brown University ($-$$$)*

Kabob & Curry – Indian
by Chloe – Vegan burgers & salads
Heng – Thai
Denden – Korean BBQ
Andreas – Greek
Flatbread – Pizza
Bajas – TexMex to go
East Side Pockets – Falafel & shawarma to go

**2019 Meeting Organizers**

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Nate Snell (Grad. Student, Barnea Lab, Brown)
Mukulika Ray (Grad. Student, Larschan Lab, Brown)
Erica Larschan (Asst. Prof. of Biology, Brown)
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# ABSTRACTS

**Microtubules stabilize intercellular contractile force transmission during ventral furrow formation**
Clint Ko, Martin Lab, Massachusetts Institute of Technology

During development, forces transmitted between cells are critical for sculpting epithelial tissues. Actomyosin contractility in the middle of the cell apex (medioapical) can change cell shape (e.g., apical constriction), but can also result in force transmission between cells via attachments to adherens junctions. How actomyosin networks maintain attachments to adherens junctions under tension is poorly understood. Here, we discovered that microtubules stabilize actomyosin intercellular attachments in epithelia during Drosophila mesoderm invagination. First, we used live imaging to show a novel arrangement of the microtubule cytoskeleton during apical constriction: medioapical, non-centrosomal Patronin (CAMSAP) foci formed by actomyosin contraction organizes an apical microtubule network. Microtubules were required for mesoderm invagination but were not necessary for apical contractility or adherens junction assembly. Instead, microtubules promoted the stable connection between medioapical actomyosin and adherens junctions. These results define a role for coordination between actin and microtubule cytoskeletal systems in intercellular force transmission and tissue morphogenesis.

**microRNA dependent regulatory switch that controls immune responses by regulating stem cell fate in the gut**
Pushpa Verma, Van Vacter Lab, Harvard University

Pushpa Verma[1], Stephen Cohen[2], David Van Vactor[1]
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Innate immunity is the first line of defense against infections in all metazoans and is conserved throughout evolution. An organism's ability to differentiate between good and the bad microbes and simultaneously mounting an appropriate immune response is very important for its fitness and survival. An attenuated immune system fails to effectively combat infections, while a hyperactive immune system can increase susceptibility to infection or cancer and auto-immune disease. Therefore, tight regulation of the immune responses proportional to the nature and gravity of infections is very crucial. We have discovered a microRNA, miR-980, in *Drosophila melanogaster* that prevents hyper-activation of immune responses under normal conditions, and also appears to be involved in regulating the cellular response to immune challenge. Loss of miR-980 results in the spontaneous formation of the melanotic tumors that normally encyst and destroy foreign particles. Melanotic tumors are aggregates of cell masses, formed by encapsulation and engulfment of any foreign agent by hemocytes. Interestingly, we discovered that miR-980 functions in the midgut barrier epithelium to determine the cell fate output of intestinal stem cell lineage, which then controls both local and global immune responses. This function of miR-980 in the barrier epithelium is mediated by repression of a gene called Reph (Regulator of Ephrin expression). Reph was previously shown to regulate ephrin expression in the optic lobe of the *Drosophila* (Dearborn et al, PLoS One, 2012), but its role in the immune system and gut has not been ascertained. During my talk, I'll be describing a novel regulatory mechanism involving functions of miR-980 and Reph in the gut to modulate immune responses and how they protect an organism from auto-immune responses.

**Dissecting the sharp response of a canonical developmental enhancer reveals multiple sources of cooperativity**
Jeehae Park, DePace Lab, Harvard Medical School

**Authors**: Jeehae Park, Javier Estrada, Gemma Johnson, Chiara Ricci-Tam, Meghan Bragdon, Yekaterina Shulgina, Anna Cha, Jeremy Gunawardena, Angela H DePace
**Affiliation**: Department of Systems Biology, Harvard Medical School, Boston, MA.

Developmental enhancers integrate graded concentrations of input transcription factors (TFs) to create sharp gene expression boundaries. Here we examine the hunchback P2 (HbP2) enhancer which drives a sharp expression pattern in the Drosophila blastoderm embryo in response to the transcriptional activator Bicoid (Bcd). We systematically interrogate cis and trans factors that influence the shape and position of expression driven by HbP2, and find that the prevailing model, based on cooperative binding of Bcd to HbP2 is not adequate. We demonstrate that other proteins, such as pioneer factors, mediator and histone modifiers influence the shape and position of the HbP2 expression pattern. By comparing our results to theory, we assess how higher-order cooperativity and energy expenditure impact boundary location and sharpness. Our results emphasize that the bacterial view of transcription regulation, where pairwise interactions between regulatory proteins dominate, must be reexamined in animals, where multiple molecular mechanisms collaborate to shape the gene regulatory function.

**KO or OK?: A computational method to uncover regulatory mechanisms of transcription factors during development**

Ashley Conard, Larschan Lab, Brown University

Recent studies show that chromatin remodelers are important in neuron development. We found that absence of one such chromatin remodeling transcription factor (TF) CLAMP negatively effects brain development in *Drosophila melanogaster.* To study the role of CLAMP in brain development, we performed RNA-seq experiments after removing CLAMP in different developmental stages (embryo, third instar brain, and adult brain), and in both sexes. We then developed a computational method to analyze these distant but related timepoints, surpassing the basic Venn diagram overlap evaluation. This novel method provides insight into the temporally complex regulatory mechanisms influenced by the TF of interest. Integrating ChIP-seq data enables our method to highlight direct and epistatic relationships between the TF of interest, and other genes. Our method is modular and enables data exploration. It outputs interactive plots showing automatically generated clusters of temporally differentially expressed genes, and within each cluster: automatic gene term analysis and ontology, chromosome comparison plots, condition comparison plots, motif analysis, Venn diagrams, and optional ChIP analysis.

Our results highlight two distinct clusters of gene trajectories shared by both males and females which are temporally important in neuronal projection morphogenesis and developmental growth. In the first cluster, removing CLAMP downregulates certain genes at the beginning stages of development, which changes neuronal projections. In the second cluster, removing CLAMP gradually upregulates certain genes across development. Additionally, through CLAMP-ChIP integration, we identified several genes that could potentially be epistatically regulated by CLAMP. This illustrates the importance of looking at all data in a wholistic model. We are validating our findings biologically.

Many studies produce data on biologically important yet distant timepoints where we cannot assume direct dependence between observations at consecutive timepoints. Further, many TFs have many context specific functions, requiring an analysis tool to explore the various regulatory mechanisms where it is involved. This method provides both robust exploration and characterization of TFs.

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**A myotubularin-related phosphatase that regulates autophagic flux**

Elizabeth Allen, Baehrecke Lab

Elizabeth Allen[1], Tina Fortier[1], Eric H. Baehrecke[1]
[1] University of Massachusetts Medical School

Macroautophagy (autophagy) controls cellular catabolism by trafficking cytosplasmic cargoes to lysosomes for degradation, and it underlies multiple human disorders. Pioneering work in Saccharomyces cerevisiae defined the core autophagy machinery, but animals possess autophagy regulators that were not identified in yeast. We are investigating autophagy programs in the context of animal development to identify novel regulators of this process. The midgut undergoes a developmentally-programmed cell size reduction and death that depends on autophagy. We screened the fly phosphatome for previously uncharacterized autophagy regulators in Drosophila intestinal midgut cells and identified a myotubularin-related (MTMR) phosphatase. Clonally-expressed CG3530-RNAi knockdown disrupts both Atg8a and ref(2)p autophagy reporter levels and localization, and CG3530 knockdown cells fail to undergo autophagy-dependent developmental cell size reduction. CG3530 encodes a phosphoinositide (PI) 3-phosphatase that is orthologous to human MTMRs6-8, which are catalytically active MTMRs implicated in cell death, neurodegeneration, and autophagy, respectively. How CG3530 regulates these processes is unknown, and a mechanistic role for MTMR8 in autophagy remains poorly defined. We hypothesize that CG3530 negatively regulates autophagy by maintaining appropriate levels of PIs at autolysosomal membranes, thereby mediating the rate of autophagic degradation within cells. In support of this hypothesis, we detect increased Atg8a autophagy reporter levels in feeding larval midgut and fat cells, during a time point at which autophagy levels are typically low. CG3530 RNAi-knockdown cells also accumulate large Atg8a- LysoTracker- and Lamp1-positive structures, and endocytic uptake of Texas Red-Avidin is impaired. However, CG3530 knockdown cells stain positive with Magic Red, suggesting that lysosomes possess functional cathepsin B and can readily degrade substrates. The CG3530 knockdown phenotype is conserved; siRNA-mediated MTMR8 knockdown in Cos7 cells phenocopies our findings in fly tissues. Moreover, MTMR8 knockdown produces perinuclear localization, increased size, and decreased numbers of LC3-, LysoTracker-, and Lamp1-positive spots. These data indicate that MTMR8 and the MTMR encoded by CG3530 have conserved requirements for autophagic flux.

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**_Drosophila_ DEG/ENaCs Expressed in Neurons and Muscle Impact Synaptic Transmission and Behavior**
Alexis S. Hill, Assistant Professor, Department of Biology, College of the Holy Cross

Members of the non-voltage gated Degenerin/Epithelial Sodium Channel (DEG/ENaC) family play important roles in regulating ionic gradients across epithelial barriers. Several family members are also expressed in the nervous system, where their functions remain poorly understood. Our previous work demonstrated that mutants for the *Drosophila* DEG/ENaC gene *ppk29* display abnormal larval movement, have altered glutamate receptor levels and postsynaptic response to neurotransmission at the neuromuscular junction (NMJ). More recently, we have found that in addition to expression in muscle, *ppk29* is expressed in the central nervous system. Using a behavioral screen and RNAi transgenes, we have identified several DEG/ENaC subunits that are required in neurons, muscle, or both, for normal larval movement. Along with data from other groups, this suggests that DEG/ENaCs play important pre- and postsynaptic roles at the NMJ. Ongoing research aims to determine how expression of specific DEG/ENaCs in both neurons and non-neuronal cells impact behavior and neurotransmission at both the NMJ and central nervous system synapses.

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**A conserved role for the N-glycosylation pathway in sleep and seizures**
Brittany Leger, Walker Lab, Massachusetts General Hospital

In a human GWAS of ~500,000 subjects, common variants near both ALG10 and ALG10B associate with sleep timing, duration, and naps. ALG10 and ALG10B are paralogous ALG enzyme coding genes. ALG enzymes function in N-Glycosylation, and mutations that alter these enzymes' functions cause congenital disorders of glycosylation (CDG), a family of rare metabolic disorders. Though poorly characterized, symptoms of these conditions often include hypertonia, developmental disability, cognitive impairment, underdevelopment of the cerebellum, and cardiac abnormalities. However, ALG10 and ALG10B have no known associated CDG.

A patient with an ultra-rare quadruple KO mutation for ALG10 and ALG10B showed ataxia, progressive myoclonic epilepsy, and cerebellar and cortical atrophy. To determine the directionality, extent, and mechanism of these phenotypes, we used the fruit fly, Drosophila melanogaster, as a model system. Though Drosophila have only one Alg10 gene, no phenotype has previously been reported for it. Alg10 knockdown using two unique RNAi lines led to sleep defects, seizures, and cardiac phenotypes. To generate independent genetic evidence supporting a role for Alg10 in inducing these traits, we are using CRISPR with Alg10 sgRNA to generate a series of Alg10 mutants. Additionally, we are generating transgenic flies expressing wildtype and patient mutant human ALG10 and ALG10B to address whether they can rescue the fly RNAi phenotypes.

To further understand the mechanism of Alg10 function, we have screened RNAi lines for the other ALG enzymes, and have consistently found cardiac, sleep, and seizure phenotypes. We are using chemical biological tools to identify the target protein(s) whose glycoregulation is disrupted in the Alg10 KD flies. We are also conducting electrophysiology on Alg10 KD flies to determine the nature of their seizures and neuronal morphology. Finally, we are screening anti-epileptics and specific sugar diets in Alg10 KD flies as a potential treatment for altered Alg10 function.

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**Early integration of multimodal chemosensory stimuli in the Drosophila larva**
Jess Kanwal, Samuel Lab, Harvard University

Jess Kanwal, Jim Truman, Ben de Bivort, Aravi Samuel

The brain integrates information from different sensory modalities in order to enhance detection and perception of external stimuli and to respond in the most efficient manner. For a Drosophila larva, this means detecting chemosensory cues to locate the most nutritious food source in its environment. How the larva innately integrates olfactory and gustatory cues, at both the neuronal and behavioral levels, remains largely unknown. To assess the larva's behavioral strategy for chemosensory integration, we compared its behavior on either an attractive olfactory or gustatory gradient alone to that on simultaneous presentations of both gradients in parallel or in conflict. Larvae show multisensory enhancement in their navigation efficiency towards the most attractive region of their chemosensory environment when both gradients are in parallel compared to either one alone. Placing the two gradients in conflict reveals that neither sensory system gates the other. Silencing specific local interneurons (LNs) and projection neurons (PNs) in the antennal lobe (AL), the first olfactory processing center of the larval brain, reveals a multiglomerular PN that modulates the degree of multisensory enhancement. Using in *vivo* calcium imaging of AL neurons in response to olfactory and gustatory stimuli, we identify several LNs and PNs that respond to both odors and tastes or are modulated by odor-taste mixtures. To our knowledge, these results indicate for the first time that neurons in antennal lobe have multisensory responses. Our findings support the idea that multisensory integration occurs at early stages of sensory processing and begin to address how this convergence enhances perception of the natural environment.

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**Behavioral features of alcohol reinforcement in *Drosophila***
Jamie Catalano, Kaun Lab, Brown University

Catalano J.[1]*, Mei N.[2], Azanchi R.[3], Song S., Blackwater T., Mahmud F., Kaun K.R.[3]
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Understanding ethanol's complex effects on reward and motivation circuits in the brain is critical for the development of better biologically informed therapies for ethanol abuse and addiction. Recent advances in neurogenetics have highlighted Drosophila melanogaster as an exciting model to study the effects of ethanol at the circuit and single neuron levels. However, methods for assessing motivation for drugs like ethanol are lacking in the Drosophila field. To address this methodological gap, we have developed a runway based operant conditioning assay to investigate the motivational drive for vaporizable stimuli like ethanol. Our results suggest that Drosophila demonstrate both seeking and avoidance behaviors for ethanol. Furthermore, machine learning software provides a high-resolution view of more subtle features of motivated behavior in this model. Future studies will assess the necessity and sufficiency of specific neuronal circuits in ethanol mediated seeking and avoidance. This experimental paradigm for estimating motivational drive will allow for circuit, single neuron, molecular, and genetic analyses of ethanol's motivational effects.

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**Identifying hygrotaxis circuit elements in Drosophila**
Tatevik Sarkissian, Garrity Lab, Brandeis University

Tatevik Sarkissian, Paul Garrity
*Brandeis University*

Humidity sensation, also known as hygrosensing, is critical for helping insects maintain proper water balance and, in vectors like mosquitoes, find hosts for blood-feeding. Hygrosensory behaviors involve the integration of external and internal cues, including external humidity and internal hydration state. In *Drosophila*, humidity is perceived by sensory neurons in the antenna and relayed to brain centers involved in sensory integration, including the mushroom body (MB) and lateral horn (LH). While the primary sensory neurons that detect humidity are known, how their input is combined with internal sensory input reflecting the fly's hydration state remains unclear. Furthermore, the internal sensors that cause a fly to switch from dry-seeking to moist-seeking upon dehydration remain unknown. We aim to identify these circuit(s) and sensors to begin to understand how they mediate state-dependent hygrotactic behavior.

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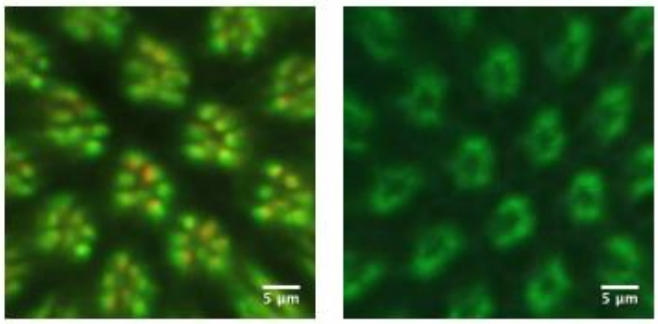
**Vitamin A deprivation as a novel approach to identify neuroprotective molecules**
Jens Rister, Principal Investigator, University of Massachusetts – Boston

Animals are frequently challenged by varying diets that deprive them of essential nutrients. For instance, vitamin A deprivation severely damages photoreceptors and is the leading cause of preventable childhood blindness. Using genetics, transcriptomics, proteomics (with A. Shevchenko), electrophysiology (with R. Hardie), and behavioral analysis, my lab studies how vitamin A deprivation affects photoreceptors on the molecular, structural, and functional level. Our central goal is to identify protective proteins that prevent retinal degeneration and preserve vision. We identified a novel transmembrane protein that is dramatically upregulated (>140-fold!) in vitamin A-deprived retinas and localizes to the light-sensing compartments of the photoreceptors. We discovered that this protein stabilizes damaged photoreceptors, as the deprived mutant shows severe anatomical (see Fig. below), behavioral, and electrophysiological defects. Taken together, this approach has the potential to identify mechanisms that stabilize damaged photoreceptors under dietary stress and could open new avenues for treating human eye diseases.




Vitamin A deprived wild type  Vitamin A deprived mutant

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**Killing time: Decoupling developmental apoptosis and neuroblast proliferation in *Drosophila***
Katherine Harding, White Lab, Massachusetts General Hospital

Katherine Harding[1] & Kristin White[1]
[1]Massachusetts General Hospital, Cutaneous Biology Research Center, Harvard Medical School, Boston MA 02129 USA

Cell proliferation and cell death are opposing but fundamental aspects of development that must be tightly controlled to ensure proper tissue organization and organismal health. Developmental apoptosis of abdominal neuroblasts in the *Drosophila* ventral nerve cord is controlled by multiple upstream spatial and temporal signals, which have also been implicated in control of cell proliferation. It has therefore remained unclear whether developmental apoptosis is linked to active cell proliferation. Previous investigations into this topic have focused on the effect of cell cycle arrests on exogenous induction of apoptosis, and thus have not addressed whether potential effects of the cell cycle lie with the sensing of damage signals or the execution of apoptosis itself. In this report, we show that developmental apoptosis is not inhibited by cell cycle arrest, and that endogenous cell death occurs independently of cell cycle phase. We also find that ectopic neuroblasts rescued from cell death retain the competency to respond to quiescence cues at the end of embryogenesis. This study demonstrates that upstream control of neuroblast proliferation and apoptosis represent independent mechanisms of regulating stem cell fate, and that execution of apoptosis occurs in a cell cycle-independent manner.

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**Generation of neuronal diversity in the *Drosophila* optic lobes through development and evolution**
Nikos Konstantinides, Desplan Lab, New York University

How neuronal diversity is generated is a major question in developmental neurobiology. We use Drosophila to understand how neurons develop after specification to acquire their terminal features that allow them to fulfill their function in neural circuits and control specific behaviors. First, we used single-cell sequencing to gain access to the transcriptome of adult Drosophila optic lobe cell types and machine learning to identify transcription factors responsible for inducing specific terminal differentiation features, in particular neurotransmitter identity. We are currently expanding our analyses to the very first stages of neuronal development to understand how neurons are specified from their progenitors. We are also studying how this neuronal diversity has evolved. Collectively, these data provide a deep understanding of the developmental and functional specification of a complex brain structure and new insights about its evolutionary history.

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**Structure-function analysis of β-arrestin Kurtz reveals a critical role of receptor interactions in downregulation of GPCR signaling *in vivo***
Fei Chai, Veraksa Lab, University of Massachusetts – Boston

Fei Chai[1], Timothy Musoke[1], George Tarabelsi[1], Steven Assaad[1], Jason Freedman[1], Rachel Peterson[1], Katarzyna Piotrowska[1], Jarrett Byrnes[1], Stephen Rogers[2] and Alexey Veraksa[1]

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β-arrestins are key regulators of signaling via the G protein coupled receptors (GPCRs), serving both as signal terminators and transducers. Previous studies identified various structural elements in β-arrestins that were shown or predicted to affect their function. However, the importance of these elements *in vivo* is still unclear, and the developmental roles of β-arrestins are not well understood. We carried out *in vivo* structure-function analysis of Kurtz (Krz), the single ortholog of mammalian β-arrestins in the *Drosophila* genome. We found that the Krz-KKVL/A mutant which is defective in both the GPCR-phosphosensing and receptor-binding finger loop regions acts as functional null. Endosome recruitment and bioluminescence resonance energy transfer (BRET) assays revealed that the Krz-KKVL/A mutation completely abolished the GPCR-binding ability of Krz. The GPCR Mist (also known as Mthl1) is activated by its ligand Folded gastrulation (Fog) and is responsible for cellular contractility and epithelial morphogenesis in *Drosophila* development. Embryos lacking *krz* or carrying mutant variants of Krz that are deficient in GPCR binding exhibited gastrulation defects that were similar to those observed in embryos with hyperactive Fog signaling. Krz-mediated downregulation of Fog-Mist signaling extended into the morphogenesis of the wing. Unexpectedly, mutations predicted to disrupt the binding of Krz to endocytic regulators did not affect Krz function. Our results have revealed that the direct binding between β-arrestin Krz and activated GPCRs is a critical regulatory step *in vivo*, which likely functions to uncouple the receptor from the G proteins and thus inhibit the pathway. These interactions are critical for controlling Fog-Mist signaling and epithelial morphogenesis in *Drosophila* development.

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**Pigment dispersing factor (PDF) signaling: A novel pathway for memory regulation by the circadian clock**
Johanna Flyer-Adams, Griffith Lab, Brandeis University

Johanna G. Flyer-Adams, Leslie C. Griffith
Department of Biology, Volen National Center for Complex Systems and National Center for Behavioral Genomics, Brandeis University, Waltham, MA

The behavioral link between your circadian clock and memory is clear: disruptions to your clock, such as jetlag and shiftwork, generate cognitive deficits which scale with the severity and duration of the disruption. However, the neural circuits involved in regulation of memory by the clock are unknown. In *Drosophila*, both the circadian clock and memory circuitry are individually well-described, providing a starting point to investigate their linkage. We find that the clock-specific signaling neuropeptide pigment dispersing factor (PDF) and its only known receptor Han are both required for normal appetitive olfactory short term memory (STM). Produced solely in the ventrolateral clock neurons, PDF canonically signals within the clock circuit through Han to entrain the circadian clock and to regulate daily patterns of locomotor activity. We show that intra-clock PDF signaling through Han can support circadian locomotor activity patterns but not normal STM, implying that a PDF target outside the clock regulates memory. Furthermore, while Han is required for appetitive STM, it is dispensable for aversive STM despite a continued requirement for the PDF ligand itself, suggesting the existence of a novel PDF receptor. Taken together, our data show that PDF from within the clock signals outside the clock to regulate memory, and does so in a valence-specific manner, likely through discrete receptors. Identification of memory-relevant PDF target neurons outside the clock is currently underway to definitively illustrate the first neural circuit by which the circadian clock regulates memory.

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**Glial α-synuclein promotes neurodegeneration characterized by a distinct transcriptional program in vivo**
Abby Olson, Feany Lab, Brigham & Women's Hospital

Olsen, Abby L.[1] and Feany, Mel B.[2]
[1] Department of Neurology, Brigham and Women's Hospital, Massachusetts General Hospital, Harvard Medical School.
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α-synucleinopathies are neurodegenerative diseases that are characterized pathologically by α-synuclein inclusions in neurons and glia. The pathologic contribution of glial α-synuclein in these diseases is not well understood. Glial α-synuclein may be of particular importance in multiple system atrophy (MSA), which is defined pathologically by glial cytoplasmic α-synuclein inclusions. We have previously described *Drosophila* models of neuronal α-synucleinopathy, which recapitulate key features of the human disorders. We have now expanded our model to express human α-synuclein in glia. We demonstrate that expression of α-synuclein in glia alone results in α-synuclein aggregation, death of dopaminergic neurons, impaired locomotor function, and autonomic dysfunction. Furthermore, co-expression of α-synuclein in both neurons and glia worsens these phenotypes as compared to expression of α-synuclein in neurons alone. We identify unique transcriptomic signatures induced by glial as opposed to neuronal α-synuclein. These results suggest that glial α-synuclein may contribute to the burden of pathology in the α-synucleinopathies through a cell type specific transcriptional program. This new *Drosophila* model system enables further mechanistic studies dissecting the contribution of glial and neuronal α-synuclein *in vivo*, potentially shedding light on mechanisms of disease that are especially relevant in MSA but also the α-synucleinopathies more broadly.

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**Regulation of endosomal Microautophagy in Drosophila**
Ana Mesquita, Jenny Lab, Albert Einstein College of Medicine

Ana Mesquita[1] and Andreas Jenny[1,2]
[1] Department of Developmental and Molecular Biology; [2] Department of Genetics, Albert Einstein College of Medicine, New York, NY, 10461, USA

Autophagy delivers cytosolic components to lysosomes for degradation and is thus essential for cellular homeostasis and to cope with different stressors. As such, autophagy counteracts various human diseases, and its reduction leads to aging like phenotypes. Macroautophagy (MA) can selectively degrade organelles or aggregated proteins, but selective degradation of single proteins has only been described for Chaperone-mediated autophagy (CMA) and endosomal Microautophagy (eMI). These two autophagic pathways, described to date only in mammals, are specific for proteins containing KFERQ-related targeting motifs.

Using a KFERQ-tagged fluorescent biosensor, we have identified an eMI-like pathway in the genetically easily tractable model organism Drosophila melanogaster. Upon starvation, this biosensor localizes to late endosomes/lysosomes in an Hsc70- and ESCRT machinery dependent manner. Currently, we are characterizing the physiological role of eMI in flies

with a focus on what types of cellular stress and by what mechanism eMI is activated. Our data suggest that oxidative stress and DNA damage, but not ER stress can elicit an eMI response, implying a selectivity of the process.

The DNA damage response is well studied and acts both as a sensor for damage and as effector to help the cell contain said damage and is thus critical to prevent cancer, neurodegeneration, and heart disease. Based on the initial observation that eMI is DNA damage inducible in flies, we are testing the hypothesis that this activation occurs through the ATM/ATR DNA damage response pathway. The ATM/ATR kinases are responsible for controlling DNA repair mechanisms, cell cycle arrest and apoptosis. Using genetic analyses, our initial results show that indeed ATM/ATR kinases can act as eMI modulators that likely control the recycling of proteins through multi-vesicular body formation in late endosomes.

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**Selective Serotonin Reuptake Inhibitors as Potential Therapeutics for MEGF10 Myopathy**
Isabelle Draper, Principal Investigator, Tufts Medical Center

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MEGF10 myopathy is a rare inherited muscle disease that is named after the causative gene, *MEGF10*. As only supportive care is available, we performed a drug screen and follow-up studies in search of a novel therapy. The primary screen, based on assessment of cellular proliferation patterns in Megf10-deficient myoblasts, yielded five candidate compounds. Secondary evaluations of hits were done using myoblasts derived from *Megf10*$^{-/-}$ mice, induced pluripotent stem cell (iPSC)-derived myoblasts from MEGF10 myopathy patients, mutant *Drosophila* that are deficient in the homologue of MEGF10 (Drpr), and *megf10* mutant zebrafish. Two selective serotonin reuptake inhibitors (SSRIs), sertraline and citalopram, consistently showed efficacious rescue of defects, and were selected as finalists. In depth follow-up analyses demonstrated that sertraline was highly effective in alleviating abnormalities across multiple models of MEGF10 myopathy. Sertraline thus shows promise as a potential therapeutic for this devastating disease. The mechanism of action may involve the Notch pathway.

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**A new GFP-tagged cell line resource from the DRSC**
Stephanie Mohr, Perrimon Lab / DRSC, Harvard Medical School

CRISPR technology provides us with a new opportunity to develop modified *Drosophila* cell lines. At the *Drosophila* RNAi Screening Center (DRSC), we have long been interested to develop a resource of fluorescent-tagged cell lines for use in lareg-scale image-based screens, proteomics analyses, and other studies. Recently, we developed a production pipeline to make GFP-tagged *Drosophila* S2R+ cultured cells. The approach includes use of a Cas9-positive cell line, a guide RNA, and a single-stranded DNA donor that provides GFP as an artificial exon. In collaboration with the Bellen lab, we successfully developed, optimized, and applied this GFP knock-in approach to build a set of cell lines in which a variety of sub-cellular organelles and compartments are tagged with GFP. The molecular workflow, validation, and resulting cell lines will be presented.