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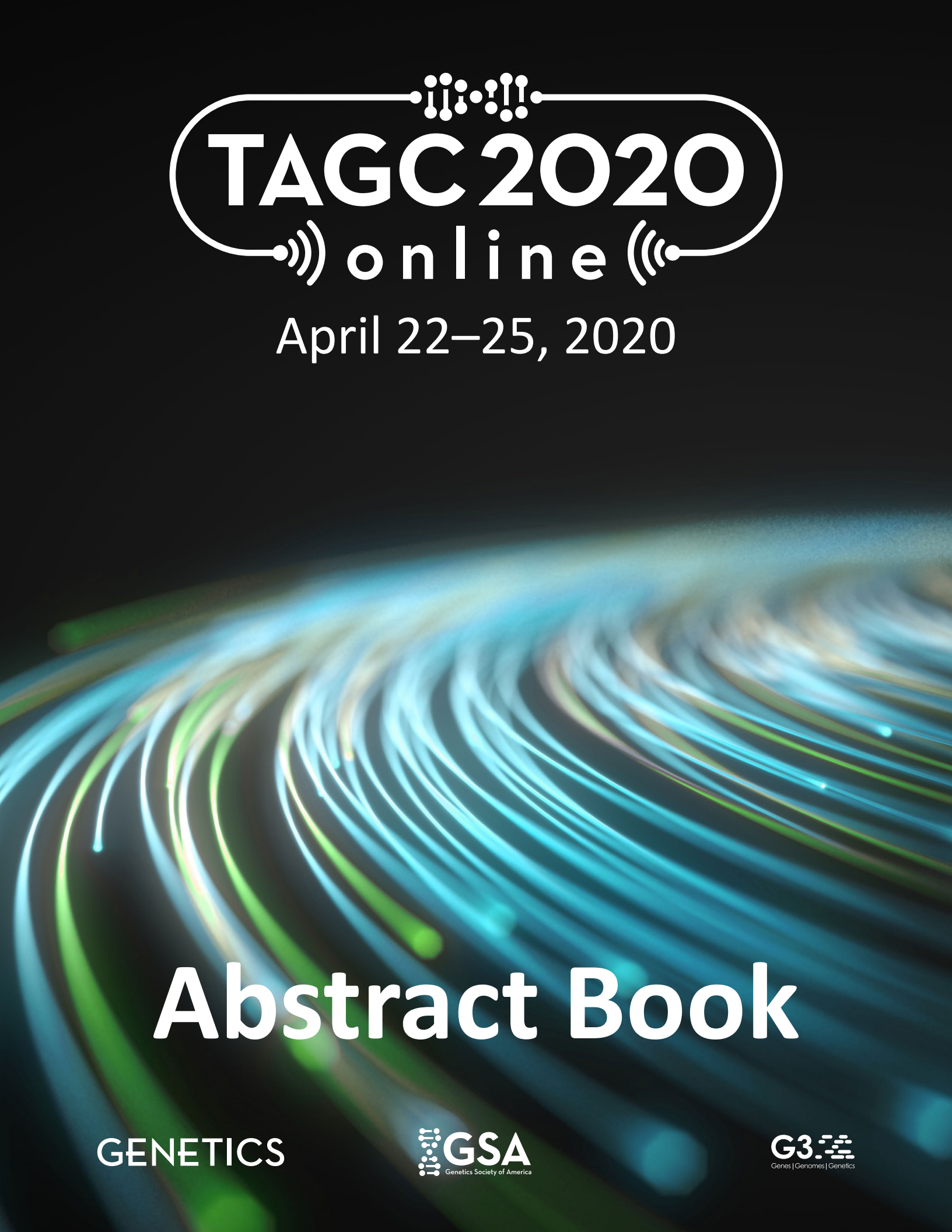

Abstract Book

GENETICS

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Thursday, April 16 2:00 PM - 6:00 PM

Mammalian Trainee Symposium

531C Replicability and reproducibility of genetic analysis between different studies using identical Collaborative Cross inbred mice *Yanwei Cai*[1], Merrie Mosedale[2,3], William Valdar[1,4] 1) Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Institute for Drug Safety Sciences, University of North Carolina at Chapel Hill, Research Triangle Park, NC; 3) Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, Chapel Hill, NC; 4) Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Replicability of experiments is an essential part of the scientific method. Paradoxically, replicability of large genetics experiments is rarely tested. This is because a strict replication study, ie, redoing the same experiment, is often viewed as both economically unviable in the sense that it is unlikely to attract funding and scientifically uninteresting because the replicability of results is can simply be assumed. By combining research records from several large scale genetics experiments with mostly identical designs, we have a dataset that to a large extent mimics the process of strict replication, making it a good resource for putting the assumption of replicability to the test.
The organisms used in these studies are mice from the Collaborative Cross (CC), a large panel of recombinant inbred mouse strains that combines both high genetic diversity for genetic analysis and the ability to generate biological replicates. In hand, we have data from three independent studies of the CC strains. Each study investigated drug-induced liver injury on a different drug but using the same treated vs control design. Thus, the control CC mice from the three studies, which are genetically identical for a given strain, underwent very similar vehicle-only dosing, so that these studies can be considered independent replicating experiments on identical subjects. Although this dataset is not perfectly controlled in its experimental pipeline and environment conditions, it matches well with real-life scenarios of replicability issues.
This is, to our knowledge, the first comparative study investigating replicability of genetic effects of a genetic reference population. From this dataset, we applied replicability comparison of both phenotypic and expression data and concluded that: 1) the genetic analysis method for this type of study design is valid, including methods to estimate genetic effect, heritability, gene-by-study effect, and QTL mapping; 2) there is a strong association between high replicability and high genetic effect of a phenotypic traits, so that heritable phenotypes are more likely to be replicated; 3) certain highly replicable gene expression data can be applied to other CC studies without expression data. We find these results supportive of CC mice being, in general, is a good resource for reproducibility analysis and therefore for rigorous genetic experiments.

538A No more paywalls: cost-benefit analysis across scRNA-seq platforms reveals biological insight is reproducible at low sequencing depths Kathryn S. McClelland[1], Oswaldo A. Lozoya[2], Suzanne N. Martos[2], Brian N. Papas[2], Jian-Liang Li[2], Douglas A. Bell[2] 1) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK/NIH); 2) National Institute of Environmental and Health Sciences (NIEHS/NIH).

The major hurdle that single-cell RNA-seq (scRNA-seq) technologies face in reaching mainstream status is cost - including money, computational footprint, and statistical effort. In trying to disseminate these technologies, the usual concern pertains representation: how many reads must be produced to capture an informative picture of single-cell transcriptomes? Instead, we approach scRNA-seq optimization from a different question: does deeper sequencing improve data sparsity? Here, we show the answer is no. First, we produced matched scRNA-seq data at various sequencing depths in different systems (Illumina and IonTorrent), from scRNA-seq libraries assembled with different technologies (10X Genomics and sci-RNA-seq), across biological models (human PBMCs and mouse embryonic kidney stroma), and each with at least three independent replicates of over 10,000 cells per specimen. Then, we implemented expression matrix focusing and SALSA (doi: 10.1101/551762) to compare gene detection rates, barcode re-incidence, and clustering reproducibility within each specimen at increasing sequencing depths. Overall, high-depth sequencing (NOVAseq, NextSeq) detected over 4x more barcodes than low-depth (MiSeq, Ion 530) for droplet-based technologies (10X Genomics), and over 3x more compounded barcodes for combinatorial-indexing techniques (sci-RNA-seq); yet, most barcodes added at high depths represented ambient RNA or gDNA debris; in contrast, all barcodes scoring as single cells at low sequencing depths were rescored as such at higher ones. Newly detected UMIs at high-depth sequencing aligned to constitutive genes detected in all barcodes or rare transcripts from discarded barcodes. Also, markers for single-cell clusters lost statistical support during differential expression analyses at high sequencing depths. These results confirm that over-sequencing of scRNA-seq libraries provides no benefit regarding data sparsity, and instead admits higher rates of “false” single-cell barcodes and transcripts the more UMI-appended debris becomes readout. In sum, statistical insight from scRNA-seq data tracks with library complexity regardless of scRNA-seq technique or sequencing platform. Our findings posit a new paradigm to extract reproducible biological insight from scRNA-seq experiments in which minimal (and inexpensive) sequencing depths, with as many cells supplied per assay as possible, are always best. (* KSM and OAL joint first author).

563B Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline Hannah A. Grunwald[1], Valentino M. Gantz[1], Gunnar Poplawski[2,4], Xiang-Ru S. Xu[1], Ethan Bier[1,3], Kimberly L. Cooper[1,3] 1) Division of Biological Sciences, University of California San Diego, La Jolla, CA, USA; 2) Department of Neurosciences, University of California San Diego, La Jolla, CA, USA; 3) Tata Institute for Genetics and Society, University of California San Diego, La Jolla, CA, USA; 4) Department of Medicine, National University of Singapore, Singapore, Singapore.

A gene drive biases the transmission of one of the two copies of a gene such that it is inherited more frequently than by random segregation. Highly efficient gene drive systems that have recently been developed in insects, which leverage the sequence-targeted DNA cleavage activity of CRISPR–Cas9 and endogenous homology- directed repair mechanisms to convert heterozygous genotypes to homozygosity. If implemented in laboratory rodents, similar systems would enable the rapid assembly of currently impractical genotypes that involve multiple homozygous genes (for example, to model multigenic human diseases). To our knowledge, however, such a system has not yet been demonstrated in mammals. Here we use an active genetic element that encodes a guide RNA, which is embedded in the mouse tyrosinase (*Tyr*) gene, to evaluate whether targeted gene conversion can occur when CRISPR–Cas9 is active in the early embryo or in the developing germline. Although Cas9 efficiently induces double-stranded DNA breaks in the early embryo and male germline, these breaks are not corrected by homology- directed repair. By contrast, Cas9 expression limited to the female germline induces double-stranded breaks that are corrected by homology-directed repair, which copies the active genetic element from the donor to the receiver chromosome and increases its rate of inheritance in the next generation. These results demonstrate the feasibility of CRISPR–Cas9-mediated systems that bias inheritance of desired alleles in mice and that have the potential to transform the use of rodent models in basic and biomedical research.

573C A GxE QTL on Chromosome 15 underlies susceptibility to air pollution-induced lung injury in mice *Adelaide Tovar*[1], Gregory J. Smith[1], Joseph M. Thomas[1], Kathryn McFadden[1], Samir N. P. Kelada[1] 1) The University of North Carolina at Chapel Hill.

Interactions between genetic and environmental factors (GxE) are proposed to account for much of the as-yet unexplained variance in disease risk among individuals. However, parsing their contributions to disease phenotypes remains challenging and thus, few definitive GxE have been identified and validated within human studies. Model organisms are a powerful resource for GxE discovery, owing to their defined genetic backgrounds which can be studied in controlled and contrasting environments. Reproducible inter-individual differences in susceptibility to air pollution-induced toxicity has been observed in both human populations and across inbred mouse strains, suggestive of GxE. Here, we use a panel of genetically diverse recombinant inbred mouse strains, namely the Collaborative Cross (CC), to identify GxE quantitative trait loci (QTL) underlying variation in lung injury following exposure to a model air pollutant, ozone (O_3). We exposed 10- to 12-week-old female and male mice from 56 CC strains to filtered air (n=4/strain) or 2 ppm O_3 (n=6/strain) in matched pairs for three hours and sacrificed animals 21 hours later. Subsequently, we measured total protein concentration in the lung lavage fluid (a marker of lung injury) and defined the O_3 response phenotype as the fold-change of protein concentration (O_3 value/FA value). We performed QTL mapping for log-transformed fold-change value and identified a significant QTL on Chromosome 15 (peak location = 48.133 Mb; 95% confidence interval = 36-57 Mb). Estimation of the haplotype effects at this locus indicated a strong positive effect of two of the eight CC founder strain haplotypes (C57BL/6J and CAST/EiJ) on the O_3 response phenotype. Inspection of high-density SNP data of CC founder strains revealed a ~2.5-Mb region of *Mus musculus domesticus* introgression in the CAST/EiJ genome within the locus interval, suggesting a shared genetic basis with C57BL/6J for the similar haplotype effects on phenotype. Merge analysis revealed candidate genes and variants within the locus for future validation.

882C Control of target gene specificity in Wnt signaling by transcription factor interactions Aravinda-Bharathi Ramakrishnan[1], Lisheng Chen[1], Kavya Adiga[1], Ken M. Cadigan[1] 1) University of Michigan, Ann Arbor.

How the Wnt signaling pathway elicits different transcriptional responses in different cells is poorly understood. The “standard model” of Wnt gene regulation is that the stabilization of β-catenin allows it to accumulate and translocate to the nucleus, where it complexes with transcription factors (TFs) of the TCF family to drive Wnt target gene expression. An interplay between TCFs and non-TCF TFs is frequently invoked to reconcile the cell type-specificity of Wnt target genes with the central role of TCFs in the Wnt pathway. We examined a set of Wnt-dependent cis-Regulatory Elements (WREs) to better understand their cis-regulatory grammar.
The first WRE is a novel enhancer near *AXIN2*, a frequent target and feedback inhibitor of Wnt signaling. It is activated by Wnt signaling in several human cell lines and is also highly active in human intestinal organoids. Systematic saturation mutagenesis of this enhancer uncovered a previously uncharacterized DNA motif (termed CAG sites) and binding sites for CDX family TFs as important regulatory elements. We confirmed the role of CDX1 in regulating this enhancer with RNAi and ChIP. We designed point mutations in TCF1 to abolish a reported interaction with CDX TFs and found that in addition to DNA binding, TCF-CDX interactions mediate enhancer activity. Our bioinformatic analysis suggests that co-regulation by CDX and CAG sites is a feature of several WREs.
The second WRE is the promoter of human defensin *5* (*DEFA5*), a gene specifically expressed in Paneth cells of the small intestine. Paneth cells are known to have high levels of both Wnt signaling and SOX9. While most WREs are repressed by the TF SOX9, the *DEFA5* promoter is synergistically activated by SOX9 and Wnt signaling. As expected, it contains functionally essential binding motifs for both TCFs and SOX9. We also identified a TCF-SOX9 protein-protein interaction, and mutations in SOX9 predicted to abolish this interaction prevent it from activating this WRE. Finally, we found a recurring novel CT-rich motif in the *DEFA5* promoter. When combined with TCF and SOX9 binding sites, it results in a synthetic WRE which mimics the regulation of the natural *DEFA5* promoter.
Put together, our results suggest that the identity of non-TCF TFs expressed in each cell and their ability to interact with TCF family members are key determinants of which genes become Wnt targets.

887B Divergence in KRAB zinc finger proteins is associated with pluripotency spectrum in mouse embryonic stem cells *Candice Byers*[1,2], Catrina Spruce[1], Christopher Baker[1,2] 1) The Jackson Laboratory; 2) Tufts Graduate School of Biomedical Sciences.

Noncoding regulatory elements coordinate cell type specific gene expression programs. The majority of disease-associated variants are concentrated in noncoding regulatory elements; however how genetic variation impacts regulatory variation is poorly understood. To begin to address this we developed a cellular systems genetics approach using mouse embryonic stem cells (mESCs). We derived a mapping panel of mESCs from 33 BXD recombinant inbred lines originating from a cross between C57BL/6J (B6) and DBA/2J (D2) mouse strains; which differ in their ability to acquire and maintain naïve pluripotency. Measuring chromatin accessibility (ca) and gene expression (e) in this mapping population maintained in naïve pluripotency, we identified large-scale *cis* and *trans* quantitative trait loci (QTL). Many eQTL and caQTL map to six major distal loci, indicating a common regulatory system driving changes in chromatin and gene expression in *trans*. One prominent distal QTL on Chromosome 13 (Chr 13) has previously been associated with multiple developmental and disease-related phenotypes including auto-immune disorders, craniofacial abnormalities, and limb development. In mESCs, the Chr 13 locus distally controls differential expression of *Obox6* (Chr 7), a gene that supports pluripotency, resulting in high expression when B6 at the QTL and repressed when D2. Using (B6xD2) F1 hybrids and a mouse strain made congenic for the Chr 13 QTL, we confirmed the D2 allele represses distal targets, including *Obox6*. Interestingly, the Chr 13 QTL contains a cluster of genes encoding KRAB zinc finger proteins (KZFPs), which function to repress chromatin. TRIM28, a KZFP co-factor, preferentially binds chromatin at the Chr 13 QTL target sites in D2, including an *Obox6* enhancer, and importantly, lacks binding in B6. This newly emergent Chr 13 KZFP cluster is hyper variable across common inbred strains suggesting rapid evolution. We find that B6, D2, and C57BL/6NJ (NJ) genomes have a ~1Mb structural variant within the QTL. A deletion in NJ and duplication in D2 encompasses a KZFP identified by mediation analysis as the best candidate for *Obox6* regulation. Subsequently, chromatin accessibility at an upstream enhancer and expression of *Obox6* are consistent with an allelic series of copy number variation at Chr 13. Current efforts are focused on confirming the candidate KZFP, determining the role of Chr 13 on 3D genome organization, and testing a functional role of Chr 13 QTL on cell state transitions.

2103C Gene Editing *ELANE* in Human Hematopoietic Stem and Progenitor Cells Reveals Variant Pathogenicity and Therapeutic Strategies for Severe Congenital Neutropenia *Shuquan Rao*[1], Josias Soares de Brito[2], Yao Yao[1], Anna Serbin[3], Qiuming Yao[1], Kevin Luks[2], Yuxuan Wu[1], Jing Zeng[1], Chunyan Ren[1], Ruth Watkinson[4], Myriam Armant[5], Luca Pinello[6], Akiko Shimamura[5], Benhur Lee[4], Scot Wolfe[2], Peter Newburger[2], Daniel Bauer[1] 1) Cancer and Blood Disorders Center, Dana Farber and Boston Children’s Hospital, Boston Children's Hospital / Dana-Farber Cancer Institute, Cambridge, MA ; 2) University of Massachusetts Medical School, Worcester, MA; 3) Harvard College, Cambridge, MA; 4) Mount Sinai School of Medicine,

New York, NY; 5) Boston Children's Hospital, Harvard Medical School, Boston, MA; 6) Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Severe congenital neutropenia (SCN) is a life-threatening disorder of insufficient granulocytes. The most common etiology of SCN is germline *ELANE* mutation, which preserve expression but alter the structure of the neutrophil elastase protein product resulting in misfolding and/or mislocalization with excessive cell death at the promyelocyte/myelocyte stage. However, the underlying mechanisms remain elusive, largely hindering the development of novel therapeutic strategies.
In order to correlate frameshifts in *ELANE* with neutrophil maturation potential *in vitro*, we performed CRIPSR/Cas9 screening with guide RNAs targeting the entire *ELANE* coding region in primary human hematopoietic stem and progenitor cells (HSPCs). We demonstrated that frameshifts within early exons of *ELANE*, which can trigger nonsense mediated decay (NMD) of the *ELANE* transcript, predicted normal neutrophil differentiation, suggesting a universal, highly efficient and simple therapeutic approach for *ELANE*-associated SCN. Using HSPCs from four *ELANE* mutant SCN patient donors, we demonstrated that exon 2 targeting ribonucleoprotein (RNPs) achieve highly efficient editing exceeding 95% indel frequency, trigger *ELANE* transcript decay, and rescue promyelocyte stage maturation arrest. In contrast to *ELANE* early exon editing, frameshifts in *ELANE* late exons, which are supposed to escape nonsense mediated decay, faithfully recapitulated neutrophil maturation arrest.
Using xenograft of NBSGW recipients, we found that indels in *ELANE* exon 5 produced profound neutrophil maturation block, with reduction from 13.4% neutrophils in controls, and 13.7% in *ELANE* exon 2 targeted recipients, to 0.5% neutrophils in *ELANE* exon 5 targeting, with otherwise indistinguishable human monocyte, lymphoid, and erythroid reconstitution. Surprisingly, we found that only -1 frame and not -2 frame induced by gene editing with NHEJ repair led to the SCN-like phenotype, consistent with the clinical observation that all 23 reported naturally occurring SCN-associated *ELANE* frameshift mutations result in -1 but not -2 bp frameshifts. Further investigation indicated that frame -2 mutations in exon 5 results in dramatic protein reduction possibly due to translational repression, suggestive of another promising therapeutic strategy.
Together these results demonstrate that to decrease mutant *ELANE* expression, due to either nonsense mediated decay or translational repression, serves as a highly efficient and universal therapy for *ELANE* mutant SCN, feasible with existing gene editing technology. Moreover, by late exon *ELANE* gene editing we have developed arobust new model of SCN using primary human HSPCs that recapitulates neutropenia *in vivo* following xenotransplant, refines themolecular genetics of mutant *ELANE* induced neutrophil maturation arrest, and offers opportunities to explore novel therapeutic approaches.

2217C Evolutionary genomics of centromeric satellites in House Mice *(Mus)* *Uma Arora*[1,2], Caleigh Charlebois[1], Raman Lawal[1], Beth Dumont[1,2] 1) The Jackson Laboratory, Bar Harbor, ME; 2) Tufts University, Boston, MA.

Centromeres execute a conserved role in kinetochore assembly and chromosome segregation. Despite their important functional roles, centromeres remain gapped on every high-quality mammalian genome assembly. Their repetitive sequence content makes them refractory to analysis using short-read sequencing methods, providing a significant barrier to understanding the scope of sequence diversity across these genomic domains and its functional consequences. To address these critical knowledge gaps, we developed a bioinformatic strategy using whole genome shotgun read libraries to quantify centromere copy number and sequence variation from diverse house mice (genus *Mus*). Our *k*-mer based approach combines the advantages of using a consensus sequence and reference-independent *k*-mer quantification to quantify variation within centromeric satellite sequences. We applied this approach to a sample of 15 laboratory mouse strains and 67 wild-caught mice from 9 diverse mouse (*Mus*) populations and two divergent Mus species (*Mus caroli* and *Mus pahari*). Inbred laboratory strains exhibit striking differences in the relative copy number of core centromere satellite repeats (minor satellite) in their genomes. Surprisingly, centromere satellite copy number divergence does not mirror the known phylogenetic relationships between inbred mouse strains, suggesting a high rate of structural instability that erodes signals of common ancestry. In addition, we observed a higher abundance of centromeric satellite repeats in inbred strains compared to wild caught populations which points towards inbreeding influencing satellite sequence evolution. In addition to copy number differences, our analysis uncovers centromere satellite sequence polymorphisms among house mouse strains and subspecies, revealing substantial turnover of centromere satellite repeat composition on short evolutionary time scales. We were able to *de-novo* assemble centromeric sequences for distant species *Mus caroli* and *Mus pahari* and are the first to report centromeric consensus sequences for *Mus pahari*. Lastly, we uncovered phenotypic associations by correlating chromosomal instability and male reproductive phenotypes with centromeric satellite copy number. This investigation highlights the power of *k*-mer based methods for inferring variation in sequence content and structure of repetitive and dynamic genomic regions and provide the first in-depth, phylogenetic portrait of centromere sequence evolution across *Mus*.

Elevated canonical WNT signalling disrupts heart development and may underlie cases of human Heterotaxy *Kristen Barratt*[1], Kelsey Walsh[1], Koula Diamand[1], Alaa Alzahrani[1], Jehangir Ahmed[1], Kyle Drover[1], Ruth Arkell[1] 1) The Australian National University.

Congenital heart disease (CHD) can occur in isolation or as part of a syndrome such as Heterotaxy, in which the laterality of internal organs is disrupted. Many cardiovascular abnormalities are associated with low heritability, hindering investigations into the underlying genetic causes of CHD. Heterotaxy, the most highly heritable cardiovascular abnormality, is frequently shown to arise from mutation of the ciliome. Mutation of the X-linked transcription factor ZIC3, a member of the Zic family of transcriptional regulators, is associated with both isolated CHDs and Heterotaxy but the cellular and molecular causes underlying ZIC3-associated Heterotaxy remain unknown.

A genetic screen for mutations that affect murine embryogenesis identified a novel null allele of *Zic3*, called katun (*Ka*). *Ka* mutant embryos exhibit Heterotaxy and also incompletely penetrant, partial (posterior) axis duplications and anterior truncation, with the latter two phenotypes redolent of elevated canonical WNT signalling. Previous work has shown that ZIC proteins can interact with TCF proteins to inhibit WNT/β-catenin mediated transcription in model systems. This raises the possibility that dysregulated WNT signalling may underlie some cases of Heterotaxy and CHD.

Using mouse genetics, we found that (i) ZIC3 loss-of-function leads to elevated WNT signalling and (ii) elevated WNT signalling is consistently associated with L-R axis and cardiac situs abnormalities in the absence of pronounced cilia defects. Detailed phenotyping and preliminary RNA-seq analysis of *Ka* mutant embryos showed that, during gastrulation, prospective definitive endoderm (DE) cells egress from the nascent mesoderm but fail to complete the mesoderm to epithelial transition and do not assemble basal basement membrane. Subsequently, DE cells do not completely clear from the emerging node, disturbing node morphogenesis and, presumably, impairing nodal flow resulting in L-R axis and cardiac situs abnormalities.

This work reveals that a specific dose of WNT activity is required for correct DE formation and is a pre-requisite for L-R axis establishment. Furthermore, it implicates genes involved in WNT signalling and DE formation as novel candidates for human CHD variants.

Exploring the Genetic Basis for Atrioventricular Septal Defects in Down Syndrome yicong li[1], peter anderson[1], xihe liu[1], anna moyer[1], sean murphy[1], liliana florea[1], chulan kwon[1], roger reeves[1] 1) Johns Hopkins University School of Medicine.

AVSD is the most frequent form of congenital heart disease in individuals with trisomy 21 and occurs through malformation of the Dorsal Mesenchymal Protrusion (DMP) in the heart. The DMP is derived from a group of migratory cardiac progenitor cells in the second heart field (SHF). The trisomic gene or genes that affect this process are not known. Down-regulation of the Sonic hedgehog (SHH) signaling pathway also interferes with normal DMP formation. In this study, we have suppressed hedgehog signaling pathway via removal of one or both copies of the pathway activator, *Smo*, specifically in the SHF of a Down syndrome mouse model. The Trisomic; *Smo-/-*genotype provides the first model in which 100% of pups develop AVSD ("AVSD mice"). AVSD mice overcome the problem of incomplete AVSD penetrance, that is, in other models it cannot be determined which E9.5 embryos will have AVSD and which will not. We used this model to understand the genetic basis for AVSD mice in which genetically marked, migrating anterior SHF cells were isolated by FACS for RNAseq. We identified 1615 transcript that are significantly different between AVSD mice and those that will form a normal DMP. Twelve of these genes were among 37 AVSD risk genes identified in the GeneCard database.
Anterior SHF cells expressed 66 of the 115 trisomic genes. Among these, transcripts of two genes, *Grik1* and *Ripk4*, are elevated substantially beyond the expected 1.5 fold level in AVSD mice. When *Grik1* and/or *Ripk4* cDNAs were transfected into cardiac progenitor cells, the SHH pathway was down-regulated. When transfected into C3H10T cells, SHH-dependent differentiation was diminished, suggesting a causative role of these genes in SHH down regulation due to trisomy.

Mouse models predict drug combinations to target oncogenes and tumor suppressors *Tyler Peat*[1,2], Snehal Gaikwad[1], Megan Andres[1], Andrew Taylor[3], Andrew Girvin[3], John Simmon[4], Craig Thomas[5], Aleksandra Michalowski[1], Beverly Mock[1] 1) Laboratory of Cancer Biology and Genetics, CCR, NCI, Bethesda MD; 2) Department of Comparative Pathobiology, Purdue University, West Lafayette IN; 3) Palantir Technologies, Palo Alto CA; 4) Personal Genome Diagnostics, Baltimore MD; 5) Chemical Genomics Center, Division of Preclinical Innovation, NCATS, Bethesda MD.

Multiple Myeloma (MM) is a neoplasm involving plasma cells in the bone marrow. Drug resistance and progression are common, underscoring the need for new drug combinations. Herein, we utilized an NCATS high-throughput screen of ~1900 small molecules to limit growth of 47 human MM cell lines. *In silico* robust regression analysis of drug responses revealed 43 potential cooperative combinations. We hypothesized that effective combinations would reduce oncogene expression and/or enhance tumor suppressor gene activity based on our earlier genetic studies which identified CDKN2A (p16INK4a) and MTOR as tumor susceptibility/resistance loci. Candidate combinations were evaluated for cooperative reductions in MYC protein expression in MM cells treated at IC50 doses of each drug. Ten combinations synergistically reduced MYC expression, which is frequently over-expressed in MM; these combinations affected several targets (CDK, HDAC, AURKA, HSP90AA1, ITK, TUBB, PLK1, TOP2A, MCL1, EGFR). Cooperative reductions in viability were observed with three top combinations in proteasome inhibitor-resistant and sensitive MM cell lines but did not limit normal fibroblast viability at similar concentrations. The combinations cooperatively increased p16INK4a and RB activity in MM cell lines, while also enhancing cleaved caspase 3, leading to increased apoptosis. To expand on the *in vitro* findings, combination-association survival was evaluated in a transplantable Ras-driven allograft model of advanced MM which closely recapitulates myeloma in humans. All three combinations significantly prolonged survival in sublethally irradiated C57BL/6 mice injected intracardiac with donor MM cells compared to control mice (n = 6). Furthermore, the top combination was assessed via 5-day target engagement and long-term survival in NSG mice bearing subcutaneous MM xenografts, the same transplantable Ras-driven model of advanced MM, and in BALB/c-Bcl-xL transgenic mice bearing plasmacytomas. The combination cooperatively reduced MYC expression in xenografts while increasing RB/p16INK4a activity and histone H3 acetylation. Furthermore, and most importantly, the combination prolonged survival compared to single agents and control in all three mouse models. These data identify potentially useful drug combinations for preclinical evaluation in drug-resistant MM and may ultimately reveal novel mechanisms of combined drug sensitivity.

Phase separation of YAP reorganizes genome topology for long-term YAP target gene expression *Danfeng Cai*[1], Peng Dong[1], Daniel Feliciano[2], Natalie Porat-Shliom[2], Zhe Liu[1], Jennifer Lippincott-Schwartz[1] 1) Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA; 2) Thoracic and GI Malignancies Branch, National Cancer Institute, National Institute of Health, Bethesda, MD.

Yes-associated protein (YAP) is a transcriptional co-activator that regulates cell proliferation and survival by binding to a select set of enhancers for target gene activation. How YAP coordinates these transcriptional responses is unknown. Here, we demonstrate that YAP forms liquid-like condensates in the nucleus. Formed within seconds of hyperosmotic stress, YAP condensates compartmentalized YAP's transcription factor TEAD1 and other YAP-related co-activators, including TAZ, and subsequently induced transcription of YAP-specific proliferation genes. Super-resolution imaging using Assay for Transposase Accessible Chromatin with photoactivated localization microscopy (ATAC-PALM) revealed that YAP nuclear condensates were areas enriched in accessible chromatin domains organized as super-enhancers. Initially devoid of RNA Polymerase II (RNAPII), the accessible chromatin domains later acquired RNAPII, transcribing RNA. Removal of YAP's intrinsically-disordered transcription activation domain (TAD) prevented YAP condensate formation and diminished downstream YAP signaling. Thus, dynamic changes in genome organization and gene activation during YAP reprogramming is mediated by liquid-liquid phase separation.

Proteomics reveals the role of translational regulation in ES cells *Selcan Aydin*[1], Duy Pham[1], Daniel A. Skelly[1], Matthew Pankratz[2], Devin K. Porter[2], Tian Zhang[3], Ted Choi[3], Steven Gygi[2], Laura Reinholdt[1], Christopher L. Baker[1], Gary A. Churchill[1], Steven C. Munger[1] 1) The Jackson Laboratory for Mouse Genetics, Bar Harbor, ME; 2) Predictive Biology, Inc., Carlsbad, CA; 3) Harvard Medical School, Boston, MA.

Genetic background is known to affect pluripotency in embryonic stem cells (ESCs) but most studies to date have been conducted on a limited number of cell lines. We recently performed a genetic analysis of gene expression and chromatin accessibility in a large panel of ESCs derived from genetically heterogeneous Diversity Outbred (DO) mice, and identified thousands of loci that were associated with differences in chromatin state and transcript abundance among DO ESC lines. Here, we are further integrating the genetic analysis of the ESC proteome to extend our investigation into the role of genetic variation on pluripotency. We used multiplexed proteomics to measure global protein abundance in each DO ESC line, and compared protein and transcript abundance across genes and lines. Overall, protein abundance was highly variable across cell lines, similar to our observation in the transcriptome. We identified genetic background and sex as major drivers of this variation. We integrated genotyping data to our proteomic dataset, and mapped thousands of quantitative trait loci that affect protein levels (pQTL). Most local pQTL also had a corresponding expression QTL (eQTL), suggesting a variant with transcriptional effects that can be observed at both the transcript and protein levels. In contrast,

only a small number of distant pQTL showed similar effects on transcript abundance. Of note, we identified several pQTL "hotspots", loci where many distant QTL co-localize and likely harbor a shared regulator. Some QTL hotspots were observed in both datasets, while others were pQTL-specific and likely indicate post-transcriptional regulatory mechanisms. Next, we applied mediation analysis to predict the causal genes modulating pQTL hotspots. We identified *Lifr* expression as the causal regulator of a large shared eQTL/pQTL hotspot on Chr 15, validating our previous finding that a single enhancer variant affected *Lifr* expression and ground-state pluripotency. Moreover, mediation analysis identified *Eef1a1* protein abundance as the top candidate regulator for a pQTL-specific hotspot on Chr 9 that controlled the abundance of many protein with roles in translation. Further, *Eef1a1* protein abundance was strongly inversely correlated with several regions of open chromatin near the pQTL hotspot, suggesting that increased EEF1A1 and/or translation-related proteins may feedback negatively on local chromatin state. Future efforts will experimentally validate other candidate regulators and better define their specific roles in pluripotency maintenance. This analysis combining measurements across molecular levels from a large number of genetically diverse ESCs will allow us to improve our understanding of the robust regulatory circuitry governing pluripotency and differentiation, and characterize the effects of genetic variation on these critical cellular processes.

Reference quality mouse genomes reveal complete strain-specific haplotypes and novel functional loci *Mohab Helmy*[1], Anu Shivalikanjli[1], Jingtao Lilue[1], David J. Adams[2], Thomas M. Keane[1] 1) European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge, UK; 2) Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK.

For over a century, different mouse strains have been used to model human disease, leading to many fundamental discoveries about mammalian biology and the development of new therapies. However, until recently only the C57BL/6J strain was subject to complete genome sequencing despite the presence of a substantial amount of phenotypic variations between strains. Using second generation sequencing, we previously reported draft *de novo* genome assemblies for 16 widely used mouse strains and found extensive strain-specific haplotype variation. We identified and characterised 2,567 regions on the current mouse reference genome exhibiting the greatest sequence diversity. These regions are enriched for genes involved in pathogen defence and immunity and exhibit enrichment of transposable elements and signatures of recent retrotransposition events. Combinations of alleles and genes unique to an individual strain are commonly observed at these loci, reflecting distinct strain phenotypes. Recent advances in sequencing technologies made it feasible to obtain significantly longer reads that can be used to produce highly contagious genomes. We present the first results of the *de novo* assembly of reference quality genomes for the 16 mouse strains using third generation long read sequencing. These assemblies are three orders of magnitude more contiguous than current draft assemblies and provide accurate and complete representation of the most complex regions of the mouse genome, illustrating striking coding and regulatory variation. The availability of these sequences can maximise the potential of mouse strains as animal models as well as providing novel insights into their evolutionary history.

Wednesday, April 22 10:45 AM - 1:10 PM

Opening Keynote Session and GSA Award Presentations

1 Cellular Biographies: Reconstructing zebrafish development *Alex Schier*[1] 1) Harvard University.

abstract is not available at the time of print

2 Detecting and Correcting Errors in Mitosis *Sue Biggins*[1] 1) Fred Hutchinson Cancer Research Center.

abstract is not available at the time of print

3 2019 Elizabeth Jones Award for Excellence in Education *Bruce Weir*[1] 1) University of Washington.

abstract is not available at the time of print

4 2019 George Beadle Award *Michael Snyder*[1] 1) Stanford University.

abstract is not available at the time of print

5 Haystack to needle: moving from quantitative to developmental genetics of a reproductive trait *Cassandra Extavour*[1] 1) Harvard University.

abstract is not available at the time of print

6 How can biology and breeding contribute to improving food systems and climate change? *Edward Buckler*[1] 1) Cornell University.

The demands of food production, fuels, nutrition, and climate change are going to require that thousands of species undergo genomic selection over the next two decades. The approaches for making genomic selection models are too inefficient for scaling to thousands of species. Here, we propose how to use evolution and machine learning of the functional elements to begin developing robust models that work across species. Evolutionary comparisons over 20 million years are efficiently identifying key distal regulatory elements. Maize and Arabidopsis diverged 140 million years ago, yet our initial machine learning models predicting gene expression have significant transferability. Finally, models trained in bacteria and archaea are showing promise for understanding plant protein adaptations. The most significant obstacle to building transferable models may be the social aspect of getting disparate communities of scientists to make mutually interoperable and transferable models.

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Development and Cell Biology (*C. elegans*)

7 Different paths to the same cell type *Karolina Mizeracka*[1, 2], Julia Rogers[1], Shai Shaham[3], Martha Bulyk[1], Maxwell Heiman[1,2] 1) Boston Children's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) The Rockefeller University, New York, NY.

In the classical view of cell differentiation, a given cell type arises from a unique lineage through the execution of a unique transcriptional program. In contrast, during the poorly understood process of "convergent differentiation", the same cell type is produced by distinct, unrelated develop-

mental lineages. Convergent differentiation has recently been discovered to be prevalent across species, and has been appreciated for decades in *C. elegans*. For example, many glial cell types are radially symmetric, with the dorsal, lateral, and ventral equivalents arising from divergent lineages. Despite this longstanding observation, the fate specification paths that lead to the production of the same cell type remain unknown. Through an unbiased genetic screen, we found that the conserved Forkhead transcription factor UNC-130 is required for the specification of the dorsal inner labial socket (ILsoD) glia, but not for their ventral or lateral counterparts which arise from different lineages. UNC-130 is also important for other cell types produced by the ILsoD sublineage, including another glial type and sensory neurons as has been shown previously. We identified point mutations in the UNC-130 DNA-binding domain that revealed that UNC-130 binds a canonical Forkhead motif and that graded defects in UNC-130:DNA binding correspond to partial specification defects. Although cell differentiation is often controlled by transcriptional activators, we show that UNC-130 acts as a transcriptional repressor via two independent domains to promote fate specification. In its absence, regulators of alternative fates, UNC-86 and RNT-1, may be spuriously mis-expressed, as loss of these factors in *unc-130* mutants partially rescues glial specification defects. In addition, UNC-130 can be functionally replaced by its human homolog, FoxD3. Interestingly, FoxD3 also acts in a lineage-specific manner in vertebrates, promoting the specification of glia and sensory neurons as in *C. elegans*. Thus, in contrast to "terminal selectors," which are required to specify discrete cell types, UNC-130 acts as a "lineage selector," enabling progenitor cells in a specific lineage to generate the correct assortment of cell types. These findings provide evidence that the same cell type can be achieved through multiple, lineage-specific differentiation trajectories.

8 The role of cell cycle in invasive differentiation behavior of the *C. elegans* anchor cell Taylor N. Medwig-Kinney[1], Jayson J. Smith[1], Nicholas J. Palmisano[1], Sujata Tank[1,2], Sydney S. Sirota[1], Wan Zhang[1], David Matus[1] 1) Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY; 2) Science and Technology Research Program, Smithtown High School East, St. James, NY.

The ability of cells to invade basement membranes is a key part of fertility, development, immunity, and disease. Our group has previously shown that cell cycle arrest is required for cell invasion. However, the mechanism of how cell cycle control interfaces with the transcriptional programs that regulate cell invasive differentiation is not well understood. Basement membrane invasion by the *C. elegans* anchor cell during uterine-vulval morphogenesis provides a genetically tractable and visually amenable model in which to study invasive differentiation *in vivo*. Using endogenously-tagged transcription factors paired with RNA interference, we dissected the gene regulatory network that underlies anchor cell invasion. We find that *egl-43* (EVI1/MEL) and *hlh-2* (E/Daughterless) form a feed-forward loop with *nhr-67* (NR2E1/Tailless/TLX) to maintain the anchor cell in a post-mitotic state through regulation of CKI-1 (p21/p27). This circuit is largely parallel to the cell cycle-independent pro-invasive pathway involving *fos-1* (Fos). However, prior to specification of the anchor cell, *egl-43*, *hlh-2*, and *nhr-67* function independently to regulate the Notch-mediated cell fate decision that specifies two alternative uterine fates, the invasive anchor cell and a stem lineage for the ventral gonad, the ventral uterine fate. Together, these results demonstrate that a gene regulatory network can be rapidly assembled to reinforce the pro-invasive, post-mitotic state of the anchor cell. Finally, as recent published work has shown that the asymmetric onset of *hlh-2* expression is predictive of ventral uterine cell fate, we explore if a similar asynchrony in controlling *nhr-67* expression and cell cycle state is predictive of anchor cell fate.

9 Single-zygote analysis of protein quantitation reveals high robustness of cell polarisation and asymmetric division to perturbations in PAR protein abundance *Nelio Rodrigues*[1], Tom Bland[1], Nate Goehring[1,2] 1) Francis Crick Institute, London; 2) MRC Laboratory for Molecular Cell Biology, University College London, London.

Early development of the *C. elegans* embryo relies critically on the ability of early blastomeres to polarize and thereby drive asymmetric divisions required to specify divergent cell fates. Cell polarity is best understood in the zygote, where PAR proteins orchestrate a characteristic asymmetric division that results in a highly reproducible difference in the size and fate of resulting daughter cells AB and P1. PAR polarity in the zygote depends on the balance between two opposing sets of PAR proteins – aPARs and pPARs – that define the anterior and posterior of the zygote, respectively. As predicted by this model, RNAi and genetic suppression experiments show that polarity is sensitive to the relative balance between aPAR and pPAR protein abundance. At the same time, heterozygous *par* mutants show minimal defects in polarity and are viable, suggesting that polarity in the zygote is highly robust. We hypothesized that this robustness could arise either through the homeostatic control of PAR protein levels (i.e. dosage compensation), through an intrinsic robustness of the polarity network itself or in how PAR polarity is interpreted by downstream pathways. One challenge in testing these hypotheses is the inability to directly assess protein abundance in individual zygotes. We have developed a simple microscopy-based method for protein dosage quantification, where we can gauge and discard the contribution of autofluorescence observed in individual embryos expressing fluorescently-tagged proteins. This allows us to carefully relate protein abundance to phenotype on an embryo-to-embryo basis. Our data argue against dosage compensation as an explanation for robustness to perturbations in gene expression. Our data indicate that robustness is engineered into the cell polarity and fate specification network at multiple levels, rendering network output highly insensitive to significant changes in the PAR protein balance.

10 PAR polarity proteins direct intracellular tube expansion through apical recruitment of the exocyst complex *Joshua Abrams*[1], Jeremy Nance[1] 1) Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, NY.

Organs are comprised of various tubes with distinct cellular compositions. The smallest of these tubes are unicellular and can be seamless, with a lumen extending through the center of the cytoplasm. To initiate and expand their lumen, seamless tubes are thought to direct vesicles to the luminal surface. But how vesicles are targeted to the luminal surface is poorly understood. We show that seamless tube expansion in the *C. elegans* excretory canal relies on a precise interplay between PAR polarity proteins (PAR-3, PAR-6, PKC-3, and CDC-42) and the exocyst vesicle-tethering complex at the luminal surface. Previously, we found that PAR proteins and exocyst are both enriched at the luminal surface of the canal. However, their roles during seamless tube growth could not be addressed because embryos lacking PAR proteins or the exocyst arrest. To overcome this, we devised a strategy to conditionally deplete these proteins in the excretory canal using the ZF1 degron method (Armenti et al. 2014). We endogenously tagged PARs and exocyst with the ZF1 domain and conditionally degraded members of each protein complex to determine their role during lumen growth. Canal-specific depletion of PAR-6 caused severe tube outgrowth defects, whereas PAR-3 depletion caused less severe early defects that resolve over time. Depletion of SEC-5, a core exocyst protein, resulted in severe tube defects similar to PAR-6, suggesting that PAR-6 may act to position the exocyst complex. To test whether PAR-6 recruits the exocyst to the luminal surface, we employed a heat shock strategy to degrade PAR-6 after canal development completed, and then examined exocyst localization. Indeed, PAR-6 depletion resulted in a loss of apical exocyst localization at the lumen. Interestingly, PAR-6 itself was reduced at the apical membrane in the absence of PAR-3, suggesting that PAR-3 may contribute to PAR-6 positioning at the lumen. In addition, depletion of the small GTPase CDC-42 caused tube defects similar to loss of PAR-6, and loss of EXC-5 (a CDC-42 GEF) caused mislocalization of the PAR-6-binding protein PKC-3. These observations suggest that active CDC-42 recruits PAR-6/PKC-3 apically

and PAR-3 acts independently to further enrich PAR-6/PKC-3 along the apical membrane. The exocyst complex is then recruited to these luminal sites where vesicle tethering and seamless tube expansion proceeds. These findings provide a molecular pathway for intracellular lumen extension.

11 Multi-tissue patterning drives anterior morphogenesis in the *C. elegans* embryo *Alisa Piekny*[1], Stephanie Grimbert[1], Karina Mastronardi[1], Ryan Christensen[2], David Fay[3] 1) Concordia University; 2) NIH; 3) University of Wyoming.

We provide the first detailed description of anterior morphogenesis in *C. elegans*. Morphogenetic events are driven by coordinated cell shape changes, migration and adhesion, which are challenging to study when multiple cell-types are involved. *C. elegans* is one of the few systems where morphogenesis can be studied at the cellular level. For example, prior studies revealed how epidermal morphogenesis is regulated by cues from the neuroblasts and muscle tissue. However, anterior morphogenesis is currently a black box. As the ventral epidermal cells enclose the embryo, neuroblasts are exposed at the anterior. The epidermal cells migrate over them and ultimately align and connect with the developing pharynx to form the anterior lumen. But it was not known how all of these cell types are coordinated. We used different types of microscopy including sweptfield confocal and diSPIM imaging to characterize the movements and patterns of neuroblasts, pharyngeal and epidermal cells from the onset of ventral enclosure to the 1.7-fold stage. We found that some of these patterns control the placement and polarization of the anterior lumen.
The first noticeable mark of the future lumen is a bright focal point labeled by PAR-6 that appears at the onset of anterior epidermal cell migration. We propose that this focal point corresponds to projections from a subset of the pharyngeal arcade cells; a small group of PHA-4+ve cells which are initially close to the surface, but then move in to a depth of ~4-5 um and form a stable rosette. Neuroblasts also migrate and form distinctive patterns; as the epidermal cells migrate anteriorly, a subset of neuroblasts move into the embryo. Another subset of neuroblasts marked by PAR-6 form a line of foci that are ventrally positioned, while other PAR-6+ve foci form two pentagons that are more dorsally positioned. The ventral epidermal cells migrate toward, but never cross the ventral line, while the dorsal epidermal cells migrate in close proximity with the pentagons. These foci ultimately align to form two proximal rows, with the central focal point in the middle, surrounded by rings of epidermal cells. Disrupting the neuroblasts that form the ventral line or pentagons cause a range of phenotypes that includes a delay in epidermal cell migration, and failure to polarize the anterior lumen. However, disrupting the epidermal cells causes mis-positioning of the anterior lumen. We are now positioned to explore the detailed mechanisms controlling the observed cell patterns.

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Disease Models and Aging (Mammal)

17 Generation of a robust and clinically-relevant mouse model of Cerebral Cavernous Malformations *Douglas Marchuk*[1], Matthew Detter[1], Christopher Benavides[1], Rhonda Lightle[2], Catherine Neilson[1], Robert Shenkar[2], Thomas Moore[2], Issam Awad[2] 1) Duke University; 2) University of Chicago.

Cerebral cavernous malformations (CCMs) are ectatic, capillary-venous vessels with disrupted endothelial barrier leading to hemorrhage, seizures, and focal neurological deficits. CCMs develop sporadically or in autosomal-dominant inheritance due to germline mutation in one of three genes. By examining surgically-resected CCMs, we have previously shown that individual malformations develop after bi-allelic inactivating mutations in one of the genes. This two-hit paradigm has led to the development of mouse models of CCM using cre/loxP technology for temporal (tamoxifen-activated CreERT2) and endothelial cell specific Ccm gene deletion. The rapid development of CCMs enables mechanistic studies of CCM development and has identified several signaling pathways dysregulated in CCM formation. The drawback of this model is that the induced CCMs do not develop all of the pathologic features observed in human CCMs. The CCMs in the current inducible model do not hemorrhage, are anatomically restricted to the cerebellum, and result in early lethality. We have now generated a variation of the cre/loxP inducible model that results in CCMs with many of the desired characteristics lacking in the original model. The development of CCMs nearly exclusively in the cerebellum of the existing mouse model is attributed to an “angiogenic window,” between postnatal day 0 and 3, when deletion of Ccm genes induces a robust phenotype. By utilizing the same transgenic mice, but inducing gene loss slightly after this angiogenic window, we have created a novel inducible mouse model that develops CCMs throughout the brain, brainstem, and spinal cord while also improving viability. These malformations also exhibit many of the same pathologic features as severe human CCMs, including a multi-cavernous morphology, hemorrhage, and an inflammatory infiltrate. This improved model will be a valuable resource for investigating a critical, and previously understudied period of disease – CCM maturation. The improved robustness, reproducibility, and viability of this model will enable us to administer a pharmacologic inhibitor after, rather than before, CCMs have developed. We have tested one potential therapy using this model and several more are underway.

18 *ZNF423* patient variants, truncations, and in-frame deletions in mice define an allele and domain-dependent series of midline brain abnormalities. *Bruce Hamilton*[1], Ojas Deshpande[1], Dorothy Concepcion[1], Raquel Lara[1], Oliver Zhang[1] 1) UC San Diego.

Interpreting rare variants remains a challenge in personal genomics, especially for disorders with multiple causal genes and for genes that contribute to multiple disorders. *ZNF423* encodes a 30-zinc finger protein that interacts with lineage-dependent (EBF) and signaling-dependent (SMAD, RAR, NICD) transcription factors to regulate developmental choices, including SHH response, in several precursor cell populations. *ZNF423* is implicated in Joubert syndrome related disorders, consistent with midline brain defects in *Zfp423*-mutant mice, but pathogenic potential of most patient variants remains uncertain. We engineered patient-derived variants and small deletions into the highly-conserved mouse ortholog and examined neuroanatomical outcome measures across nearly 800 littermate pairs. Three substitutions previously asserted pathogenic appeared benign, while a fourth (H1277Y) was effectively null. An early premature termination codon (PTC) variant (D70Vfs*6) escaped nonsense mediated decay to produce a partial protein and hypomorphic function. All other PTC variants, including patient frameshift variants with humanized coding potential, behaved as simple nulls. PTC heterozygotes showed mild haploabnormality independent of position, consistent with loss-of-function intolerance inferred from human population data, but inconsistent with a proposed dominant negative activity. In-frame deletions of select zinc fingers showed mild to moderate brain abnormalities, as did variants that reduced protein expression. These results affirm the need for functional validation of rare variants and demonstrate cost-effective modeling of neuroanatomical abnormalities.

19 Mouse models of an undiagnosed pediatric neurodegenerative disorder *Jay Vivian*[1,2], Katelin Gibson[1], Wenjing Fausnett[1], Michelle Winter[1], Linda Eggiman[1], Sarah Tague[1], Sarah Soden[2], Neil Miller[2], Kenneth McCarson[1], Peter Smith[1] 1) University of Kansas Medical Center, Kansas City KS; 2) Children's Research Institute, Children's Mercy Kansas City, Kansas City MO.

High-throughput genomic screening of patients have been instrumental in helping us understand the underlying basis for many rare disorders. Efforts must also focus on functional genomics approaches and the development of models that replicate phenotypes at the whole organism level. This study emanates from clinical assessments of a child enrolled in the NIH Undiagnosed Disease Program with a progressive neurodegenerative phenotype. This manifested as early motor delays that progressed to loss of language and motor skills and ultimately death at 9 years of age due to respiratory compromise. Our analysis of the patient's and family's genomes identified two novel variants in the *ACER3* locus in this patient, as a compound heterozygote: T133I missense variant; and a SNP variant in the splice acceptor of a downstream exon. ACER3 is a member of a family of alkaline ceramidases, and is broadly expressed. These family members maintain sphingolipid homeostasis by catalyzing the conversion of ceramides to sphingosine.
To further understand the functional consequences of these *ACER3* variants and their potential role in this patient's neurological deficits, we have recently generated mouse models, via CRISPR-Cas9 genome editing, which harbor point mutations that recapitulate the patient-derived ACER3 variants. These *Acer3* mutant mice display a progressive loss of motor function and coordination assessed via whole animal behavioral testing, including deficits in rotarod and beam walk tests. RNASeq and immunofluorescence analysis of spinal cords of the ACER3 mutant mice revealed a strong upregulation of genes associated with a neuroinflammatory response, including microglial activation and astrogliosis. These mutant mice provide compelling evidence that the *ACER3* variants are deleterious the likely cause of the patient's neurodegeneration, and suggest neuroinflammation may be a component of the progressive disorder. These studies provide a mechanistic roadmap for how an altered ceramide-sphingosine biochemical axis disrupts neuronal homeostasis, neuroinflammatory response, and motor function. As these mutant mice are relevant disease models for this and possibly other neurodegenerative disorders, we are exploring their use in preclinical studies to guide potential therapeutic interventions.

20 Analyzing Hematology by Complete Blood Count in a Genetically Diverse Mouse Population: Changes with Age and Impacts on Mortality Risk
Andrew Deighan[1], Ron Korstanje[1], Luanne Peters[1], Gary Churchill[1] 1) Jackson Laboratory, Bar Harbor, ME.

The complete blood count (CBC) is a simple and routinely performed procedure that provides general information on the hematologic status of the subject. Changes in CBC parameters with age and their usefulness as risk factors for mortality and morbidity have been extensively documented in humans. Here we characterize how CBC parameters change with age in diversity outbred (DO) mice and how they impact survival. The parameters that affect mortality risk in DO mice are similar to those in humans. As with humans, we are able to make informed predictions on short- and medium-term mortality, but the data are less informative regarding and long-term mortality. Finally, we use the the genetic diversity present in the DO stock to identify genetic loci for several CBC parameters.

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Plenary Session and Larry Sandler Award Lecture (*Drosophila*)

12 Meiotic drive and satellite DNA in *Drosophila melanogaster* Xiaolu Wei[1], Ching-Ho Chang[2], Beatriz Navarro Dominguez[2], Marion Herbette[3], Raphaelle Dubruille[3], Benjamin Loppin[3], *Amanda Larracuente*[2] 1) University of Rochester Medical Center, Rochester, NY; 2) University of Rochester, Rochester, NY; 3) University of Lyon, Lyon, France.

Conflicts arise within genomes when genetic elements are selfish and fail to play by the rules. Meiotic drivers are selfish genetic elements found in a wide variety of taxa that cheat meiosis to bias their transmission to the next generation. One of the best-studied drive systems is an autosomal male driver found on the 2nd chromosome of *Drosophila melanogaster* called *Segregation Distorter* (*SD*). Males heterozygous for *SD* and sensitive wild type chromosomes transmit *SD* to >95% of their progeny, whereas female heterozygotes transmit *SD* fairly, to 50% of their progeny. *SD* is a sperm killer that targets sperm with large blocks of tandem satellite repeats (called *Responder*) for destruction through a chromatin condensation defect after meiosis. The molecular mechanism of drive is unknown. We combine genomic, cytological, and molecular methods to study the interactions between the driver and the target satellite DNA in this system. We uncover defects in the histone-to-protamine transition and effects on satellite-derived transcripts in *SD* testes. We will discuss the impact of *SD* on the regulation of the *Responder* satellite, and how this provides insight into mechanisms of drive.

13 GCNA preserves genome integrity and fertility across species Varsha Bhargava[1], Courtney Goldstein[1], Logan Russell[2], Lin Xu[1], Murtaza Ahmed[1], Wei Li[3], Amanda Casey[1], Kelly Servage[1], Rahul Kollipara[1], Zachary Picciarelli[2], Ralf Kittler[1], Alexander Yatsenko[2], Michelle Carmell[4], Kim Orth[1], James Amatruda[5], Judith Yanowitz[2], *Michael Buszczak*[1] 1) UT Southwestern Medical Center, Dallas, TX; 2) Magee-Womens Research Institute and University of Pittsburgh School of Medicine, Pittsburgh, PA; 3) Tsinghua University, Beijing, PRC; 4) University of Massachusetts Medical School, Worcester, MA; 5) Keck School of Medicine of the University of Southern California, Los Angeles, CA .

The propagation of species depends on the ability of germ cells to protect their genome from a variety of exogenous and endogenous threats. While these cells employ ubiquitous repair pathways, specialized mechanisms that ensure high-fidelity replication, chromosome segregation, and repair of germ cell genomes remain incompletely understood. We have identified Germ Cell Nuclear Acidic Peptidase (GCNA) as a conserved regulator of genome stability in flies, worms, zebrafish and human germ cell tumors. GCNA contains an acidic intrinsically disordered region (IDR) and a protease-like SprT domain. In addition to chromosomal instability and replication stress, *Gcna* mutants accumulate DNA-protein crosslinks (DPCs). GCNA acts in parallel with a second SprT domain protein Spartan. Structural analysis reveals that while the SprT domain is needed to limit DNA damage, the IDR controls proper chromosome segregation in germ cells and early embryos. This work shows that GCNA protects germ cells from various sources of damage, providing insights into conserved mechanisms that promote genome integrity across generations.

14 Larry Sandler Award Presentation *Barbara Mellone*

abstract is not available at the time of print

15 Larry Sandler Award Talk *Balint Kacsoh*[1] 1) Perelman School of Medicine, University of Pennsylvania.

abstract is not available at the time of print

16 Active genetics comes alive *Ethan Bier*[1] 1) Univ California, San Diego.

Active genetic elements are transmitted during reproduction at greater than expected Mendelian frequencies. Such "super-Mendelian" inheritance can be used for a variety of applications including: gene-drive systems for disseminating beneficial traits throughout insect or mammalian populations (e.g., spreading immunizing factors that prevent mosquitoes from transmitting malarial parasites or immunizing endangered mammalian species against prevalent pathogens), reversing insecticide resistance, devising elements that can eliminate or inactivate gene drives, creating active genetic modifications that facilitate breeding by bypassing constraints imposed by independent assortment and linkage, and development of self-amplifying systems in bacteria to scrub antibiotic resistance factors from the environment or potentially from patients with chronic bacterial infections, and engineering next-generation genetic circuits for synthetic biology.

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Yeast Genetics Meeting Lifetime Achievement Award and the Yeast Gimme a Break: Chromosome Stability in Stress and Development Session

28 Introduction of Nancy Kleckner for the Yeast Genetics Meeting Lifetime Achievement Award *Eric Alani*[1] 1) Cornell University.

abstract is not available at the time of print

29 Meiotic and Mitotic Chromosomes *Nancy Kleckner*[1] 1) Harvard University.

abstract is not available at the time of print

30 GLOE-Seq – a new genomic tool to map replication patterns and DNA lesions with nucleotide resolution Annie M. Sriramachandran[1], Giuseppe Petrosino[1], María Méndez-Lago[1], Axel J. Schäfer[1], Liliana S. Batista-Nascimento[1], Nicola Zilio[1], Helle D. Ulrich[1] 1) Institute of Molecular Biology gGmbH (IMB).

DNA single-strand breaks (SSBs) are among the most common lesions in the genome, arising both spontaneously and as intermediates of many DNA transactions. Nevertheless, in contrast to double-strand breaks, their distribution in the genome has rarely been addressed in a meaningful way. We now present a technique based on the Genome-wide Ligation of 3'-OH Ends (GLOE-Seq) designed for mapping SSBs with nucleotide resolution, but versatile enough to be applied to any lesion convertible into a free 3'-OH terminus. We have assessed the accuracy and sensitivity of GLOE-Seq with *in vitro* digested DNA and benchmarked its performance by comparison with an established method for the mapping of base lesions. We demonstrate its applicability to physiological DNA damage, introduced *in vivo* by ultraviolet (UV) irradiation or by a site-specific endonuclease in budding yeast. In addition, we show how the unique feature of GLOE-Seq, its ability to map pre-existing SSBs, can be used to analyze Okazaki fragments, spontaneous breaks and nicks as well as DNA repair intermediates. Our analysis validates GLOE-Seq as a versatile method for the genome-wide high-resolution mapping of a range of DNA lesions that promises to provide insight into the yet poorly understood characteristics of SSBs in the genome.

31 Systemic Aneuploidization of the Yeast Genome Lydia R Heasley[1], Ruth Watson[1], J Lucas Argueso[1] 1) Colorado State University.

The rate and patterns by which cells acquire mutations profoundly shape their evolutionary trajectories and phenotypic potential. Darwinian paradigms posit that genomic change occurs incrementally and gradually. Consequently, we usually interpret the genomic variations observed within and between populations as the result of small changes acquired randomly and independently over very long periods of time. While this linear mode of mutation certainly contributes to genome evolution, cells can also experience mutagenic processes that drive *rapid genome evolution*. One such process manifests as *saltational bursts of genomic instability,* in which during a transient episode, a genome acquires multiple mutations simultaneously, but then returns to a state of stability. Recent genomic analyses of expanding populations support the emerging model that these bursts play a substantial role in creating intra-population genetic diversity. But, the molecular causes, mutational signatures, and phenotypic outcomes of these events remain entirely unknown, in part because a suitable experimental system has been lacking. We have identified and characterized bursts of mutagenesis in the budding yeast *Saccharomyces cerevisiae*. Specifically, we have discovered that *multiple structural variations can be acquired during transient, non-independent episodes of genomic instability*. We will present new findings from our studies of whole-chromosome aneuploidization, during which we have identified cell clones who have become aneuploid for multiple chromosomes. Our analyses indicate that these multi-chromosome gain/loss events are unlikely to have been acquired sequentially, as Darwinian models would predict. Instead, our observations fit more closely with a model in which a cell becomes highly aneuploid during a short and transient burst of genomic instability, and then resumes stable propagation with a severely altered and novel karyotype in the subsequent generations. Moreover, these findings indicate that mutational processes can act transiently and systemically such as to rapidly alter the structure of the entire genome.

32 Multiple origins of large insertions at chromosomal breaks *Yang Yu*[1], Xin Wang[2,3,4], Ruofan Yu[1,5], Weiwei Dang[1,5], Kaifu Chen[2,3,4], Grzegorz Ira[1] 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Center for Bioinformatics and Computational Biology, Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX; 3) Institute for Academic Medicine, Houston Methodist Hospital, Houston, TX; 4) Weill Cornell Medical College, Cornell University, Department of Cardiothoracic Surgery, New York, NY; 5) Huffington Center on Aging, Baylor College of Medicine, Houston, TX.

Insertions of mitochondrial DNA, mobile elements and fragments of nuclear chromosomes at DNA double-strand breaks (DSBs) threaten genome integrity and are common in cancer. Insertions of nuclear chromosome fragments at V(D)J recombination loci can increase antibody diversification. Recently, we revealed a yeast mutant, lacking evolutionarily conserved Dna2 nuclease, which shows frequent insertions of sequences between approximately 0.1 and 1.5 kb in length into DSBs, with many insertions involving multiple joined DNA fragments. Sanger sequencing of around 500 DNA inserts reveals that they originate from Ty retrotransposons, ribosomal DNA (rDNA) and from throughout the genome. We proposed a model in which alternative processing of DNA structures arising in Dna2-deficient cells can result in the release of extrachromosomal DNA fragments and their capture at DSBs by nonhomologous end-joining (NHEJ) to generate duplication. We hypothesized that similar DNA insertions at DSBs can occur in any cells with linear extrachromosomal DNA fragments. To test this we set up a high throughput approach to study the large DNA insertions at DSBs. Using this approach, we screened many cellular conditions and many mutants that are defective in genome maintenance. We observed the large insertions in multiple mutants and in replicative aging cells. The majority of large insertions are from rDNA, Ty retrotransposons and mitochon-

drial DNA in replicative aging cells. Unlike in Dna2 deficient cells, the largest hotspot is in the region of *RDN18* with clear boundary and direction polarity. Sequencing revealed a number of other types of genome instability including small inversions formed likely by microhomology-mediated break-induced replication (MMBIR). Our results obtained from high throughput sequencing indicate the large insertions have multiple origins and occur in many cellular conditions. These results can help elucidating how similar events are formed in cancer cells or at V(D)J loci in human and may provide insights on conditions that activate the pathway sensing extrachromosomal and cytoplasmic DNA, c-GAS.

33 Translational control of methionine and serine metabolic pathways underpin the paralog-specific phenotypes of Rpl22 ribosomal protein mutants in cell division and replicative longevity Nairita Maitra[1], Chong He[2], Heidi Blank[1], Matt Kaeberlein[3], Birgit Schilling[2], Rodolfo Aramayo[1], Brian Kennedy[2,4], *Michael Polymenis*[1] 1) Texas A&M Univ; 2) Buck Institute for Research on Aging; 3) University of Washington; 4) National University of Singapore.

A long-standing problem is how cells that lack one of the highly similar ribosomal proteins (RPs) often display distinct phenotypes. Some may reflect general effects due to lower growth rate and ribosome levels, but a number of diverse phenotypes cannot be explained through this mechanism. Yeast and other organisms live longer when they lack specific ribosomal proteins, especially of the large 60S subunit of the ribosome. However, longevity is neither associated with the generation time of RP deletion mutants nor with bulk inhibition of protein synthesis. Here, we comprehensively queried actively dividing RP paralog mutants through the cell cycle. Our data link transcriptional, translational, and metabolic changes to phenotypes associated with the loss of paralogous RPs. We uncovered specific translational control of transcripts encoding enzymes of methionine and serine metabolism, which are part of one-carbon (1C) pathways. Cells lacking Rpl22Ap, which are long-lived, have lower levels of metabolites associated with 1C metabolism. Loss of 1C enzymes, such as the serine hydroxymethyltransferase Shm2p increased the longevity of wild type cells. These results provide a molecular basis for paralog-specific phenotypes in ribosomal mutants and underscore the significance of 1C metabolic pathways in mechanisms of cell division and cellular aging. 1C pathways exist in all organisms, including humans, and targeting the relevant enzymes could represent longevity interventions.

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Genetic Control of Development and Regeneration (Zebrafish)

34 *foxm1* is required for cardiomyocyte proliferation after zebrafish cardiac injury *Daniel Zuppo*[1], Maria Missinato[1], Lucas Santana dos Santos[2], Panayiotis Benos[2], Michael Tsang[1] 1) Department of Developmental Biology, University of Pittsburgh, Pittsburgh, PA; 2) Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA.

Mammalian hearts fail to regenerate damaged tissue after cardiac injury because adult cardiomyocytes (CM) do not proliferate sufficiently. However, recent findings demonstrate these CMs can dedifferentiate, proliferate, and redifferentiate near the site of injury. It is imperative to discern the mechanisms regulating CM proliferation and to determine how they affect regeneration. Unlike mammals, adult zebrafish CMs can robustly proliferate after injury which allows for the identification of genes and pathways that control heart regeneration. We compared the transcriptome profile of uninjured and ventricular resectioned hearts and identified a number of candidate genes implicated in cell cycle regulation. Expression of *foxm1*, a forkhead-binding transcription factor, was identified in CMs at 3 days post-amputation (dpa) when CM proliferation initiates. Foxm1 is a mitotic regulator and is expressed during mammalian cardiac development, but its role in cardiac regeneration has not been characterized. We hypothesized that *foxm1* is critical for CM division, and that loss of its activity will result in heart regeneration failure. Indeed, we observed a significant decrease in PCNA+ CMs at 7dpa and increased scar tissue at 30dpa in *foxm1* mutants compared to WT controls. Transcriptome profiling of *foxm1* mutant hearts at 3dpa showed decreased cell cycle gene expression. Specifically, several G2/M phase cell cycle genes were decreased in mutant hearts suggesting that Foxm1 is required for mitotic progression through activation of genes involved in cytokinesis. This was supported by the decreased expression of *centromere protein f*(*cenpf),* a gene involved in chromosome segregation, *g2/m-phase specific E3 ubiquitin protein ligase (g2e3),* and *protein regulator of cytokinesis 1 (prc1)* in *foxm1* mutant hearts. Previous research revealed *cenpf* expression and CM proliferation in the developing heart persists until P7 in neonatal mice, so we hypothesized *cenpf* is required for mitosis. To confirm that *cenpf* is critical for CM cell division,we analyzed *cenpf* mutant hearts after ventricular resection and noted a failure to regenerate after injury. Moreover, by 20dpa *cenpf*-mutant hearts showed an abundance of binucleated CMs indicative of incomplete cytokinesis. These findings reveal that *foxm1*is critical for proper CM proliferation after injury through activation of G2/M phase cell cycle genes.

35 Enhancers and the uneven distribution of regenerative capacities in vertebrates *Wei Wang*[1, 2], Chi-Kuo Hu[3], An Zeng[1], Dana Alegre[1], Deqing Hu[1], Kirsten Gotting[1], Augusto Ortega Granillo[1], Yongfu Wang[1], Sofia Robb[1], Robert Schnittker[1], Shasha Zhang[1], Dillon Alegre[1], Hua Li[1], Eric Ross[1], Ning Zhang[1], Anne Brunet[3, 4], Alejandro Sánchez Alvarado[1, 2] 1) Stowers Institute for Medical Research; 2) Howard Hughes Medical Institute; 3) Department of Genetics, Stanford University; 4) Glenn Laboratories for the Biology of Aging.

Even though regeneration has been the subject of extensive phylogenetic, developmental, cellular, and molecular studies, the mechanisms underlying the broad disparity of regenerative capacities in animals remains elusive. Here we report on a comparative epigenomic and transcriptomic approach which identified an evolutionarily conserved regeneration response program (RRP) in vertebrates. By defining the *cis*-regulomes and transcriptomes of early stages of regeneration in the distantly related zebrafish *Danio rerio* and the African killifish *Nothobranchius furzeri*, we were able to discriminate between species-specific and evolutionarily conserved genomic responses to amputation. Importantly, functional testing by systematic transgenic reporter assays of the conserved *inhibin beta A* (*inhba*) regeneration responsive enhancer (RRE) from zebrafish, killifish, and humans identified species-specific variations. Furthermore, deletion of the killifish *inhba* RRE significantly perturbed caudal fin regeneration and completely abrogated cardiac regeneration. We also show that *inhba* RRE activity requires the presence of predicted binding motifs for the Activator Protein 1 (AP-1) complex. Interestingly, AP-1 binding motifs can be identified in the conserved and non-conserved teleost RREs reported in this study, indicating that AP-1 may be required for both injury and regeneration responses. We propose that changes in RREs driven by natural selection are likely a crucial source of loss of regenerative capacities in vertebrates, including humans.

36 *Robo2* and Type-IV Collagen function in a common molecular pathway to promote target-specific axon regeneration *Patti Murphy*[1], Michael Granato[1] 1) University of Pennsylvania, Philadelphia, PA.

After nerve injury, regenerating axons must navigate through a complex environment to reform functional connections with appropriate targets. Functional recovery is rare because even when axons regrow robustly, they often select inappropriate paths at guidance choice-points. Despite their importance for restoring function, the cellular and molecular mechanisms that direct pathway choice by regenerating axons are not well understood. Using laser nerve transection in larval zebrafish, we identified *robo2* as a key regulator of pathway selection by regenerating motor axons. After transection, *islet1*$^{+}$ motor axons, which innervate dorsal muscles, regenerate and correctly select the dorsal path at a stereotyped choice-point ~80% of the time. In contrast, regenerating *islet1*$^{+}$ axons in *robo2* mutants select ventral and other inappropriate paths ~50% of the time. *Islet1*$^{-}$ motor axons, which innervate ventral targets, do not require *robo2* to select their ventral path during regeneration. However, overexpression of *robo2* in *islet1*$^{-}$ motor neurons is sufficient to promote dorsal path selection by *islet1*$^{-}$ axons in regeneration. This suggests that *robo2* promotes dorsal regeneration by preventing ventral regeneration. Previously, we found that the extracellular matrix collagen *col4a5* is also required to guide regenerating dorsal (*islet1*$^{+}$), but not ventral (*islet1*$^{-}$), motor axons. During regeneration, a small subset of Schwann cells at the transection site upregulate *col4a5* as well as the Robo2 ligand *slit1a* (Isaacman-Beck, et al. 2015). Using our *robo2* overexpression assay, we find that *robo2* signals through *col4a5* and its Slit-binding domain to prevent ventral regeneration by motor axons. Together, these results suggest that Schwann cell-derived cues direct regenerating *robo2*$^{+}$ axons toward dorsal muscle targets after injury. We will present ongoing work to determine the role of heparan sulfate proteoglycans, which function as Slit-Robo coreceptors, in this motor axon guidance pathway during regeneration.

37 bCharacterizing mechanisms of conserved skin appendage formation at single-cell resolution *Lauren Saunders*[1], Andrew Aman[2], Sanjay Srivatsan[1], Cole Trapnell[1], David Parichy [2] 1) University of Washington, Seattle, WA; 2) University of Virginia, Charlottesville, VA.

Vertebrate skin is endowed with diverse skin appendages, including hair, feathers and scales. The study of skin appendage development provides insight into fundamental aspects of developmental biology including tissue patterning and morphogenesis. The signaling events that govern the formation of skin appendages are generally conserved among vertebrates, but the participating cell types and molecular targets of contributing signaling pathways, including Wnt/ β-catenin, Ectodysplasin (Eda) and Fibroblast growth factor (Fgf), are poorly understood. Zebrafish scale development relies on a precise sequence of organized events across multiple dermal and epidermal cell types, beginning with regularly patterned groups of dermal fibroblasts and culminating in a coat of overlapping scales. To uncover the cell types and mechanisms underlying this process, we profiled the transcriptomes of 150,000 cells representing more than 25 cell types from whole skins during scale development. Reconstructing developmental trajectories of dermal and epidermal cell types during scale formation revealed gene expression dynamics and signaling events across multiple cell populations that drive scale development. And through orthogonal *in situ* hybridization and super resolution microscopy analyses, we relate single cell transcriptomes to their spatial origin, providing additional resolution of this spatially organized developmental process. In addition to characterizing normal scale development, we sought to gain a better understanding of dermal and epidermal changes in fish models of human skin disease. Thus, we profiled cells from zebrafish harboring a mutation in *eda*, a TNF-kB signaling ligand implicated in hereditary ectodermal dysplasia, a condition in which afflicted individuals lack skin appendages and teeth. We additionally profiled the skin of fish lacking thyroid hormone; hypothyroidism in humans leads to a range of disorders from dry skin to myxedema, a debilitating skin disease. Importantly, both *eda* mutants and hypothyroid fish fail to develop scales properly. By comparing normal and disrupted scale formation at single-cell resolution, we resolved cell-type specific contributions and signaling events over the course of scale development. Taken together, our results provide a high-resolution view of zebrafish scale formation during both normal and disrupted scale formation, providing a deeper understanding of the ancient, conserved process of skin appendage formation.

38 Single-cell transcriptomic analysis of embryonic vasculogenesis identifies the conversion of Etv2-deficient vascular progenitors into skeletal muscle Brendan Chestnut[1], Satish Casie Chetty[1,2], Andrew Koenig[1,2], *Saulius Sumanas*[1,2] 1) Cincinnati Children's Hospital Medical Center; 2) University of Cincinnati.

During vertebrate embryogenesis, vascular endothelial cells originate in the lateral plate mesoderm (LPM) next to the progenitors of skeletal muscle. It is currently not clear what prevents vascular progenitors from responding to the adjacent signals that promote muscle development. An ETS transcription factor Etv2 functions as an evolutionarily conserved master regulator of vasculogenesis. Here we performed single-cell transcriptomic analysis of hematovascular development in wild-type and *etv2* mutant zebrafish embryos. Distinct transcriptional signatures of different types of hematopoietic and vascular progenitors were identified using an *etv2*^{ci32Gt} gene trap line, in which Gal4 transcriptional activator has integrated into the *etv2* gene locus. Unexpectedly, a cell population with the skeletal muscle signature was observed in *etv2*-deficient embryos. We demonstrate that multiple *etv2*^{ci32Gt}*; UAS:GFP* cells migrate into the somites, elongate and differentiate as skeletal muscle cells instead of contributing to vasculature in *etv2*-deficient embryos. Wnt and FGF signaling promoted the differentiation of these putative multipotent *etv2* progenitor cells into skeletal muscle cells. We conclude that *etv2* actively represses muscle differentiation in vascular progenitors, thus locking these cells into vascular endothelial fate. We also identified the transcriptional signature of putative multipotent progenitors within the LPM that may give rise to vascular progenitor and skeletal muscle cells. Finally, we demonstrate that arterial progenitors co-express multiple arterial and venous markers during early stages of vasculogenesis, suggesting multi-potency of early vascular progenitors.These findings will be important in understanding the ontogeny of different mesodermal lineages and will help in designing *in vitro* differentiation strategies to generate vascular, muscle and other types of progenitors for therapeutic purposes.

39 MicroRNA-mediated control of developmental lymphangiogenesis Hyun Min Jung[1], Ciara Hu[1], Alexandra Fister[1], Andrew Davis[1], Daniel Castranova[1], Vsn Pham[1], Lisa Prioce[1], *Brant Weinstein*[1] 1) National Institute of Child Health and Human Development, NIH.

Although transcriptional programs directing lymphatic vessel formation have been described in recent years, post-transcriptional mechanisms contributing to molecular regulation of developmental lymphangiogenesis and lymphatic network assembly are still not well understood. We used high throughput small RNA sequencing to identify miR-204, a highly conserved microRNA dramatically enriched in lymphatic vs. blood endothelial cells, and we demonstrate that this microRNA plays an essential role during lymphatic development. Suppressing miR-204 leads to loss of lymphatic vessel formation, while endothelial-autonomous overexpression of miR-204 accelerates lymphatic vessel formation, suggesting a positive role during developmental lymphangiogenesis. We also identify the NFATC1 transcription factor as a key conserved target for post-transcriptional regulation by miR-204 during lymphangiogenesis. While miR-204 suppression leads to loss of lymphatics, knocking down its target NFATC1 leads to lymphatic hyperplasia, and the loss of lymphatics in miR-204-deficient animals can be rescued by NFATC1 knockdown. Together, our results highlight a miR-204/NFATC1 molecular regulatory axis required for proper lymphatic development.

40 Investigating interactions between the actin and microtubule networks in the yolk cell during zebrafish morphogenesis Ashley Bruce[1], *Haoyu Wan*[1] 1) University of Toronto.

Epiboly is an essential gastrulation movement during zebrafish development. It is defined as the thinning and spreading of the blastoderm, a multi-layered cell mass, that sits on top of a large syncytial yolk cell. During epiboly, the blastoderm spreads towards the vegetal pole to enclose the yolk cell. The motive force for epiboly comes primarily from acto-myosin based motors in the yolk cell. The yolk cell also contains a microtubule network oriented along the animal-vegetal axis. When epiboly is delayed, the yolk microtubule and actin networks are often disrupted. While the role of each network during epiboly has been studied, interactions between them have not been examined. We hypothesize that yolk actin and microtubules interact to facilitate normal epiboly and we developed methods to co-visualize them in live embryos. When yolk microtubules are stabilized or depolymerized, changes in actin distribution are apparent, however the acto-myosin contractile ring still forms and epiboly is only mildly affected. By contrast, actin depolymerization, severely delays epiboly and disrupts the microtubule network. To distinguish between the loss of actin from the loss of acto-myosin contractility, dominant-negative (DN) Rho A was over-expressed. DN-Rho A delays epiboly, but yolk microtubule organization was not strongly affected. These results suggest that there might be structural interactions between actin and microtubules. In previous work, we identified what appear to be non-centrosomal microtubule organizing centers present at mid-epiboly stages in the yolk. In addition, it is known that the CAMSAP/Nezha/Patronin family member Camsap2, a microtubule minus-end stabilizing protein, is expressed in the yolk during epiboly. We postulate that Camsap2 might function to stabilize non-centrosomal microtubule minus-ends. Given that microtubule minus ends are in close proximity to the marginal actin ring, it is possible that structural interactions between actin and microtubules are required to regulate microtubule minus-end dynamics via Camsap2. Morpholino knockdown of *camsap2* results in disrupted yolk actin and microtubules, as well as severely delayed epiboly. We have successfully generated guide RNAs to knock-out *camsap2* and these experiments on on-going. Future work will focus on the role of Camsap2 in mediating functional interactions between yolk actin and microtubules during epiboly.

41 Ectopic *kcnh2a* slows niche-to-mesenchyme transitions to prolong fin outgrowth and disrupt organ scaling of *longfin* zebrafish Scott Stewart[1], Heather Le Bleu[1], Gabriel Yette[1], Astra Henner[1], Joshua Braunstein[1], Jad Chehab[1], Michael Harms[1], *Kryn Stankunas*[1] 1) University of Oregon.

Adult zebrafish fins regenerate to their original form irrespective of the extent or type of damage, providing a striking example of organ size control. Current models speculate fin cells maintain grid-like "positional identities" that instruct differential outgrowth after injury. We propose an alternative "Transpositional Scaling" model for robust fin size restoration whereby the positional information is stored in skeletal geometry. We show the amount of Wnt produced by a pool of distal "niche" cells determines the extent of fin outgrowth. The niche forms from mesenchyme populating the inside of the eighteen differentially sized and tapered bony rays. The niche, and therefore Wnt production, then gradually depletes by the net re-differentiation of niche cells back into a mesenchymal cell state. Regenerated ray lengths become a function of ray widths at the injury position with the skeletal geometry inherently restored by the depleting niche. Aided by mathematical modeling, we show *longfin*^{t2} zebrafish regenerate extraordinarily long fins due to a broken niche "countdown timer". We determine the *longfin*^{t2} phenotype is caused by cis-ectopic expression of the *kcnh2a* voltage-gated potassium channel. *kcnh2a* expression is ectopically expressed in *longfin*^{t2} intra-ray mesenchyme, where it likely disrupts activity of the Ca^{2+}-dependent phosphatase calcineurin. We use blastula transplantations to show *longfin*^{t2}-ectopic *kcnh2a* expression acts autonomously within the intra-ray mesenchyme/niche lineage. We propose skeletal geometry and niche-to-mesenchyme cell state transitions promoted by membrane depolarization and Ca^{2+}/ calcineurin signaling restore zebrafish fin size and shape.

Wednesday, April 22 1:30 PM - 3:30 PM

Technologies, Resources and Genomics (*Xenopus*)

25 GEO Data, Human Disease, and Phenotypes on Xenbase: New Tools and Features *Malcolm Fisher*[1], Mardi Nenni[1], Praneet Chaturvedi[1], Christina James-Zorn[1], Stanley Chu[2], Joshua Fortriede[1], Troy Pells[2], Vaneet Lotay[2], Virgilio Ponferrada[1], Kevin Burns[1], Ying Wang[2], Dong Zhou[2], Erik Segerdell[3], Sergei Agalakov[2], Brad Arshinoff[2], Kamran Karimi[2], Peter Vize[2], Aaron Zorn[1] 1) Division of Developmental Biology, Cincinnati Children's Hospital, OH, USA; 2) Department of Biological Sciences, University of Calgary, Alberta, Canada; 3) Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA.

Xenbase, the Xenopus knowledgebase, continues to develop tools and support to accelerate basic research and human disease modeling. A major goal is to make the large amount of data generated by Xenopus research more accessible to the research community. To this end we have developed support to improve user understanding and learning with new tools to explore and visualize the biological data. Our newest support focuses on Next Generation Sequencing (NGS) data, human disease associations, and Xenopus phenotypes. Xenbase has developed a standardized pipeline of curation and bioinformatic processing for all standard RNA-Seq and ChIP-Seq Xenopus NGS data in the NCBI Gene Expression Omnibus (GEO) repository, with work ongoing to allow the processing of other sequencing based assays. This allows diverse datasets to be aligned to the latest genomes rendering them consistent and comparable. These data are viewable in the Xenbase instance of the JBrowse genome browser, along with a variety of other annotation tracks. Secondary analyses of the processed data are available as tables of differential expression and a variety of visualizations. Xenopus data is curated using ontologies, controlled vocabularies of terms with defined relationships, which are essential to making many types of complex data standardized and computer searchable. Recognizing researchers may not be familiar with ontologies, Xenbase has implemented an interactive tree viewer of the Xenopus Anatomy Ontology (XAO) and Human Disease Ontology (DO) allowing navigation of the ontology structure within Xenbase. A Xenbase Disease Page provides a summary of the genes and literature associated with the disease and provides links to related human and model organism resources. Further support for human disease is ongoing with the development of the Xenopus Phenotype Ontology (XPO) in collaboration with developers from the uPheno project. The XPO will be used in phenotype curation, with phenotypes from human disease models as a top priority. Incorporation of the DO allows direct linkage of a Xenopus phenotype with human diseases making the data more accessible and provides gene-disease-phenotype associations which will be made available and shared with other disease model resources. Xenbase is funded by a Biotechnology Resource grant from the NICHD.

26 A genome editing laboratory course for undergrads using CRISPR in butterflies and frogs *Arnaud Martin*[1] 1) The George Washington University DC.

"*The things we hear about in other biology courses about genome editing were actually performed in this class and we got to see real organisms*

with the result of the injections we did [...]*. This class revealed how important it is for us to contribute to the right sort of conversation in scientific innovations as we graduate from college."*
Anonymous student feedback

CRISPR offers new opportunities for biology students to perform inspiring research that explores the gene-to-phenotype relationship in depth. It is also crucial to introduce the future generation of biologists, practitioners and other protagonists to the technical and societal aspects of gene editing. In this talk, I will share my experience leading an undergraduate laboratory class for the past 6 semesters, where biology senior undergraduates formulated hypotheses regarding the roles of candidate genes, designed guide RNAs targeting them, and each injected CRISPR in hundreds of embryos, targeting developmental genes such as *abdominal-A, STAT92E, WntA, optix*, and *slc45a2*, in two strategically chosen vertebrate and invertebrate organisms: the *Vanessa cardui* butterfly and *Xenopus laevis* frog. Both frogs and butterflies are commercially available and outstanding teaching tools as they provide scalable numbers or readily fertilize, large eggs that can be injected using cheap microinjection devices. Each semester, students consistently generated spectacular mosaic knockout morphological phenotypes with key insights in developmental biology and functional genomics. The class also includes discussions, readings, student presentations and essays on the Bio-Ethics of Genome Editing. Student feedback and subsequent applications to PhD programs indicated the approach fostered the student interests for research careers.

27 Differential embryonic gene activation across the subgenomes of Xenopus laevis *Wesley Phelps*[1], Anne Carlson[1], Miler Lee[1] 1) University of Pittsburgh, Pittsburgh, PA.

Embryogenesis is a highly regulated process that guides a single-celled zygote to a multicellular organism. During the earliest developmental stages, the embryo is transcriptionally silent and must rely on maternal factors to carry out all cellular processes. Subsequently, maternal transcription factors activate the embryonic genome to produce de novo gene expression. Embryonic genome activation is a crucial event during early development that occurs throughout most animals and plants and is required for embryonic viability. Here, we present our efforts to discover the mechanisms underlying genome activation in *Xenopus laevis*, taking advantage of the unique configuration of the *X. laevis* genome: *X. laevis* is allotetraploid, encoding two homeologous subgenomes called L and S, diverged by ~34 million years. Using RNA-seq in early-stage *X. laevis* embryos, we have identified homeologous L and S gene pairs with differential embryonic expression levels, demonstrating divergent regulation of the two subgenomes in the early embryo. To deduce the mechanisms that drive differential gene activation, we have performed a subgenome-wide comparison of regulatory sequences. We adapted the low-input assay CUT&RUN for *X. laevis* embryos and found widespread differential histone modification across homeologous promoter and enhancer regions. Using ATAC-seq, we further found differential chromatin accessibility in these regions, correlating with enrichment of specific transcription factor binding motifs, including ones predicted to be recognized by the pluripotency-inducing factors OCT4 and SOX2. These results are consistent with a transcriptomic analysis of the egg, which reveals that *pou5f3.3* and *sox3* are among the most highly maternally contributed mRNA. Together, these results strongly suggest that maternal Pou5f3.3 and Sox3 differentially activate the *X. laevis* subgenomes. Future loss-of-function analyses will confirm their roles in the early embryo. By further understanding the mechanisms underlying embryonic genome activation, we will gain further insight into how transcriptomes are reprogrammed and how pluripotency is induced across vertebrates.

Wednesday, April 22 1:30 PM - 3:30 PM

Demographic Inference (PEQG)

21 The Genomic Landscape of Neanderthal Ancestry in Modern Humans *Arun Sethuraman*[1], Melissa Lynch[1] 1) California State University San Marcos.

Genomes of modern human populations outside of Africa share 1-5% of identical by descent (IBD) haplotypes with Neanderthals, owing to multiple bouts of gene flow from this now extinct `ghost' population. The recent availability of high quality archaic genomes has greatly improved our knowledge of the landscape of Neanderthal genomic variation in modern humans. However, our understanding of the evolutionary history and functional consequences of putatively introgressed Neanderthal genomic regions is still developing. Here we attempt to understand the combined effects of differential introgression and linked natural selection across genomic regions of populations of Central European and Han Chinese ancestry that are introgressed from Neanderthals under an Isolation with Migration framework using a new parallelized Bayesian MCMC method called MigSelect. We compile a composite genomic dataset from Altai Neanderthals, and unrelated Central Europeans from Utah (CEU), and Han Chinese (CHS/CHB) individuals from the 1000 Genomes Project, and estimate the evolutionary history across putatively introgressed regions. Our findings reveal (a) no significant ($p > 0.05$) differential introgression across all loci analyzed (i.e. no selection for or against introgression across all Neanderthal haplotypes), (b) no significant ($p = 0.07$) linked natural selection effects across all loci analyzed, but (c) greater support for some loci to have lower effective population sizes, and hence potentially under linked natural selection effects, including loci linked to the MUSK and GKAP1 genes, which are preferentially expressed in animal testes.

22 Fast estimation of effective migration surfaces *Joseph Marcus*[1], Wooseok Ha[2], Rina Foygel Barber[3], John Novembre[1,4] 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Statistics, University of California, Berkeley, CA; 3) Department of Statistics, University of Chicago, Chicago, IL; 4) Department of Ecology and Evolution, University of Chicago, Chicago, IL.

Understanding the relationship between geography and genetic differentiation is a long-standing problem in population genetics. A widespread feature in population genetic data is "isolation by distance," where genetic differentiation tends to increase as populations become more geographically distant. While spatial models of "isolation by distance" have provided powerful predictions of population structure, they do not capture heterogeneous migration that occurs when geographic features can slow down or enhance gene-flow in localized regions. Recently, Petkova et al. 2016 developed a statistical method called Estimating Effective Migration Surfaces (EEMS) for inferring heterogenous effective migration rates using a coalescent inspired model for gene-flow on a dense spatial graph. EEMS has been applied to many systems, including non-model organisms, and has allowed for the discovery and interpretation of geographic features that might have restricted or enhanced gene-flow. Most importantly, EEMS is a flexible tool for users to visualize geographic structure in their data. EEMS uses Markov Chain Monte Carlo (MCMC) to obtain posterior samples of these migration rates, employing a spatial prior using a Voronoi tessellation of the focal geographic region. Here we draw inspiration from EEMS by utilizing a similar graph-based likelihood, allowing for heterogeneous gene-flow, but take an optimization-based approach. We take advantage

of sparse linear algebra routines for fast gradient computation and impose a new type of spatial regularization in the effective migration rates. Our approach allows for fast model exploration and iteration. We accurately recover migration surfaces for within-model and coalescent-based simulations. Applications to population genetic datasets from humans and non-model organisms from around the globe perform comparably to EEMS but are fit orders of magnitude faster. We see our method as being a useful complementary tool alongside EEMS, expanding the ability for users to quickly visualize and interpret spatial structure in their data.

23 Reconstructing spatio-temporal patterns of admixture in human history using present-day and ancient genomes *Manjusha Chintalapati*[1], Nick Patterson[2], Neel Alex[1], Priya Moorjani[1] 1) University of California, Berkeley, CA ; 2) Broad Institute of Harvard and MIT, Cambridge, MA .

Recent analysis of present-day and ancient genomes have shown that gene flow or admixture has been pervasive throughout human history. Understanding the timing and signatures of admixture is important for studying the genomic, evolutionary and functional impact of admixture, as well as uncovering the historical context in which the mixture occurred. While a number of methods exist for characterizing population mixture in contemporary populations, they are less applicable to sparse data available from ancient DNA specimens (samples with low coverage and large proportions of missing information). Here we introduce a new method, *DATES* (Distribution of Ancestry Tracts of Evolutionary Signals) which leverages ancestry covariance patterns measured in a single diploid genome to infer the ancestry proportions and the time since admixture. By performing simulations, we show that *DATES* provides reliable results under a range of demographic scenarios, including for samples with large amounts of missing data and cases when only data for distinctly related ancestral reference populations is available. We show *DATES* outperforms other available methods like ALDER, especially for ancient DNA samples which have very sparse data. To illustrate the utility of *DATES*, we applied it to worldwide data from 5000 present-day and 2000 ancient samples. We identified pervasive evidence of admixture in many human populations and confirmed dates of previously documented events such as Arab Slave Trade, spread of Mongol ancestry across Eurasia and recent mixtures in the Americas. We further applied *DATES* to reconstruct the population shifts and mixtures that accompanied the Neolithic farming, uncovering the dynamics of the mixtures between the Hunter-gatherers, Anatolian farmers and Steppe-pastorialists in Europe. We also applied *DATES* to show that the timing of the formation of the Myceneans populations correlated to mixture between Minoans and bronze age Europeans, rather than earlier times. These analyses highlight the reliability of *DATES*, its utility for making inferences under various demographic scenarios, providing complementary insights to archaelogical and linguistic evidence about human migrations in the past 10,000 years.

24 Recurrent Collection of *Drosophila melanogaster* from Wild African Environments and Genomic Insights into Species History Quentin Sprengelmeyer[1], Suzan Mansourian[2], Jeremy Lange[1], Daniel Matute[3], Brandon Cooper[4], Erling Jirle[2], Marcus Stensmyr[2], *John Pool*[1] 1) University of Wisconsin - Madison; 2) Lund University; 3) University of North Carolina; 4) University of Montana.

A long-standing enigma concerns the geographic and ecological origins of the intensively studied vinegar fly, *Drosophila melanogaster*. This globally distributed human commensal is thought to originate from sub-Saharan Africa, yet until recently, it had never been reported from undisturbed wilderness environments that could reflect its precommensal niche. Here, we document the collection of 288 *D. melanogaster* individuals from multiple African wilderness areas in Zambia, Zimbabwe, and Namibia. The presence of *D. melanogaster* in these remote woodland environments is consistent with an ancestral range in southern-central Africa, as opposed to equatorial regions. After sequencing the genomes of 17 wilderness-collected flies collected from Kafue National Park in Zambia, we found reduced genetic diversity relative to town populations, elevated chromosomal inversion frequencies, and strong differences at specific genes including known insecticide targets. Combining these genomes with existing data, we probed the history of this species' geographic expansion. Demographic estimates indicated that expansion from southern-central Africa began ~10,000 years ago, with a Saharan crossing soon after, but expansion from the Middle East into Europe did not begin until roughly 1,400 years ago. This improved model of demographic history will provide an important resource for future evolutionary and genomic studies of this key model organism. Our findings add context to the history of *D. melanogaster*, while opening the door for future studies on the biological basis of adaptation to human environments.

Wednesday, April 22 3:45 PM - 5:45 PM

New Technology and Resources in Development (*C. elegans*)

42 Lineage-specific analysis of proliferation-differentiation control in *C. elegans* *Sander van den Heuvel*

abstract is not available at the time of print

43 Cultivating relationships: genetics and genomics microbiome form and function Adrien Assie[1], Fan Zhang[1], Jessica Weckhorst[1], *Buck Samuel*[1] 1) Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston TX USA.

The gut microbiome extends the capabilities of its host and alters its physiology. Together with diet, host genetic landscapes shape microbiome form and function in the animal gut. Despite its importance, the essential functions that drive microbiome assembly and stability in remain largely elusive. To address this challenge, we leverage the nematode Caenorhabditis elegans to explore how microbiomes assemble in different host genetic backgrounds. This system has several advantages, including: (1) a simple microbiome that can rapidly removed (bleaching) and replaced in high-throughput gnotobiotic experiments; (2) highly conserved intestinal physiology, metabolism and innate immunity; and (3) shared microbial functions for host gut persistence.

To examine the natural variation in acquisition of the microbiome in C. elegans, we first established the natural core microbiome and assembled a functionally redundant, model core microbiome of bacteria (BIGbiom) in the lab. Then a panel of 38 fully genome sequenced *C. elegans* wild strains were made 'germ-free' and colonized with BIGbiom to assess strain-level microbiome composition (16S) and levels (CFU) longitudinally using a high-throughput pipeline. The strains clustered tightly into three distinct groups: (1) a highly-selective group that differed greatest from the surrounding environment [*Ochrobactrum*-dominant]; (2) a 'dysbiotic' group [*Bacteroidetes*-dominant, 30-fold more CFUs]; and (3) a 'non-selective' group. All strains tested retain deterministic selection and/or control, the two programs for microbiome regulation. By GWAS-, RNAi- and RNAseq-based approaches, we identified ~1000 candidate regulators in highly conserved pathways (>60%). Insulin signaling pathways specifically regulate Ochrobactrum colonization, as impaired daf-2/IGFR signaling (mutants or RNAi of wild strains) limits its colonization.

Since we saw dramatic host-to-host differences in microbiome composition, we next sought to examine alterations in microbiome function. To do this, we sequenced and annotated >100 bacterial genomes [BIGbiom plus others in natural microbiome]. Virtual metagenomes were constructed for each C. elegans microbiome and indicate broad microbiome functions are shared, but also point to many emergent functions among the three host groups. Our study highlights the potential a robust platform to identify conserved host and microbial determinants that may underlie assembly and stability of the microbiome.

44 Bicistonic tagging and severing (BiTS): a new gene editing tool in C. elegans using endogenous trans-splicing pathways. *Ryan Littlefield*[1] 1) University of South Alabama.

In C. elegans, mRNAs processing often includes endogenous spliced leader (SL) trans-splicing pathways that add short, 5' capped SLs and resolve polycistronic mRNAs into separate messages. Intercistronic regions (ICRs) efficiently direct trans-splicing of polycistronic messages and have been utilized for transgene co expression. Here, we demonstrate that an ICR is able to direct trans-splicing of endogenous genes when inserted by CRISPR Cas9 homologous recombination. Modifying either the 5' or 3' ends of the protein coding sequence generates bicistronic messages that minimally alters the endogenous gene yet allows for the co expression of a fluorescent protein (FP) reporter. Efficient trans-splicing occurred for all experimentally tested genes, including genes that are normally not trans-spliced to a spliced leader and genes within naturally occurring operons. In addition, we precisely insert an ICR within the myosin heavy chain myo3 coding sequence between protein domains to generate a novel 'severed' allele, in which the catalytic ATP sensitive actin binding head domain is physically separated by the coiled coil filament forming tail domain. Our results show that BiTS is a versatile and efficient tool for the reliable expression of transcriptional reporters, knock in of exogenous genes under precise control of endogenous promoters, and gain of function alleles, where endogenous genes are expressed under exogenous promoters. Moreover, the rapid generation of 'severed' alleles provides a unique way to investigate a wide range of potential scaffolding and mechanical roles for different protein domains.

45 Dietary serine enhances chemotherapeutic toxicity through altering the metabolism of the microbiota *Wenfan Ke*[1], Jake Saba (co-first)[1], Cong-Hui Yao[2], Michael Hilzendeger[1], Anna Drangowska-Way[1], Chintan Joshi[3], Vinod Mony[1], Shawna Benjamin[1], Sisi Zhang[2], Jason Locasale[4], Gary Patti[2], Nathan Lewis[3], Eyleen O'Rourke[1] 1) The University of Virginia, Charlottesville, VA; 2) Washington University, St. Louis, MO; 3) University of California, San Diego, La Jolla, CA; 4) Duke University, Durham, NC.

Fluoropyrimidines are widely used in the clinic to treat colorectal and other prevalent cancers. However, the efficacy and toxicity of these drugs is highly variable, and the variant response is largely independent of the patient's genetics. On the other hand, environmental factors such as diet and microbiota, are critical to such variations in efficacy and toxicity. Nevertheless, the molecular mechanisms of such diet–drug–microbe–host 4-way interaction remain to be elucidated. In this study, we established a diet–5'-fluorodeoxyuridine(FUdR)–*E. coli*–*C. elegans* 4-way system. Through 3-way (drug-microbe-host) compound screens and 4-way (diet-drug-microbe-host) genetic screens of *E. coli* Keio KO library, followed by metabolomics and biochemistry analysis, we uncovered the remarkably distinct mechanisms by which dietary thymidine and serine increase the toxicity of the FUdR (>10 fold) in a microbiota genetics dependent manner. We identified unique gene sets in *E. coli* responsible for thymidine or serine enhanced FUdR toxicity, which leads to the discovery of two different toxic mechanisms: 1) dietary thymidine promotes bioconversion of prodrug FUdR to toxic 5-fluorouridine-5'-monophosphate (FUMP) through pyrimidine salvage pathway; 2) dietary serine enhances FUdR toxicity via promoting folate-synthesis, and with that enabling the 5-Fluoro-2'-deoxyuridine 5'-monophosphate(FdUMP)-mediated inhibition of *E. coli* thymidylate synthase, which in turn deprives *C. elegans* from its major source of thymidine. Moreover, 4-way genetics screens for host genes mediating the toxicity and cellular mechanistic studies revealed distinct host (*C. elegans*) responses to microbiota (*E. coli*) mediated FUdR-FUMP bioconversion and thymidine starvation. FUMP targets mitochondrial RNAs and DNA. Mitochondrial lipid signals and AMPK alert of this mitochondrial dysfunction, ultimately activating lethal levels of autophagy. By contrast, thymidine starvation does not target mitochondrial nucleic acids, and autophagy protects against death in this condition. Our study highlights that one single dietary amino acid (serine) can change the microbe's genetic pathways mediating chemotherapeutic drug toxicity, and alter or reverse the host genetic and cellular response to drugs. Altogether, the 4-way interactions dissected here demostrate the complexity of microbe-host co-metabolism and emphasize the value of genetically tractable systems to enable deconstructing this complexity.

46 Rapid Self-Selecting and Clone Free Integration of Transgenes into Engineered CRISPR Safe Harbor Locations in *C. elegans* *Megan Moerdyk-Schauwecker*[1], Zachary Stevenson[1], Brennen Jamison[1], Patrick Phillips[1] 1) University of Oregon, Department of Biology, Institute of Ecology and Evolution, Eugene, OR .

Precision editing of the genomes of model organisms has revolutionized the functional analysis and validation of a wide variety of molecular systems. To date, the capacity to insert transgenes into the model nematode *Caenorhabditis elegans* has focused on utilizing either transposable elements or CRISPR-based safe harbor locations. These methods require laborious screening processes that result in false positives due to generation of heritable extrachromosomal arrays or rely on co-CRISPR markers to identify likely edited individuals. As a result, verification of transgene insertion requires anti-array selection screening methods and/or PCR genotyping. These approaches also rely on constructing and cloning plasmids for the insertion of large transgenes. Here, we present a novel, safe harbor CRISPR-based integration strategy that utilizes engineered insertion locations containing a synthetic guide RNA target and a split-selection system to eliminate false positives from array formation. This integration-specific selection approach allows the experimenter to confirm an integration event has taken place without molecular validation. We show that this method is capable of producing integrated transgenic lines in as little as five days post injection. Split selection sites using high efficiency guides have been engineered into multiple genomic locations, allowing for insertion of multiple transgenes for more complex modeling of systems. In order to further increase the speed of transgene generation, we bypassed the need for pre-construction of plasmids by utilizing the *C. elegans* native microhomology-based recombination to assemble transgenes *in-situ*. We show that complete transgenes can be assembled and inserted into our split-selection safe harbor locations starting from simple PCR products. Overall, this combination of approaches provides an economical and rapid system for generating highly reproducible complex transgenics in *C. elegans*.

47 Driving with caution: lessons learned from TIR1 promoters and TIR1 receptor function *Michael Martinez*[1], Guinevere Ashley[2], Tam Duong[3], Max Levenson[2], Hannah Saeger[2], Brittney Davidson[2], Matthew Ragle[2], Daniel Dickinson[3], David Reiner[4], David Matus[1], Jordan Ward[2] 1) Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY ; 2) Department of Molecular, Cell, and Developmental Biology, University of

California-Santa Cruz, Santa Cruz, CA; 3) Department of Molecular Biosciences, University of Texas at Austin, Austin, TX; 4) Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX.

Auxin-inducible degron (AID) technology was recently introduced to the *Caenorhabditis elegans* community. This powerful and rapid technology allows for spatiotemporal control of protein degradation through a plant-specific F-box protein, transport inhibitor response 1 (TIR1). In *C. elegans*, TIR1 relies on SKR-1/2, CUL-1, and RBX-1 to form a functional SCF^{TIR1} complex, targeting AID-tagged proteins for proteasomal degradation in the presence of the hormone auxin (IAA or NAA). With the popularity of the AID system rising in the *C. elegans* community, there is a need to: 1) expand the current library of published TIR1 promoters; and 2) limit the basal activity of existing TIR1 proteins. Here, we explore issues related to tissue-specific promoters, and solutions for basal degradation without auxin. We envision a more efficient means of degrading target proteins by leveraging *C. elegans* machinery and seeking alternative methods to limit unwanted secondary effects of TIR1 in a cell- and tissue- specific manner.

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Gene Regulation (Yeast)

62 Introduction of Doug Koshland for the Lee Hartwell lecture *Phil Hieter*[1] 1) University of British Columbia.

abstract is not available at the time of print

63 Lee Hartwell Lecture: Higher order chromosome structure in yeast, an informative oxymoron then and now *Douglas Koshland*

Higher-order chromosome structure is critical for chromosome segregation, DNA repair, and gene expression. Thirty years ago, the gross cytological differences between chromosomes from budding yeast and other larger eukaryotes led to the notion that budding yeast likely lacked higher-order chromosome structure. However, once sister chromatid cohesion and condensation were visualized in budding yeast by my colleague, my laboratory and many others exploited yeast to discover and characterize the SMC complexes, the small family of proteins that mediate chromosome architecture in all kingdoms of life. More recently, techniques of chromosome conformation capture had revealed loops and topologically associated domains in mammalian chromosomes that were absent from budding yeast, once again leading to the notion that fundamental features of higher-order chromosome structure were not conserved. My laboratory with the help of the Darzacq laboratory have used micro-C to visualize individual chromosome loops throughout the yeast genome as well as topologically associated domains (TADs). Because of the robust signal for these features compared to other eukaryotes and the wealth of information about the cis and trans factors that modulate genome function, budding yeast is poised to help elucidate the molecular mechanisms that underlie the formation of these chromosome folds and their biological function. While the exploitation of budding yeast to elucidate fundamental aspects of cell biology like higher-order chromosome structure will continue to be important, shifting the emphasis from what is happening inside yeast to how they interact with their natural environment will lead to the most surprising and novel biological insights.

64 Control of the nucleo-cytoplasmic localization of the yeast mRNA decapping complex Kiril Tishinov[1], *Anne Spang*[1] 1) Biozentrum, University of Basel.

In yeast, mRNA is mostly degraded from the 5' to 3' end by removal of the 5' 7-methylguanosine cap and subsequent 5'-3' exonuclease digestion in processing bodies (PBs). The cap removal itself is an irreversible tightly controlled process, which requires the concerted action of the decapping complex Dcp1/Dcp2 with an ensemble of auxiliary protein factors. Our data indicate that two of these proteins, the translational repressor Scd6 and the decapping activator Edc3 are directly responsible for the proper distribution of the decapping complex between the cytosol and the nucleus by linking Dcp1/2 to the other components of the cytoplasmic mRNA decay machinery. In mutants deleted for either or both *EDC3* and *SCD6*, Dcp1/Dcp2 was enriched in the nucleus and failed to relocalize it to the cytoplasm to form PBs even under strong stresses, resulting in decreased fitness and reduced growth. Increasing the cytosolic levels of Dcp2 by overexpression or by anchoring Dcp2 to on ER membrane alleviates these effects. Importantly, anchoring Dcp2 onto mitochondria reduced the rescue ability drastically, indicating that the proper Dcp2 localization within the cytoplasm is important. The nuclear localized Dcp1/2 complex is not linked to nuclear mRNA degradation. Our data rather indicate that nuclear localization functions as a ready releasable storage pool for Dcp1/Dcp2, which could be moved immediately to the cytoplasm under stress.

65 A stress response allows highly mutated eukaryotic cells to survive and proliferate *Rebecca Zabinsky*[1], Jonathan Mares[1], Daniel Jarosz[1] 1) Stanford University, Stanford, CA.

Mutations accumulate slowly as cells age but can accumulate quickly in the absence of DNA repair, as is the case of hypermutating cancers. Despite the deleterious effects of individual mutations, cancers and pathogens can survive surprisingly high mutation loads. We investigated whether accumulating mutations, regardless of their identity, induced a stress response by propagating dozens of independent *Saccharomyces cerevisiae* mutator lineages in a mutation accumulation framework. This created clonal cell lineages that each harbor thousands of distinct mutations. Across these lineages, the fitness cost per mutation was high for initial mutational events, but lower for later events even though the mutation spectrum did not differ. This change in fitness cost coincided with induction of a gene expression program that is distinct from previously characterized stress responses. Inhibition of this response, which we term EMBR (eukaryotic mutation burden response), selectively killed the lineages with high mutation burden but not their unmutated ancestors. We identified the transcriptional regulator *UME6,* which recruits the histone deacetylase complex Rpd3L, as a likely EMBR activator. Multiple lines of evidence implicate protein misfolding as a source of toxicity associated with mutation burden, and we found that both *UME6* and the Rpd3L scaffold *SIN3* are necessary for tolerating chronic protein misfolding stress. Our results establish that eukaryotic cells can mount a stress response that buffers the cost of accumulating mutations and suggest that the capacity to survive mutation burden is a vulnerability that could be targeted therapeutically.

66 ER stress sensor Ire1 deploys a divergent transcriptional program in response to lipid bilayer stress *Guillaume Thibault*[1,3], Nurulain Ho[1], Haoxi Wu[1,4], Jiaming Xu[2], Wei Sheng Yap[1], Jhee Hong Koh[1], Chong Shu Chen[1], Stefan Taubert[2] 1) School of Biological Sciences, Nanyang Technological University, Singapore, 637551; 2) Centre for Molecular Medicine and Therapeutics, BC Children's Hospital Research Institute, Department of Medical Genetics, The University of British Columbia, Vancouver, British Columbia, Canada, V6H 3N1; 3) Institute of Molecular and Cell Biology, A*STAR, Singapore, 138673; 4) Current address: Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309, USA.

The unfolded protein response (UPR), a complex adaptive stress response of the endoplasmic reticulum (ER), is compromised in disease and aging. Typically activated by the accumulation of misfolded proteins within the ER lumen, the UPR is similarly activated by alterations of fatty acids and lipid composition at the ER. Chronic UPR activation by ER lipid aberrations, which we termed lipid bilayer stress-induced UPR (UPR^{LBS}) is associated to the development of the metabolic syndromes. However, most studies to dissect the UPR^{LBS} mechanisms employ exogenous lipids or omit precursors of lipid biosynthesis. To systematically understand pathways that contribute to UPR activation, we performed a genome-wide genetic screen in *S. cerevisiae* and an RNAi screen in *C. elegans* to identify mutations that activate the UPR though LBS. Several unexpected cellular perturbations were identified to induce the UPR through LBS. As one of the strongest hits inducing the UPR, we further characterise the activation mechanism of Ire1 in Δ*opi3* where lack of phosphatidylcholine (PC) synthesis induces LBS. We found that the luminal domain (LD) of Ire1, which senses misfolded peptides, is dispensable to activate the UPR by LBS while LD overexpression was sufficient to uncoupled LBS-induced to proteotoxic-induced UPR. Conversely, we mutated Ire1 residue R537 located at the interface of the amphipathic and transmembrane helices rendering it insensitive to LBS while retaining the capacity to activate the UPR by proteotoxic stress. Furthermore, transcriptomic and ChIP-qPCR data revealed that the UPR program diverges if activated by LBS or proteotoxic stress. Together, our data point to the UPR as a broad-spectrum compensatory pathway in which LBS and proteotoxic stress-induced UPR deploy divergent transcriptional programs.

67 Generating new orthogonal tRNA and aminoacyl-tRNA synthase pairs in yeast to engineer translation *Stephanie Zimmerman*[1], Bianca Ruiz[1], Stan Fields[1] 1) Department of Genome Sciences, University of Washington, Seattle, WA.

Orthogonal tRNA and aminoacyl-tRNA synthase (aaRS) pairs are useful reagents for engineering translation, as they do not interact with the native translational machinery of the cell. They have been widely used for genetic code expansion to install non-canonical amino acids site-specifically in proteins by codon reassignment. However, previous work in eukaryotes has focused on the development and application of only a few well-characterized aaRS/tRNA pairs, and nearly always to suppress the amber stop codon, limiting the number of sites that can be reassigned at once. Our goal is to expand the diversity of orthogonal aaRS/tRNA pairs available in eukaryotes and engineer these pairs to reassign many codons. First, we have developed a high-throughput strategy to screen tens of thousands of potential new orthogonal aaRS/tRNA pairs in yeast. In this method, a library of tRNA genes with a nonsense suppressor anticodon are combined with a library of barcoded aaRS genes. Yeast containing these pairs are sorted for their ability to suppress a stop codon in GFP, and the tRNA and aaRS barcode are sequenced to identify tRNAs with suppressor activity only in the presence of their cognate aaRS. We tested this method by combining *E. coli* aaRS genes with a library of tRNA genes from *E. coli* and the Archaea *Methanocaldococcus jannaschii* and *Methanosarcina mazei*. We show that this method can recover the known *E. coli* TyrRS/$tRNA^{Tyr}$ and LeuRS/$tRNA^{Leu}$ orthogonal pairs, as well as identify the most active $tRNA^{Tyr}$ and $tRNA^{Leu}$ sequences in their families. Currently, we are using this strategy to comprehensively screen all aaRS/tRNA pairs from multiple bacteria and Archaea species for activity and orthogonality in yeast. Second, we are screening orthogonal tRNAs with all possible anticodon sequences to identify those that can reassign sense codons. To multiplex these screens, we take advantage of the fact that tRNAs with anticodons that produce mistranslation at sense codons are frequently toxic in cells and become depleted in the population. Using this approach, we show that *E. coli* $tRNA^{Leu}$ can be engineered to reassign nearly any non-leucine codon, and that *E. coli* $tRNA^{Tyr}$ has less plasticity but can still reassign many sense codons. We expect that these new reagents will be portable to other eukaryotes, and will be useful for expanding the diversity of non-canonical amino acids that can be installed in proteins.

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Neurogenetics (Zebrafish)

68 Retinoic acid organizes the vagus motor topographic map via spatiotemporal regulation of Hgf/Met signaling *Adam Isabella*[1], Gabrielle Barsh[1,2], Jason Stonick[1], Cecilia Moens[1,2] 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) University of Washington, Seattle, WA.

The accurate transmission of information through the complex cellular networks of the nervous system requires that the pattern of connectivity within these networks be extremely well organized. A common organizational motif is the topographic map, in which the positions of neurons' cell bodies corresponds to the positions of their axon targets. Topographic organization is established by the targeted extension of axons to their targets during embryonic development; while this is believed to rely on spatial identity differences within neurons and target tissues, the mechanisms that promote topographic axon targeting are, in many cases, not understood. We have identified a novel mechanism of topographic map development in the highly conserved zebrafish vagus nerve. The vagus nerve forms a cluster of neurons in the brain that extends four axon branches to pharyngeal arches (PAs) 4,5,6, & 7 in the neck. We have used single-cell mapping to precisely map the innervation of the vagus nerve, revealing a topographic organization in which more anterior neurons extend axons to more anterior branches. This complex pattern is important to carry out the multiple functions of the vagus nerve, but how it is appropriately organized during development is not known. Using live imaging of nerve growth and single-cell transplantation, we found that both the spatial position of a neuron, and the time at which it extends its axon, influence which branch it innervates, suggesting complex spatiotemporal control of axon targeting decisions. To identify the *spatial* neuronal identity differences that control axon targeting, we performed spatially-resolved RNAseq and single-neuron genetic manipulation, and found the culprit to be differential Retinoic Acid (RA) signaling levels across the A-P axis. We further found, using pharmacological, genomic, and mutant analysis, that RA controls targeting through regulation of the axon guidance receptor *met*. To identify the *temporal* identity differences that control axon targeting, we examined the dynamics of these factors over time and observed that RA drives a anterior-to-posterior wave of *met* expression in vagus neurons. Finally, we found that pharmacologically manipulating the timing of met signaling is sufficient to specify axon targeting. These data reveal a novel mechanism in which precise spatiotemporal control of chemoattractant signaling guides complex axon targeting decisions and topographic nerve organization.

69 The autism- and epilepsy-associated gene Neurobeachin regulates electrical synapse formation via interactions with an intracellular synaptic scaffold *Anne Martin*[1], Jen Michel[1], Adam Miller[1] 1) University of Oregon.

Electrical synapses are ubiquitous connections between neurons mediated by gap junction channels. They are required for initial circuit wiring and chemical synapse formation and are used broadly in neural circuits throughout adulthood. Recent progress in our lab has identified asymmetric electrical synapse protein arrangements which suggest complex electrical synapse composition and development, yet the mechanisms guiding electrical synapse form and function are largely unknown. While the biochemical makeup of electrical and chemical synapses are distinct, suggesting unique molecules and processes regulate their development, the autism- and epilepsy-associated gene Neurobeachin is required for the formation

of both types of synapses. Neurobeachin is proposed to organize chemical synapse receptor trafficking and synapse stabilization, but how it regulates electrical synapse formation is completely unknown. Here, we investigated Neurobeachin function within zebrafish Mauthner neurons as they make easily identifiable and stereotyped electrical and chemical synapses in developing embryos. We find Neurobeachin directly binds with Tjp1b, a postsynaptic electrical synapse scaffolding protein, which is itself required for electrical synapse formation and function. In addition, we show Neurobeachin function is required postsynaptically, suggesting it may be involved in either dendritic trafficking or postsynaptic stabilization of Tjp1b. Moreover, we find that Neurobeachin is required for glutamatergic AMPA receptor and glycinergic receptor localization at nearby chemical synapses. These findings suggest that Neurobeachin regulates specific postsynaptic targeting of electrical and chemical synapse proteins, and highlights the need to identify the mechanism(s) by which Neurobeachin acts as a master regulator of both electrical and chemical synaptogenesis.

70 Identifying proteins that bind Hmx3a and testing their roles in spinal cord development *William Haws*[1], Samantha England[1], Gabriela Susana[1], Sophie Hernandez[1], Ginny Grieb[1], Katharine Lewis[1] 1) Syracuse University, Syracuse, NY.

One of the most important properties that neurons acquire during development is their neurotransmitter phenotype, as this determines how they communicate in neuronal circuits. In all cases examined so far, neurotransmitter phenotypes are initially specified by transcription factors. We have discovered that the homeodomain-containing protein Hmx3a is required for the glutamatergic (excitatory) neurotransmitter phenotype of at least some dI2 spinal neurons in zebrafish. As *Hmx3a* is expressed in the same cell types in mouse spinal cord, this function is likely conserved in mammals. However, we do not yet know the molecular mechanism through which Hmx3a specifies dI2 glutamatergic phenotypes. Surprisingly, our preliminary data suggest that Hmx3a does not require its homeodomain DNA-binding domain for normal function. Therefore, we hypothesized that other proteins may interact with Hmx3a, enabling it to bind DNA indirectly or have a different cellular function. As no Hmx3a binding partners were known, we performed a yeast two-hybrid screen to identify proteins that interact with Hmx3a. We then confirmed a subset of these interactions with co-immunoprecipitation using zebrafish embryo lysates and recombinant proteins. We are now testing interactions of confirmed binding partners with proteins encoded by different *hmx3a* CRISPR mutants. So far, we have identified a DNA-binding protein that binds both full-length and a truncated, but functional, mutant form of Hmx3a, but does not bind a truncated form of Hmx3a that results in abnormal spinal cord, ear and lateral line phenotypes in homozygous mutant embryos. Since both of these truncated proteins lack the homeodomain, we hypothesize that this binding partner may mediate an interaction between Hmx3a and DNA that is required for normal Hmx3a function. We have also used CRISPR interference (CRISPRi) and CRISPR mutagenesis to knock down/ knock out expression of confirmed Hmx3a binding partners to test whether they are also required for correct dI2 neurotransmitter phenotypes. Excitingly, for several of these binding partners, embryos in which the gene is mutated, or expression is knocked-down, have altered numbers of excitatory spinal cord neurons. None of these interacting proteins have previously been implicated in specifying neurotransmitter phenotypes, suggesting that this research will illuminate new mechanisms through which neurons acquire appropriate neurotransmitter phenotypes.

71 Inflammatory signaling regulates neurofibromin 1 (*nf1*)-dependent habituation learning in larval zebrafish *Andrew Miller*[1,2,3,6], Jessa Snower[2,3,6], Yeng Yang[2,3,6], Anna Huttenlocher[4,5,6], Mary Halloran[2,3,6] 1) Neuroscience Training Program; 2) Department of Integrative Biology; 3) Department of Neuroscience; 4) Department of Pediatrics; 5) Department of Medical Microbiology and Immunology; 6) University of Wisconsin-Madison, Madison, WI, USA.

Identification of the cellular signaling pathways that regulate neurofibromin 1 (*nf1*)-dependent learning is a key step toward improving the lives of patients living with Neurofibromatosis type 1 (NF1), a multi-symptom, monogenetic disorder. An increasingly recognized aspect of the disease is neurodevelopmental impairment, including learning and attention disorders. Nf1 is known to inhibit Ras signaling through a Ras-GTPase activating protein-related domain. Clinical trials with drugs that inhibit downstream Ras signaling have shown promise in reducing tumor burden in NF1 patients but have not improved learning or attention. Therefore, Nf1 may regulate other cellular signaling pathways that influence neurodevelopment and behavior.

To study *nf1*-dependent learning, we use habituation of the acoustic startle circuit in larval zebrafish. Habituation is a simple form of non-associative learning that allows organisms to filter irrelevant stimuli and reduce unnecessary behavioral responses. To identify cellular signaling pathways affected by *nf1* mutation, we took two approaches: (1) a small-molecule drug screen assessing habituation in *nf1* mutant larval zebrafish; (2) RNA-seq to measure differences in gene expression between wildtype and *nf1* mutant larval zebrafish. We found an upregulation of genes associated with the immune and inflammatory response in *nf1* mutants. To test the hypothesis that inflammatory signaling influences behavior, we asked if anti-inflammatory drugs could rescue the *nf1*-dependent habituation defect. We found that a Tnf-α inhibitor (pentoxifylline) and a Cxcr1/2 antagonist (SB225002) improved habituation in *nf1* mutants. Additionally, a Cxcr4 antagonist (plerixafor) improved habituation in *nf1* mutants with at least one wildtype copy of *nf1a* or *nf1b* but not double homozygous mutants. We are investigating the specificity of these behavioral effects to the *nf1* mutation by testing anti-inflammatory drugs on other known habituation mutants and by inducing inflammation in wildtype larvae. Lastly, we are investigating the neural circuits involved in *nf1*-dependent habituation using a whole-brain, phosphorylated ERK activity mapping approach, which allows us to identify brain regions and cell types with differential activity between wild type and nf1 mutants during spontaneous swimming, non-habituating acoustic startle, and habituating acoustic startle.

72 Dolk regulates motor behaviors through the episodic ataxia-associated protein Kv1.1 *Joy Meserve*[1], Jessica Nelson[1], Kurt Marsden[1], Jerry Hsu[1], Roshan Jain[1], Marc Wolman[1], Michael Granato[1] 1) University of Pennsylvania.

Executing controlled movements requires coordination at different sites throughout the nervous system, including the brain, spinal cord, and neuromuscular junction. Deficits in initiating or executing movements are associated with a range of neurodevelopmental and neuropsychiatric disorders. Body movements are the cumulative result of many neural circuits functioning in the brain and spinal cord, making it difficult to pinpoint which neural circuit components are disrupted in movement disorders. Even when the causative disrupted gene is identified, this gene can function in numerous cell types and neuron populations. To identify and characterize genes that regulate movement, we performed a forward genetic screen in larval zebrafish. By 5 days post fertilization, larval zebrafish perform a variety of behaviors, including slow spontaneous swimming and rapid escape responses, which utilize unique and overlapping neural circuits. Through whole genome/exome sequencing, we identified eight genes that regulate kinematic movement parameters of movement. These genes act at different loci, including in muscle, at the neuromuscular junction, and within spinal interneurons, to control movement. Here we focus on two genes, mutants of which display exaggerated movements. We identify one mutant to be caused by a mutation in *dolichol kinase* (*dolk*), a key regulator of the glycoprotein biosynthesis pathway. Many proteins require glycosylation

for proper function, including the potassium Shaker-like channel subunit Kv1.1. We found through our genetic screen that mutation of *kcna1a*, which encodes Kv1.1, causes the same exaggerated movements observed in *dolk* mutants. This behavior is similar to behaviors observed in patients with episodic ataxia, which is caused by mutations in *KCNA1*. We demonstrate that Kv1.1 protein is mislocalized in *dolk* mutants, suggesting they act in the same genetic pathway to regulate movement. We further demonstrate that the motor defect in *dolk* and *kcna1a* mutants is independent of known hindbrain circuitry driving locomotion, suggesting their site of action is within the spinal circuitry. These results demonstrate *dolk* and *kcna1a* act together in a pathway critical for motor circuits that regulate movement magnitude.

73 Fishing for function in the evolutionary gene pool: a zebrafish model for human-specific duplicated gene *SRGAP2* *Jose Uribe-Salazar*[1,2], Brittany Radke[1], Alexandra Colon-Rodriguez[1], KaeChandra Weyenberg[1], Cole Ingamells[1], Gulhan Kaya[1], Eva Ferino[1], Pamela Lein[3], Li-En Jao[4], Megan Dennis[1,2] 1) Genome Center, MIND Institute, and Department of Biochemistry & Molecular Medicine, University of California, Davis, CA, USA; 2) Integrative Genetics and Genomics Graduate Group, University of California, Davis, CA, USA; 3) Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA, USA; 4) Department of Cell Biology and Human Anatomy, University of California, Davis, CA, USA.

Gene duplication is a fundamental source of species innovation that contributes to novel phenotypic features. *SRGAP2* is a notable example of a gene uniquely duplicated in the *Homo* lineage resulting in three partial paralogs present in modern humans that potentially contributed to neurological features exclusive to humans. Previous studies show that expression of human paralog *SRGAP2C*, a truncated form of the ancestral *SRGAP2A*, impacts cortical neuronal migration and synaptogenesis in mice, by antagonizing the function of the ancestral ortholog *Srgap2*, mirroring the traits observed in *Srgap2* knockout mice. Based on the high conservation of zebrafish ortholog *srgap2* and full-length *SRGAP2A* (74% amino acid identity), we propose to use zebrafish (*Danio rerio*) as a new higher-throughput model to quickly test gene function. We tested if the expression of human-specific *SRGAP2C* in zebrafish phenocopied *Srgap2* knockout in mice. Consistent with previous findings that link *SRGAP2A* dysfunction with epilepsy (in humans) and synaptic connectivity (in mice), *SRGAP2C*-mRNA-injected zebrafish exhibited increased locomotor activity when exposed to GABA antagonist pentylenetetrazol. *srgap2*-null larvae also showed a similar seizure-like phenotype. Conversely, larvae injected with the full-length human *SRGAP2A* mRNA showed significantly reduced activity. We also observed a significantly altered excitatory/inhibitory neuronal ratio in our *srgap2*-knockout and *SRGAP2C*-injected zebrafish, and, again, the reciprocal phenotype in *SRGAP2A*-injected larvae. To understand the nature of these defects on a molecular level, we performed RNA-seq analysis and found that genes in nervous system processes were dysregulated in the *srgap2*-null larvae (20 fold decrease, p=1.74x10^{-40}). Unexpectedly, genes related to visual perception were the most significantly downregulated (63 fold decrease, p=2.38x10^{-58}), including numerous crystalline genes, suggesting that our *srgap2* knockout lines suffer a lens defect not previously reported in other model systems. Preliminary experiments evidence that these same lens genes are upregulated in the *SRGAP2A*-injected larvae, showing again this reciprocal effect. In all, these studies show that zebrafish represents a feasible model to characterize and discover potentially new functions of a well-studied human duplicated gene and provide a roadmap to understand if/how other genes may contribute to the evolution of novel neurological traits unique to humans.

74 E4bp4-2b/Nfil3-2b contributes to circadian regulation by repressing *cryptochrome1aa* and *period2* expression via the D-box enhancer *Han Wang*[1] 1) Soochow University.

Within the core circadian clock mechanism, transcription control exerted by a small set of enhancers, is fundamental for timing function. While E-box and RRE regulation seems to be highly conserved throughout vertebrate evolution, the function of the D-box appears to have changed significantly. Specifically, the D-box enhancer appears to serve as as a clock-controlled element in mammals, whilst it also represents a directly light-driven enhancer in fish. However, our understanding of the function of the family of D-box-binding transcription factors in different vertebrate groups remains poorly understood. While the PAR bZIP transcription factors TEF, DBP and HLF have been studied extensively in mouse models, the role of E4BP4/NFIL3 transcriptional repressors remains unclear. Here we show that the zebrafish ortholog *e4bp4-2b/nfil3-2b* is rhythmically expressed in various zebrafish tissues and organs and is controlled by the circadian clock via E-box and RRE as well as being-light inducible via D-box in its promoter. In TALEN-generated *e4bp4-2b* mutants and heat-shock inducible *Tg(hsp70l:e4bp4-2b;CG)* transgenic fish we reveal that the expression of the key circadian clock genes *period2 (per2)* and *cryptochrome1aa (cry1aa)* and the phase of locomotor rhythms is significantly altered. E4bp4-2b represses *cry1aa* and *per2* transcription via binding to the D-boxes in these clock gene promoters and thereby provides a feedback mechanism for the core circadian clock mechanism. Together, these findings point to significant complexity in the regulation and function of E4BP4/NFIL3 factors and imply that they may serve as integrators of environmental timing signals in the control of core cell functions.

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Disease Models (*Xenopus*)

57 *Importance innate-like T cells in tolerance versus resistance to virus and mycobacteria in Xenopus* *Jacques Robert*[1], Sobhan Roy[2], Adil Khan[1], Martin Pavelka[1], Curtis McMurtrey[3], William Hildebrand[3], Erin Adams[2], Mattieu Paiola[1] 1) University of Rochester Medical Center, Rochester NY; 2) The University of Chicago, Chicago, IL ; 3) The University of Oklahoma Health Sciences Center, Oklahoma City, OK .

To date, *Xenopus* is the only species outside mammals where an immune surveillance system based on non-polymorphic MHC class I-like directing the development and function of unconventional innate-like (i)T cells has been characterized. Unlike adults, tadpoles rely mostly on a few prominent subsets of functionally distinct innate-like (i)T cells. We have characterized by reverse genetics and MHC tetramers technology two *Xenopus*-non-polymorphic MHC I-like (XNC) genes controlling two distinct *X. laevis* iT cell subsets. XNC10 restricts iVa6 T cells that are functional analogs to mammalian iNKT cells and critical for defenses against ranavirus (pox-like large double strand DNA virus) pathogens. XNC4 is required for iVa45 T cells development and function in host defenses against mycobacteria (*M. marinum*). Notably, mass spectrometry analysis reveals that XNC4 binds abundant and uncharacteristically long peptides (9-11mer) with a single putative anchor reminiscent of human HLA-F. Furthermore, XNC4 tetramers produced by co-culture with *M. marinum*-derived peptides, preferentially recognizes a iT cell subset in infected liver. The prominent tadpole iT cell response against *M. marinum* inoculation correlates with active non-inflammatory tolerance to pathogen accumulation, which contrasts with the adult CD8 T cell-mediated immune resistance, inflammation and granuloma formation. This is the first direct evidence of ligands associated with an MHC molecule from a basal tetrapode vertebrate. Additionally, these data provide novel insights into the evolution and structural adaptation of unconventional presentation of ligands by MHC class-like molecules, and the critical function of MHC-like and iT cells in regulating host resistance

versus tolerance against pathogens.

58 *Xenopus tropicalis* mutation in the transcription factor *six3* reveals its key role in controlling the eye gene regulatory network Sumanth Manohar[1], Takuya Nakayama[1], Marilyn Fisher[1], *Robert Grainger*[1] 1) University of Virginia.

Historically *Pax6* has often been presented as the key gene initiating eye formation, though clearly several genes play essential early roles, e.g. *Rax*, *Lhx2*, and others. While the transcription factor *Six3* and related gene family members have also been implicated in eye formation in both vertebrates and invertebrates, the headless (and thus eyeless) phenotype in *Six3* knockout mice, and even conditional mutants targeting the eye in the mouse, have not allowed the roles of this gene to be clearly understood. The *X. tropicalis six3* loss of function mutant we have generated (Nakayama et al. 2013) has a somewhat weaker phenotype fortuitously allowing us to discern prominent roles for this gene in the regulatory network controlling both lens and retina formation and to show that it acts independently of *Pax6*. We find that this gene is essential for proper lens formation: the majority of mutants have no lens and the remainder a very rudimentary one. Transplantation of lens ectoderm between wildtype and mutant embryos reveal a largely non-autonomous function for this gene, implying the mutation interferes with essential signaling from the retina that is involved in lens induction. Among the numerous downstream targets of *six3* identified in the mutant are the closely related nuclear protein genes *mab21l1* and *mab21l2*. Injection of *mab21l1* mRNA significantly rescues the lens phenotype, placing one or both of these genes in a key intermediary position in the *six3* gene hierarchy. Also affected in the mutant are BMP signaling components in the retina, which are rescued in the *mab21l1* mRNA injection experiments, supporting a hypothesis in which BMP signaling is essential for lens formation. Although BMP signaling has been implicated in lens induction previously, how this key signal is regulated has not been previously recognized. In addition to effects on the lens, the mutant fails to form the characteristic layers in the retina, a phenotype which is also at least partially rescued by *mab21l1* mRNA injections into the *six3* mutant, again supporting a key intermediary role for *mab21l1* (and/or the closely related *mab21l2*) in mediating *six3* function. Taken together studies on the *Xenopus six3* mutant support previously unrecognized roles for this gene in key steps of both lens and retina formation.

59 The mechanisms of neural crest defects in DDX3X syndrome and related genetic diseases *Shuo Wei*[1], Mark Perfetto[1, 2], Xiaolu Xu[1], Jiejing Li[2] 1) University of Delaware; 2) West Virginia University.

Mutations in the RNA helicase DDX3 have emerged as a frequent cause of intellectual disability in humans. Because many patients carrying DDX3 mutations have additional defects in craniofacial structures and other tissues containing neural crest (NC)-derived cells, we hypothesized that DDX3 is also important for NC development. Using Xenopus tropicalis as a model organism, we show that DDX3 is required for normal NC induction and craniofacial morphogenesis by regulating AKT kinase activity. Depletion of DDX3 decreases AKT activity and AKT-dependent inhibitory phosphorylation of GSK3β, leading to reduced levels of β-catenin and Snai1, two GSK3β substrates that are critical for NC induction. DDX3 function in regulating these downstream signaling events during NC induction is likely mediated by RAC1, a small GTPase whose translation depends on the RNA helicase activity of DDX3. These results suggest an evolutionarily conserved role of DDX3 in NC development by promoting AKT activity, and provide a potential mechanism for the NC-related birth defects displayed by patients harboring mutations in DDX3 and its downstream effectors in this signaling cascade.

60 Modeling Li-Fraumeni Mutations in *Xenopus laevis* *Amisheila Kinua*[1,2], Alexandria Blackburn[1,3], Mark Corkins[1], Zubaida Saifudeen[4], Rachel Miller[1,3,5] 1) Rice University, Houston, TX; 2) UTHealth McGovern Medical School, Houston, TX; 3) MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX; 4) Tulane University, New Orleans, LA; 5) University of Texas MD Anderson Cancer Center, Houston, TX.

Li-Fraumeni syndrome is caused by mutations in the tumor suppressor gene, *p53*, resulting in patient predisposition to cancer. Tumor protein 53, also known as p53, acts as a tumor suppressor and responds to cellular stressors such as DNA damage. Though p53 is well known for its role as a tumor suppressor, it is also important for normal processes during embryogenesis, including kidney development. Preliminary clinical data indicate that *p53* mutations identified in Li-Fraumeni patients are associated with an increased prevalence of urogenital anomalies, suggesting that p53 may be important for kidney development. Given that nephron structure and function are highly conserved among vertebrates, *Xenopus laevis* (frog) embryos will be used to model Li-Fraumeni patient mutations associated with urogenital anomalies. Using both morpholino and CRISPR/Cas9 strategies, we show that reduced p53 results in reduced expression of kidney markers. We express patient-identified dominant-negative mutations in the *Xenopus laevis* kidney and find that patient mutations result in a moderate reduction of kidney phenotypes that pheno-copy overexpression of wild-type human p53 above the sub-phenotypic dose. Taken together, we find that the p53 mutations in Li-Fraumeni patients may disturb kidney development.

61 The CLEAR consortium: elucidating the genetic and cellular basis of trachea-esophageal birth defects Aaron Zorn[1, 2], Talia Nasr[1, 2], *Nicole Edwards*[1, 2], Pamela Mancini[1, 2], Scott Rankin[1, 2], Zachary Agricola[1, 2], Matthew Kofron[1, 2], Paul Kingma[1], Yufeng Shen[3], Wendy Chung[3], James Wells[1] 1) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Center for Stem Cell and Organoid Medicine, Cincinnati, OH; 3) Columbia University Medical Center, New York, NY.

The trachea and esophagus (TE) arise from a common foregut tube during embryonic development. Disruptions in TE morphogenesis cause life-threatening trachea-esophageal birth defects (TEDs). TEDs occur in approximately 1 in 3500 births and their etiology is poorly understood. We have established the www.CLEARconsortium.org; a NICHD-funded multidisciplinary team of clinicians, geneticists, bioinformaticians, stem cell and developmental biologists focused on discover the genetic and cellular basis of trachea-esophageal birth defects. Here we report recent CLEAR consortium studies using *Xenopus* and mouse models to define the conserved molecular and cellular mechanisms that regulate normal TE morphogenesis from the fetal gut tube. We show how the Hedgehog/Gli pathway regulates key steps in TE morphogenesis including Rab11-dependent epithelial remodeling which when disrupted results in tracheoesophageal clefts similar to human patients. Finally, we provide an update on ongoing F0 CRISRP mutagenesis screens in *Xenopus tropicalis* testing candidate mutations (variants of unknown significance) from whole exome sequencing of human patient-parent trios. To date we have strong evidence implicating half a dozen sporadic mutations that likely cause esophageal atresia. These results significantly advance our understanding of TEDs with the goal of revealing phenotype-genotype associations that will impact clinical treatment.

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Gene Regulation (Mammal)

48 Genome-wide identification and analysis of single nucleotide variants disrupting RNA structure and function Jianan Lin[1,2,3], Yang Chen[1,2], Yuping Zhang[3], *Zhengqing Ouyang*[1,2,3] 1) The Jackson Laboratory for Genomic Medicine, Farmington, CT; 2) University of Massachusetts, Amherst, MA; 3) University of Connecticut, Storrs, CT.

Single nucleotide variants (SNVs) have been associated with numerous traits and diseases. An emerging class of SNVs, named riboSNitches, disrupt RNA structures and affect their functions. A handful of riboSNitches have been identified. However, genome-wide identification and analysis of riboSNitches is challenging as the signals of RNA structural disruption are often subtle. We introduce a computational pipeline for riboSNitch identification and analysis at the genome scale. Compared to previous approaches, our pipeline shows higher accuracy on identifying known riboSNitches captured by different experimental RNA structure probing methods including the parallel analysis of RNA structure (PARS) and the selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE). Further, our pipeline detects the experimentally validated riboSNitch that regulates human catechol-O-methyltransferase haplotypes and outputs structurally disrupted regions precisely at base resolution. In addition, we construct a database that includes the annotation and visualization of known riboSNitches and predicted riboSNitches that are expression quantitative trait loci, representing a new resource for investigating the functional roles of SNVs.

49 Structure and function of SWI/SNF complexes is regulated by RNA interactions Carl Manner[1], *Jesse Raab*[1] 1) University of North Carolina at Chapel Hill.

The SWI/SNF chromatin remodeling complex is among the most frequently mutated families of genes across all human cancers. Mutations in the complex are most frequently inactivating and presumed to lead to disruption of expression of the complex. However, several subunits have missense mutations that may lead to altered activity or disruption of specific functions of the protein. The consequence of these less severe disruptions is unclear, but mutations in the catalytic SWI/SNF subunits can have effects on chromatin accessibility and oncogene expression either directly or by influencing the function of sibling subunits.

We recently found that SMARCA4, one of the catalytic subunits of mammalian SWI/SNF, bound thousands of transcripts. Several examples of individual RNAs that interact with the SWI/SNF complex are known, but the extent of global SWI/SNF interactions with RNA were not appreciated. Additionally, previous *in vitro* studies point to a potential inhibitory role of RNA on SWI/SNF catalytic activity, while recent biochemical approaches suggest that RNA may contribute structurally to many complexes, including SWI/SNF. We sought to investigate the consequence of widespread RNA interactions with SWI/SNF on the composition and/or function of the complex. Our results suggest that RNA association with SWI/SNF occurs co-transcriptionally at thousands of transcripts. Using genomics based approaches and modulation of transcription globally and at individual loci we are identifying the function of these interactions. Immunoprecipitations of SMARCA4 in the absence of RNA support a role for RNA in regulating the composition of SMARCA4 containing complexes. Additionally, putative RNA binding domains in SMARCA4 have been described and we are characterizing the function of these regions within the protein. Notably, several cancer associated missense mutations can be found in these regions. Our work aims to uncover novel regulatory mechanisms that control SWI/SNF composition and/or function.

50 Mechanism of monoallelic expression and allelic rheostat role of DNA methylation *Alexander Gimelbrant*[1] 1) Dana-Farber Cancer Institute.

In mammalian cells, large groups of genes show epigenetically controlled unequal transcription of maternal and paternal alleles. Thousands of autosomal genes subject to monoallelic expression (MAE) comprise the largest such group. The initial random allelic choice in these loci is followed by mitotically stable transmission of the allele-specific state, leading to stable epigenetic and functional differences between clonal cell lineages. Molecular mechanisms underpinning MAE maintenance are not known. We devised and performed a drug screen for reactivation of epigenetically silenced alleles in mouse cells, using a new strategy based on targeted allele-specific RNA sequencing, which can read out multiple loci simultaneously. We found that, contrary to previous observations, DNA methylation plays key role in mitotic memory of MAE in multiple autosomal loci. Having identified the first perturbation affecting allele-specific expression in multiple loci, we assessed its genome-wide impact. We show that DNA methylation acts as an allelic rheostat, with wide variation of stable states of allelic imbalance. Our findings reveal a previously unappreciated interplay of genetic gene regulatory architecture with epigenetic control of allele-specific expression and reinforce the role of epigenetic mechanisms is maintaining differences between developmentally equivalent clonal cell lineages.

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Genetic Technology in Practice

75 Fungal genetics and automated strain engineering at Zymergen *Kenneth Bruno*[1], Brandon Pfannenstiel[1], Sachin Jain[1], Arthur Fong[1], Patrick Westfall[1], Zach Serber[1] 1) Zymergen Inc.

Industrial fermentation by bacteria and fungi convert simple sugars to enzymes, pharmaceuticals, organic acids, and other valuable commercial products. Strain improvement is critical in these organisms because the efficiency with which microbes generate these products has a direct impact on cost of production. Traditional strain improvement programs use random mutagenesis and screening to identify strains with improved characteristics, such as higher titer, yield, productivity, and other valuable tolerance characteristics (i.e. heat, pH, product toxicity etc.). However, random mutagenesis campaigns also result in deleterious changes that accumulate through consecutive rounds of of strain improvement, making it more difficult to generate strains that may approach a maximum theoretical yield. To address this problem, Zymergen has built a platform for automated and high-throughput targeted strain engineering that helps us determine which individual mutations contribute to important aspects of fermentation such as titer, yield, and productivity. Using this approach, we have uncovered unexpected epistatic genetic interactions that can be used to generate improved production strains that likely would not be attainable through rational engineering. The tools we have developed take the basic concepts and techniques of fungal genetics into automation to help rapidly improve production of valuable products that utilize filamentous fungi.

76 Making the most of our molecules - a computational framework for unified germplasm characterization and inference *Eli Rodgers-Melnick*[1] 1) Corteva Agriscience.

Haplotype frameworks provide an intuitive and evolutionarily-grounded framework for organizing genetic information within a set of populations. These may be constructed from sequenced individuals on the basis of large segments of identical-in-state markers, which are common within the context of closed breeding programs. Extending the haplotype framework to new individuals with low marker density, unknown pedigree relationships, and legacy genotyping data can present a barrier to germplasm characterization and genomic prediction. Here, an approach is presented to unify the analysis of genetic data through the construction of a common mathematical manifold that is invariant to marker platform. The approach implicitly takes advantage of both haplotype sharing from recent coancestry and short-range LD from shared population history without the need for any specification of population genetics parameters. Downstream applications of the framework are presented, including haplotype reconstruction, marker imputation, and enhanced functional genomic inference.

77 High Yield and Robust *Saccharomyces cerevisiae* Strains for Biofuel Industry *Celia Payen*[1] 1) DuPont .

The first generation of yeast-based ethanol production is to convert grain into fuel ethanol, its annual production is about 90 billion liters worldwide. It is estimated that about 70% ethanol production cost is the feedstock. Since the production volume is so big, yield improvement will have massive economic impact for the whole industry.
During industrial fermentation, yeasts are exposed to a very complex and stressful environment that negatively affects their growth, viability and ethanol productivity. Improving high yield strains to be multi-stress tolerant is crucial for their successful industrial applications. DuPont's focus is to develop very high yield yeast strains robust to a variety of stressful conditions that will have great economic and social impact for biofuel industry. In this presentation, we will discuss strategies to increase ethanol production yield, methods to identify robust traits, and approaches to construct high yield and robust yeast strains.

78 Divalent siRNA Scaffold for Robust Gene Modulation in the Central Nervous System *Julia Alterman*[1], Bruno MDC Godinho[1], Matthew R. Hassler[1], Chantal Ferguson[1], Andrew H. Coles[1], Dimas Echeverria[1], Ellen Sapp[2], Reka A. Haraszti[1], Richard Moser[3], Miguel Sena-Esteves[4], Heather Grey-Edwards[4,5], Marian DiFiglia[2], Neil Aronin[1,6], Anastasia Khvorova[1,7] 1) RNA Therapeutics Institute, University of Massachusetts Medical School, MA; 2) Dept. of Neurology, Mass General Institute for Neurodegenerative Disease, MA; 3) Dept. of Neurosurgery, University of Massachusetts Medical School, MA; 4) Horae Gene Therapy Center, University of Massachusetts Medical School, MA; 5) Department of Radiology, University of Massachusetts Medical School; 6) Department of Medicine, University of Massachusetts Medical School, MA; 7) Program in Molecular Medicine, University of Massachusetts Medical School, MA.

RNA interference (RNAi)-based gene silencing holds great promise as a therapeutic strategy for incurable, genetically-defined neurological diseases, such as Huntington's Disease (HD). However, non-toxic and efficient delivery of synthetic oligonucleotides to the central nervous system (CNS) remains a primary challenge that hinders fast progression of this technology for the treatment of CNS disorders.

Here we describe a neuroactive divalent siRNA scaffold (Di-siRNA) that allows broad and long-lasting gene silencing (up to 6 months) in the mouse brain after a single intracerebroventricular (ICV) injection. This novel fully chemically modified scaffold also demonstrated widespread distribution in the brains and spinal cords Cynomolgus macaques after a single injection. Furthermore, Di-siRNAs exhibited similar subcellular perinuclear localization, both in neurons and glia, to that observed in previous rodent studies. Potent silencing of the Huntingtin (Htt) mRNA target and protein was achieved in various regions of the non-human primate (NHP) brain, including cortex, hippocampus and striatum, but also in the spinal cord 1 month after injection. Preliminary toxicity assessments revealed no detectable pathology (bleeding, edema, etc.) and no major inflammatory response in the NHP brain. In addition, no significant changes were observed in complete blood counts and in a panel of biochemical markers, including liver enzymes and electrolytes, suggesting minimal systemic impact. Finally, RNAseq analysis of transcriptomic changes revealed no major off target effects.

Together these data validate the utility of Di-siRNAs for potent and sustained modulation of gene expression in larger mammalian brains, and greatly contribute to the advancement of RNAi-based therapeutics for neurological disorders.

79 NemaMetrix: cutting-edge genome editing and phenotyping tools in *C. elegans* and zebrafish and how we've targeted parasitic worms *Janis Weeks*[1] 1) NemaMetrix Inc..

As an expert in CRISPR genome editing, NemaMetrix creates custom genome-edited *C. elegans* and zebrafish to enable aging, developmental and disease studies. NemaMetrix also develops and manufactures genotyping and phenotyping products, including instruments, reagents and consumables to allow researchers to discover and explore new phenotypes. In addition, NemaMetrix provides services to produce data and insights for researchers aiming to quickly advance early-stage development of new compounds. NemaMetrix was founded by two neuroscience faculty at the University of Oregon in 2011. Our inaugural product combined electrophysiology and microfluidics, with the goal of making electrical recordings from *C. elegans* broadly accessible to researchers. Since then, NemaMetrix has expanded in many new directions. This presentation will provide an overview of the company and describe specifically how the electrophysiology platform developed in *C. elegans* was successfully repurposed for use with parasitic nematodes of humans and animals. Helminth infections cause a staggering burden of disease worldwide and new anthelmintic drugs are critically needed. The powerful genetic tools, custom phenotyping assays and other offerings at NemaMetrix advance basic and applied research, from combatting parasitic diseases to preclinical validation of promising compounds to studying human gene variants expressed in *C. elegans* or zebrafish.

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The Evolution of Gene Expression (PEQG)

51 Simultaneous Quantification of mRNA and Protein Levels in Single Cells Reveals *Trans*-acting Genetic Variation *Christian Brion*[1], Sheila Lutz[1], Frank Albert[1] 1) Genetics, Cell Biology, and Development, University of Minnesota, MN.

Heritable variation in gene expression is a key component of phenotypic diversity and evolutionary change. Most of this variation arises from

t*rans*-acting variants located on different chromosomes from their target genes. Previous work suggested that some *trans*-acting variants specifically affect mRNA or protein levels of a given gene. However, this conclusion rests on studies with low statistical power, conducted at different times, with different experimental designs, and in different environmental conditions. To fully understand how natural genetic variation influences gene expression, we need a strategy that couples high statistical power with simultaneous readouts of mRNA and protein.
We developed a system for quantification of mRNA and protein levels in single, live cells of *Saccharomyces cerevisiae*. We tagged genes of interest with a C-terminal GFP sequence followed by a 3'UTR harboring a CRISPR guide RNA flanked by two ribozymes. After transcription, the ribozymes release the guide RNA, which directs a dCas9 protein fused to a transcriptional activator to drive expression of an mCherry reporter gene. Thus, mCherry serves as a readout of mRNA abundance, while the GFP tag allows measurement of protein levels in the same cell.
This reporter system enabled us to use bulk segregant analysis in hundreds of thousands of recombinant cells to map the genetic basis of expression variation with high statistical power. By applying this approach to ten genes with diverse functions and regulatory architectures, we identified 99 loci affecting gene expression, all of which acted in *trans*. Only 20 loci had concordant effects on mRNA and protein levels. More than half of the loci (56) influenced the protein level of a given gene but showed no effect on its mRNA. There were 17 loci that affected mRNA but not protein. Six loci influenced both mRNA and protein of the same gene, but in opposite directions.
Within one protein specific QTL, we identified a nonsense mutation in the *YAK1* kinase gene as the causal variant. Mass spectrometry and RNA sequencing revealed that this variant affected hundreds of genes in a post-transcriptional fashion, possibly by reducing the expression of genes involved in translation.
Our strategy revealed complex and distinct genetic influences on mRNA vs. protein levels that arise from *trans*-acting loci across the genome. These results have implications for the diversity and evolution of gene expression within and between species.

52 A mutagenesis survey of a developmental enhancer using automation and robotics reveals constraints on evolvability *Timothy Fuqua*[1,2], Jeff Jordan[3], Marlize van Breugel[1,5], Aliaksander Halavatyi[1], Christian Tischer[1], Peter Polidoro[3], Namiko Abe[4], Albert Tsai[1], Chaitanya Rastogi[4], Richard Mann[4], David Stern[3], Justin Crocker[1] 1) European Molecular Biology Laboratory, Heidelberg, Germany; 2) Heidelberg University, Heidelberg, Germany; 3) Janelia Research Campus, Virginia, USA; 4) Columbia University, New York, USA; 5) Leiden University, Leiden, Netherlands.

Gene regulatory evolution provides a significant source of material for phenotypical change. However, we have only a limited understanding of what paths are possible for regulatory evolution, as most evidence is limited to either standing variation or biased perturbations of transcriptional enhancers. Using a synthetic mutation library for a developmental enhancer in *Drosophila melanogaster*, and an automated robotics pipeline, we show that most nucleotide mutations in a minimal enhancer cause changes in gene expression. These changes include transcription levels, probability, timing, and spatial patterns. We demonstrate that this pipeline can be used to identify novel transcription factor binding sites. Based on these sites, we present evidence for transcriptional cooperativity that makes the enhancer sensitive to nucleotide polymorphisms. Intriguingly, we find that sets of mutations often simultaneously change levels and locations of expression. Together, our results suggest that the parameters of gene expression are convolved within an enhancer and not independent variables and this codependency can constrain the evolvability of developmental enhancers.

53 From Codons to Ecology – Using Codon Optimization as a Proxy for Gene Expression to Identify Ecologically Adapted Metabolic Pathways *Abigail LaBella*[1], Dana Opulente[2], Chris Todd Hittinger[2], Antonis Rokas[1] 1) Vanderbilt University; 2) University of Wisconsin–Madison.

A core tenant of evolution is that organisms are adapted to their environments and that these adaptations are reflected within the genome. Genomic data, therefore, can reveal information about ecology – a framework known as reverse ecology. Reverse ecology has been used to identify ecologically relevant adaptations in microbes by associating targets of selection with ecological niches. Differential gene expression also plays a critical role in adaptation; since gene expression is energetically costly, highly expressed metabolic genes that are key to an organism's evolutionary ecology will be highly codon optimized. This raises the hypothesis that codon optimization can serve as a proxy for gene expression in a reverse ecology framework to identify metabolic pathways critical for adaptation to specific environments. We tested this hypothesis in a rich dataset spanning the diversity of the budding yeast subphylum by conducting two experiments. In the first experiment, we measured codon optimization in the genomes of 211 budding yeasts that contain a galactose utilization pathway. Consistent with our hypothesis, we found that inferred high expression of the galactose pathway is strongly associated with isolation from a dairy environment, where the ability to metabolize galactose is beneficial, and anticorrelated with isolation from the beer environment, where the ability to metabolize galactose is likely neutral or detrimental. In the second experiment, we discovered that inferred high expression of two genes involved in lactate assimilation, which are present in the major human pathogen *Candida albicans* and in the emerging pathogen *Candida auris*, are associated with isolation from humans. Lactate is both a carbon source and an anti-microbial molecule which suggests that budding yeast able to readily utilize lactate may have an advantage in the human host environment. These results highlight the significant potential of codon optimization measures for assessing metabolic adaptation to specific ecological environments in microbial eukaryotes.

54 Massively parallel identification of *cis*-regulatory variants in yeast promoters Rocky Cheung[1], Kaushik Renganaath[2], Laura Day[1], Sriram Kosuri[1], Leonid Kruglyak[1,3], *Frank Albert*[2] 1) University of California, Los Angeles, CA; 2) University of Minnesota, Minneapolis, MN; 3) Howard Hughes Medical Institute, Los Angeles, CA.

Regulatory DNA variants influence gene expression and are a key source of variation in complex traits. Thousands of genomic regions that harbor regulatory DNA variants have been identified as expression quantitative trait loci ("eQTLs"). In particular, local eQTLs arise from variants that influence the expression of genes located close to them, mostly via *cis*-acting mechanisms. The identification of individual causal variants in local eQTLs has been challenging due to genetic linkage between neighboring variants.

Here, we used a massively parallel reporter assay to identify *cis*-regulatory variants in promoters of a laboratory and a wine strain of the yeast *Saccharomyces cerevisiae*. We synthesized 17,500 synthetic DNA oligonucleotides to assay 7,005 unique variants in the promoters of 3,076 genes. This design covered half of all promoter variants in these two strains, including all variants within 144 bp immediately upstream of transcription start sites. We cloned these synthetic promoter fragments into plasmids *en masse*, such that each fragment was coupled to a reporter gene and hundreds of expressed barcodes. We quantified gene expression driven by each fragment by high-throughput barcode sequencing. We compared the expression driven by pairs of oligos that differed only at a given variant, pinpointing the effects of regulatory variation with single variant resolution.

We identified 467 causal variants at a false discovery rate of 5%. The effects of these variants correlated with the effects of local eQTLs previously mapped in these strains, suggesting that the single variants identified here contribute to natural variation in gene expression. At several local eQTLs, our assay revealed multiple causal variants with independent effects. Correspondence between variant effects in our assay and local eQTLs was stronger for variants not bound by nucleosomes in the genome, suggesting that chromatin state can alter the effects of individual variants.

Causal variants were enriched for changes in the binding motifs of dozens of transcription factors. Causal variants were less likely to be detected in promoters of essential genes, tended to have lower population frequency than non-causal variants, and were enriched at evolutionarily conserved sites, suggesting that natural *cis*-acting variants that affect gene expression tend to be under purifying selection. Our results provide insights into the mechanisms of action and the evolution of regulatory variants.

55 Quantifying absolute changes in transcription and translation over 22 years of bacterial adaptation. John Favate[1], Shun Liang[1], Srujana Samhita Yadavalli[1], *Premal Shah*[1,2] 1) Rutgers University; 2) Human Genetics Institute of New Jersey, Piscataway, NJ.

Changes in the regulatory regions of a genome can alter the transcriptional and translational landscape of an organism. As a result, these changes can be adaptive and subject to natural selection. However, the extent and molecular mechanisms by which these changes affect evolutionary processes are poorly characterized. We examined changes in gene expression in Lenski's *E. coli* Long Term Evolution Experiment (LTEE), where 12 populations of initially isogenic *E. coli* are continuously serially propagated in a minimal medium. We used genome-wide RNAseq and RIBOseq to quantify absolute and relative changes in transcription and translation in single clones from the 50,000th generation of each of the 12 evolved lines compared to the ancestors in the exponential phase. Our analysis reveals a significant degree of parallelism in both transcriptomic and translatomic changes, resulting in parallel changes to metabolic pathways. We find that changes in transcription tend to drive changes in translation, and few genes have altered ribosome densities. We also quantified absolute changes in transcript abundance and found that all evolved lineages have more RNA in their cells, and this increase in RNA abundance scales with cell-size. This work sheds light on the extent to which gene expression can change over evolutionary time scales, as well as the molecular mechanisms of adaptation in the LTEE.

56 Changes throughout a Genetic Network Mask the Contribution of Hox Gene Evolution *Yang Liu*[1,8], Margarita Ramos-Womack[2], Clair Han[3], Patrick Reilly[3], Kelly LaRue Brackett[4], William Rogers[5], Thomas Williams[5], Peter Andolfatto[6], David Stern[7], Mark Rebeiz[1] 1) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 2) Department of Ecology Evolution and Behavior, Princeton University, Princeton, NJ; 3) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 4) Department of Molecular Biology, Princeton University, Princeton, NJ; 5) Department of Biology, University of Dayton, Dayton, OH; 6) Department of Biological Sciences, Columbia University, Sherman Fairchild Center for Life Sciences, New York, NY; 7) Janelia Research Campus of the Howard Hughes Medical Institute, Ashburn, VA; 8) Department of Biology, Johns Hopkins University, Baltimore, MD.

Hox genes have long been appreciated to pattern the anterior-posterior body axis, and shifts in their expression patterns have been implicated in the evolution of animal body plans. However, it has been unclear how changes in these Hox genes, on their own, influence ultimate phenotypic evolution. To clarify the precise role of Hox genes in morphological evolution, we have dissected the molecular basis of a polygenic trait that differentiates two sister species: a striking difference in abdominal pigmentation between *Drosophila* (*D.*) *yakuba* and *D. santomea*. We discovered that this trait involves a major shift in the expression of the Hox gene *Abd-B*. Using a CRISPR/Cas9 based complementation test, we found that this shift contributed to the morphological difference between species, and identified a regulatory region of *Abd-B* that was modified during evolution. This represents the first case that evolutionary changes in a Hox gene have been pinpointed and directly connected to a phenotype.

However, our further investigations uncovered an intriguing paradox. When we introduced the *D. yakuba Abd-B* gene into *D. santomea*, we observed no effect on pigmentation, despite a complete reversal of its expression difference. Our experiments revealed that this historical change in *Abd-B* function is masked in modern-day *D. santomea* by changes at other loci. We identified four additional loci whose evolution contributed to this trait. Three of these loci have evolved expression patterns that do not respond to Abd-B, explaining the observed epistatic interactions. Our results demonstrate how body plans may evolve through small evolutionary steps distributed throughout Hox-regulated networks. The polygenicity and epistasis uncovered in this evolving Hox-regulated network highlights important challenges in resolving the molecular and genetic causes of macroevolution.

Thursday, April 23 10:00 AM - 10:15 AM

GSA Awards

80 2020 Beadle Award *Julie Ahringer*[1] 1) University of Cambridge.

abstract is not available at the time of print

81 2020 Morgan Medal *Gerald Fink*[1] 1) MIT.

abstract is not available at the time of print

82 2020 GSA Medal *Bonnie Bassler*[1] 1) HHMI/Princeton University.

abstract is not available at the time of print

Thursday, April 23 10:15 AM - 11:15 AM

COVID - 19 Keynote

83 Tracking SARS-CoV-2 using real-time phylogenetics with nextstrain *Richard Neher*[1] 1) Biozentrum, University of Basel.

The number of available SARS-CoV-2 genomes has risen rapidly from a handful in January to several thousand today. Since SARS-CoV-2 accumulates about 2 changes in its 30kb genome per month, these genomes allow us to retrace how the virus is spreading across the globe and how outbreaks in different parts of the world are connected. Compared to previous viral outbreaks, this genomic epidemiology is truly happening in real-time with delays between sample collection and analysis often less than two weeks. I will discuss the potential, pitfalls, and challenges in performing and interpreting such real-time analysis.

84 Getting to community surveillance for COVID-19 *Lea Starita*[1] 1) Brotman Baty Advanced Technology Lab, University of Washington.

The Seattle Flu Study (SFS) is a city-wide respiratory pathogen surveillance platform that launched in 2018, with the goal of preparing for precisely the situation that we now find ourselves in. The SFS integrates innovative sample collection mechanisms, large scale molecular screening and sequencing pipelines, and predictive modeling and genetic epidemiology. In the 2018-19 influenza season, a major weather event ("Snowpocalypse") allowed us to quantify the impact of social distancing on the transmission of respiratory viral pathogens. We also established the "swab and send" mechanism by which SFS participants collected and shipped their own nasal swab specimens. In the middle of the SFS's second year, the outbreak of COVID-19 led us to rapidly pivot the study to add SARS-CoV-2 screening and sequencing to the SFS pipeline, which resulted in our identification of what was at the time the first documented case of community transmission of COVID-19 in the United States, accelerating the introduction of public health mitigation measures. In recent weeks, the SFS team has partnered with Public Health Seattle King County to create a community surveillance platform (the greater Seattle Coronavirus Assessment Network or SCAN). We are also exploring alternative methods that may enable more rapid scaling of SARS-CoV-2 testing for the US population.

Thursday, April 23 11:30 AM - 1:15 PM

The Architectures of Complex Traits (PEQG)

107 Negative selection on complex traits limits genetic risk prediction accuracy between populations *Arun Durvasula*[1], Kirk Lohmueller[1] 1) Department of Human Genetics, UCLA, Los Angeles, CA.

Accurate genetic risk prediction is a key goal for medical genetics and great progress has been made toward identifying individuals with extreme risk across several traits and diseases. However, many of these studies are done in predominantly European populations. Although GWAS effect sizes correlate across ancestries, risk scores show substantial reductions in accuracy when applied to non-European populations. We use simulations to show that human demographic history and negative selection on complex traits results in population specific genetic architectures. For traits under moderate negative selection, ~50% of the heritability can be accounted for by variants in Europe that are absent from Africa. We show that this directly leads to poor performance in risk prediction when using variants discovered in Europe to predict risk in African populations, especially in the tails of the risk distribution. To evaluate the impact of this effect in genomic data, we built a Bayesian model to stratify heritability between European-specific and shared variants and applied it to 43 traits and diseases in the UK Biobank. Across these phenotypes, we find ~50% of the heritability comes from European-specific variants, setting an upper bound on the accuracy of genetic risk prediction in non-European populations using effect sizes discovered in European populations. We conclude that genetic association studies need to include more diverse populations to enable to utility of genetic risk prediction in all populations.

108 ΩComprehensive dissection of complex traits using a panel of 250,000 barcoded diploid yeast segregants *Takeshi Matsui*[1], Martin Mullis[2], Kevin Roy[1], Ian Ehrenreich[2], Sasha Levy[1] 1) Stanford; 2) University of Southern California.

Many traits of evolutionary and medical significance are genetically complex. Despite decades of research focused on characterizing the genetic basis of such phenotypes, our knowledge of how complex traits are specified remains incomplete. A major contributor to this problem is that alleles accounting for trait variation tend to have low frequencies in natural populations, making detection of loci with small and/or non-additive effects difficult using methods such as GWAS. Linkage mapping using crosses of genetically distinct isolates from model organisms offers a more complete dissection of complex traits, because in populations derived from a few founders, high frequencies of all alleles and allelic combinations can be ensured. Indeed, QTL mapping studies using yeast segregants has successfully identified thousands of loci that contribute to many complex traits in yeasts, such as growth, drug resistance, and colony morphology. However, working with large panels of segregants is often laborious and costly, as each segregant needs to be individually genotyped and phenotyped. As a result, most QTL studies in yeasts have relatively small sample size and, therefore, low statistical power to detect loci with very small effects and higher order interactions. Furthermore, by only sampling a small genotype space, rare allelic combinations that result in unexpected phenotypes may be missed.

To overcome these limitations, we have developed a high-throughput DNA barcode-based technique capable of generating extremely large panels of barcoded yeast diploid segregants (>250,000) with known genotypes that can be phenotyped simultaneously in a pool. We are using this panel to measure fitness distributions and map loci that contribute to fitness across multiple environments. Using simulations with real genotype data, we find that a forward scan for additive effects can robustly identify all additive loci, even those with very small effects (<0.01% phenotypic variance explained). In addition, we find that most loci involved in higher order interactions can also be identified by this simple forward screen, even when they do not have any additive effect by themselves. Using this technique, we expect to perform the most comprehensive analysis of the genetic architecture underlying fitness of a mixed genotype population to date.

109 Latent phenotypic complexity of adaptation in a single environment *Grant Kinsler*[1], Dmitri Petrov[1], Kerry Geiler-Samerotte[2] 1) Stanford University, Stanford, CA; 2) Arizona State University, Tempe, Arizona.

Organisms have an amazing ability to adapt to diverse challenges, yet recent observations suggest most mutations affect many traits and most of those effects are deleterious. How is adaptation possible if mutants that influence one trait in a beneficial way influence many other traits in

a deleterious way? Evolution experiments with DNA barcodes to track millions of independent evolving lineages have quantified and identified the spectrum of unique single mutations that can each help yeast adapt to glucose limitation. Using recent developments in DNA barcoding, we precisely measure the fitness of a collection of hundreds of these adaptive mutants in many subtly different environments. These data allow us to understand how these mutants interact with the environment, identify the number of fitness-relevant phenotypes affected by these mutants, and uncover the genotype-phenotype-fitness map. We find that single genetic mutations affect many phenotypes (pleiotropy). Despite this observation, we show that only a small number of these traits matter for adaptation to the original evolution condition. This finding sheds light on how adaptation can proceed despite widespread pleiotropy - the key insight being that not all phenotypes affected by mutation have fitness effects in the current environment. This work has wide-ranging implications on how adaptation proceeds in complex phenotype space, specifically the extent to which adaptation is limited by tradeoffs and how the relationship between genotype, phenotype, and fitness is environmentally-dependent.

110 Resolving the genetic basis of simple and complex traits using outbred Hybrid Swarm mapping populations *Cory Weller*[1,2], Alan Bergland[2] 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) University of Virginia, Charlottesville, VA.

Association studies seeking to describe the link between genotype and phenotype often use panels of sequenced inbred lines, such as the Drosophila Genetic Reference Panel (DGRP) or Drosophila Synthetic Population Resource (DSPR). However, inbred reference panels may not accurately represent naturally-occurring patterns of heterozygosity and nucleotide diversity, and inbred populations cannot be used to address certain biological questions (e.g. evaluating dominance effects or allele-specific expression). We developed a cost-effective method for outbred genetic association mapping, which we call the Hybrid Swarm. To generate a Hybrid Swarm population, dozens to hundreds of pre-sequenced inbred lines are randomly crossed for five generations. To reduce sequencing costs and make Hybrid Swarm genotyping financially feasible, we also developed a computational pipeline to reconstruct 99.9% accurate whole genomes from ultra-low-coverage (<0.05X) sequencing data. We then compared the GWAS performance of the Hybrid Swarm to alternative mapping populations, including inbred lines, recombinant inbred lines, F_1, F_2, and F_{50} crosses. To assess GWAS accuracy, we generated Receiver Operating Characteristic (ROC) curves for tens of thousands of simulated GWAS across various mapping population designs, effect sizes, and genetic architectures. We then compared the area under the ROC curves (AUC) to assess GWAS accuracy for the simulated populations. GWAS were most able to detect genotype-phenotype associations for recombinant inbred line populations (modeling the DSPR), which may be expected due to the lack of low-frequency (<10%) alleles in such populations. However, of the remaining populations tested, the Hybrid Swarm performed comparably to inbred lines (modeling the DGRP) and to more extensively outbred F_{50} populations. Further, while the Hybrid Swarm exhibited neutral levels of Genomic Inflation Factor, inbred lines suffered from genome-wide inflation of p-values—even on chromosomes physically unlinked to a causal allele. These results demonstrate that the Hybrid Swarm approach can be a cost-effective alternative method for genetic association studies, particularly in cases where an outbred population is desired.

111 Extent and context dependence of pleiotropy revealed by high-throughput single-cell phenotyping *Kerry Geiler-Samerotte*[1], Shuang Li[2], Charalampos Lazarus[2], Austin Taylor[2], Naomi Ziv[2], Chelsea Ramjeawan[2], Annalise Paaby[3], Mark Siegal[2] 1) Arizona State University; 2) New York State University; 3) Georgia Institute of Technology.

Pleiotropy – when a single mutation affects multiple traits – is a controversial topic with far-reaching implications. Pleiotropy plays a central role in ongoing debates about how complex traits evolve and whether biological systems tend to be modular or organized such that every gene has the potential to affect many traits. Pleiotropy is also critical to initiatives in evolutionary medicine that seek to trap infectious microbes or tumors by selecting for mutations that encourage growth in some conditions at the expense of others. Research in these fields, and others, would benefit from understanding the extent to which pleiotropy reflects inherent relationships among phenotypes that correlate no matter the perturbation (vertical pleiotropy), versus the action of genetic changes that impose correlations between otherwise independent traits (horizontal pleiotropy). We tackle this question by using high-throughput single-cell phenotyping to measure thousands of pairwise trait correlations across hundreds of thousands of cells representing hundreds of genotypes of the budding yeast, *Saccharomyces cerevisiae*. We map pleiotropic quantitative trait loci using genotypes derived from a cross between natural strains, and we separate vertical and horizontal pleiotropy by partitioning trait correlations into within- and between-genotype correlations. We investigate how pleiotropy can change by using genotypes from mutation-accumulation lines that experienced minimal selection, and by tracking trait correlations through the cell-division cycle. We find ample evidence of both vertical and horizontal pleiotropy, and observe that trait correlations depend on both genetic background and cell-cycle position. Our results suggest a nuanced view of pleiotropy in which trait correlations are highly context dependent and biological systems occupy a middle ground between modularity and interconnectedness. These results also suggest an approach to select pairs of traits that are more likely to remain correlated across contexts for applications in evolutionary medicine.

Thursday, April 23 11:30 AM - 1:15 PM

Diversity, Equity, and Inclusion Session

96 Inclusive PhD admissions: An evidence-based self-education process for faculty, staff and trainees *Scott Barolo*[1] 1) University of Michigan Medical School.

PhD admissions decisions are hard. Many life-sciences admissions faculty have a "sense" of what type of student is most likely to succeed. However, these impressions are based on our personal experiences, creating dangers of bias in favor of privileged applicants, those from over-represented groups in STEM, and those who most resemble the admissions faculty. A substantial body of scholarly literature addresses academic admissions and its biases and inequities, but many life-sciences faculty are unaware of its findings. In 2017, The Program in Biomedical Sciences (PIBS)—a large umbrella program at the University of Michigan that includes 14 participating biomedical PhD programs—underwent an evidence-based self-education and debate process on the GRE as a tool for graduate admissions. As a result of that rigorous and transparent process, we were one of the first biomedical PhD programs in the US to decide to drop the requirement for GRE test scores from our applicants—a change that has now swept across most major US PhD programs in the field.

In 2019, PIBS continued and expanded this process by assembling a group of graduate students, faculty and staff to similarly examine *every* aspect of the PhD admissions process, including each section of the application (GPA/transcript, research experience, applicant statements, letters of refer-

ence, etc.), on-campus visits, interviews, and final decisions. This group is charged with:

(1) Creating an evidence-based "Guide to Effective and Inclusive PhD Admissions" for admissions faculty that presents the strengths, weaknesses, and potential biases of each aspect of the admissions process, and provides recommendations for how to use (or not use) each piece of information as part of a rational, inclusive holistic-admissions strategy.

(2) Making recommendations to PIBS for changes to our application and admissions process to make admissions as effective and inclusive as possible.

The latest findings of this working group will be presented, along with strategies for familiarizing our admissions faculty and staff with the group's findings and for implementing their recommendations.

97 The Diversity Preview Weekend: A graduate student-led initiative to promote diversity, equity and inclusion in higher education *Andrea Darby*[1], Suzanne Pierre[2], Cait McDonald[1], Kelly Zamudio[1] 1) Cornell University; 2) University of California, Berkeley.

Graduate programs in STEM fields suffer from a lack of diversity and inclusion in the United States. Underrepresented students generally have greater obstacles and less access to resources that would prepare them to make an informed decision to pursue a graduate education. In an effort to address this need, graduate students from the Department of Ecology and Evolutionary Biology, Department of Entomology, and the School of Integrative Plant Sciences at Cornell University joined forces to build the Diversity Preview Weekend (DPW). DPW recruits people from underrepresented groups from around the United States and US territories that are interested in graduate school. Selected participants are invited to attend a fully-funded weekend at Cornell with the primary goal of empowering participants through workshops, campus tours, and familiarization with the application process. DPW also aims to provide a safe space for minorities to discuss their experiences and challenges in academia. The weekend immerses participants in a graduate community, facilitating networking with faculty, graduate students and other participants. DPW continues communication and support of past attendees through graduate student mentorship and former attendee networks. The initiative is currently in its fourth consecutive year, and is organized entirely by current graduate students and funded by department contributions and outside donors, including private industry. We demonstrate the efficacy of this type of program by showing that participants express a deeper understanding and greater confidence to navigate the graduate school application process. Out of the 62 DPW attendees from the first two years, 32 have matriculated to graduate degree programs. Similar programs have recently been initiated at several other universities. These types of programs warrant further investment to promote and maintain diversity, equity and inclusion in leadership roles in public and private institutions and industries.

98 Bridging Worlds for Diversity and Inclusion: Social Science with Biology Education Research Through the iEMBER Network *Gary McDowell*[1,2], Andrea Cobb[1,3], Jessica Lucas[1,4] 1) iEMBER; 2) Lightoller LLC, Chicago, IL, USA; 3) George Mason University, Manassas, VA; 4) Southern Illinois University, Carbondale, IL.

Retention in undergraduate biology majors is a major checkpoint hampering diversification of the biomedical academy (Meyers et al., 2018). Current reform efforts in biology education focus on promoting both biological literacy, and retention, diversity, and inclusion in biology (AAAS, 2011). Most effort has been spent on content and practices, while sociological implications that mitigate these reform elements have been overlooked.

The Inclusive Environments and Metrics in Biology Education and Research (iEMBER) Network has been created to address diversity, equity, inclusion, and social justice, and drive reform efforts in biology education from a multidisciplinary sociological perspective. This change initiative is building a network of scholars to collaborate across biology, biology education and the social sciences. iEMBER members are from all kinds of research and teaching institutions across the U.S., representing policy experts, administrators, and psychology, sociology, anthropology, biology education and biology researchers.

Our community of practice framework allows stakeholders to build relationships and share resources across institution types, actively embracing early career researchers in this work. The iEMBER group examines new ways of thinking about STEM higher education reform, taking the view of education spaces as social interaction spaces.

iEMBER is a new organization as of 2017, funded by the NSF. The group has held two conferences (Tennial et al., 2019) at which it has sparked collaborations through start-up style pitching sessions. Projects have resulted in manuscripts, new IRB-approved collaborative research projects, and formal and informal sessions at national conferences.

It is critical for STEM stakeholders to have resources and research community support necessary to understand environments and their impact on students. In this presentation, the theoretical framework underpinning communities of practice and the methods iEMBER uses to engage members and outcomes will be discussed. We will detail current activities, recent meetings and workshops and their outcomes, and will discuss why we are involved in the network. We will provide details of how participants can get involved with the network. See: iEMBER.qubeshub.org

Meyers LC et al., 2018, https://doi.org/10.1371/journal.pone.0190606
AAAS, 2011, Vision and Change in Undergraduate Biology Education: A Call to Action.
Tennial et al., 2019, https://doi.org/10.1187/cbe.18-03-0042

99 Inclusion of cultural dietary practices in genetics research diversifies the scientific workforce and addresses health disparities *Jennifer Alexander*[1], Alana O'Reilly[1] 1) Fox Chase Cancer Center.

The widening gap in health disparities is partly due to the lack of diversity among biomedical research and clinical professionals. Although racial

and ethnic diversity continues to rise within the U.S, the small number of adequately trained underrepresented groups in biomedical sciences is an alarming concern. Despite substantial evidence demonstrating that complex problems are better solved with a multiplicity of diverse perspectives, disparities continue to persist within the STEM workforce, which stagnates innovation. To combat this, our goal is to enhance scientific discovery on two fronts: by promoting cultural diversity in research and training future STEM scientists.

For centuries, cultural groups have empirically engaged in "clinical research"; often prescribing dietary nutrients for disease intervention and prevention. While the molecular function has been identified for some nutrients, such as folic acid, the mechanism of action for most nutrients remain undefined. To this end, we created a systematic and comprehensive approach for defining how nutrients impact developmental signaling pathways commonly dysregulated in cancer. In this approach, high school students from diverse backgrounds investigate their dietary cultural practices through experimentation aimed at "reverse mapping" the signal transduction pathways influenced by specific nutrients. Through these efforts, students found that selenium increases the activity of EGFR-Ras signaling in developing flies, a pathway commonly targeted in multiple cancers. This work builds on findings from our primary research demonstrating a novel signaling role for dietary cholesterol in regulating growth factor dependent oogenesis and cancers. Collectively, these discoveries may lead to the development of dietary restrictions for patients receiving targeted therapies in specific pathways.

Our approach is dually powerful: we encourage students to embrace their cultural dynamics to move biomedical research forward while simultaneously genetically mapping targets of nutrients that may aid therapeutic interventions in cancer. Moreover, we have ignited a passion in our student for biomedical research, to which majority pursue STEM related fields in college. Altogether, this creates a continuous pipeline of well –trained, diverse research scientists expediting the goal of eradicating devastating diseases, such as cancer.

100 For Us, By Us, but Not *All of Us*: building our own Indigenous biobank as a better way to ensure research equity *Krystal Tsosie*[1,2], Joseph Yracheta[1,3,4], Guthrie Ducheneaux[1,4], Timothy Watkins[1] 1) Native BioData Consortium, Eagle Butte, SD; 2) Vanderbilt University, Nashville, TN; 3) Department of Environmental Health and Engineering, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; 4) Missouri Breaks Industries Research Inc., Eagle Butte, SD.

Background: Indigenous people continue to be severely underrepresented in genomics studies, despite endeavors by the National Institutes of Health (such as *All of Us*) and other large-scale diversity projects to include more participants of non-European descent. The lack of Indigenous representation in genomic studies contributes to gaps in our knowledge of global human ancestry and, more critically, a paucity in our understanding of variants contributing to health and disease for tribal communities. Even if tribes acknowledge that their willful disengagement might exclude them from future advances in precision genomic medicine, tribal leaders and members still express concern with: 1) the requirement to deposit genomic information collected from Federally-funded research into open-access databases that circumvent tribal research oversight, 2) inclusion of Indigenous biomarkers for research without translatable benefits to tribal members, and 3) potential biocommercial exploitation of Indigenous genomic information by for-profit companies partnering with academic or government institutions.
Purpose: Recent changes to the Common Rule uphold and acknowledge the right of U.S. Federally-recognized tribal nations to govern research processes conducted within their communities. For some tribes, this is limited to a form of passive control of agency centered on the ability to establish tribal institutional review boards (IRBs) to regulate largely non-Indigenous investigator-driven protocols. However, we as Indigenous genomic researchers have established the first tribally-led biobank in the United States, a non-profit research institution called the Native BioData Consortium (NBDC). While community-engaged research models are certainly more equitable than practices of the past, we wish to usher in the next phase of tribally-driven research.
Implications: We describe a 5-year process for developing institutional allies, establishing governance procedures, and engaging the Lakota community and tribal leaders in the importance of biobanking for repatriation and genomic and data sovereignty. We also describe building an electronic portal for managing and recruiting tribal participants utilizing a dynamic consent model that also provides a venue for return of results and educating community members—as potential solutions to challenges that often burden community-based participatory research. More importantly, by centering Indigenous people as the agents of access for their own genomic data, we hope that more tribes will be more empowered to conduct research that truly benefits them.

Thursday, April 23 11:30 AM - 1:15 PM

Chromatin and Transcription

85 RSC readies the quiescent genome for rapid hypertranscription *Christine Cucinotta*[1], Rachel Dell[1], Keean Braceros[1], Toshio Tsukiyama[1] 1) Fred Hutchinson Cancer Research Center, Seattle, WA.

Quiescence is a conserved stage by which cells can reversibly exit the cell cycle for long-term survival. Quiescence reversibility is essential for differentiation, tissue regeneration, stem cell renewal, and immune cell activation. Although the non-cycling nature of quiescent cells has made their study challenging, a recently developed yeast model of quiescence produces sufficient quantities of highly purified quiescent cells. Using this model, we have found that like its mammalian counterparts, quiescent chromatin is compact and hypoacetylated. Furthermore, in quiescent cells, nucleosome-depleted regions (NDRs) are narrower than at any other cell cycle stage. Although yeast cells may be maintained in quiescence almost indefinitely, we have observed widespread transcription activation occurring within the first few minutes of nutrient repletion. Surprisingly, despite this rapid hypertranscription, complete chromatin recovery does not occur until the first G2/M phase two hours later. This suggests that transcription machinery transcribes through a highly repressive chromatin template in early exit via an unknown mechanism. We tested candidate factors that may promote transcription in this environment. Our data show that ~60% of genes are activated upon quiescence exit and most have poised RNA Polymerase II (Pol II) and the SWI/SNF family chromatin remodeling enzyme RSC bound in quiescent cells. In cycling cells, RSC promotes transcription initiation by increasing NDR width, aiding binding of transcription factors and recruiting Pol II. However, in quiescence exit, depletion of RSC causes Pol II to stall at the 5'-ends of genes and decline over time. This phenomenon is likely rendered by the high nucleosome occupancy unique to the quiescent state. RSC may therefore facilitate transcription elongation through repressive chromatin in addition to its canonical role in enabling transcription initiation. Together, these results imply a poised transcriptional program heavily dependent on RSC that prepares cells for initiating and maintaining large-scale rapid gene expression despite a globally repressive chromatin state.

86 How cell size controls genome activation and orchestrates fate decisions *Hui Chen*[1], Wenchao Qian[1], Matthew Good[1,2] 1) Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2) Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA.

A fundamental question is how embryonic cells induce genome activation and determine their fate in early embryo development. It has been proposed that genome activation is regulated by cell size through a titration mechanism dependent on DNA:cytoplasm ratio. Recently, using *Xenopus* embryos which contain a gradient of cellular dimensions we demonstrated that the onset of zygotic genome activation (ZGA) is dependent on cells reaching a critical threshold size. By physically manipulating the size of embryos, we also demonstrated that reductions in cell size is sufficient to trigger premature genome activation in a dose-dependent fashion. However, it remains unknown how cell size regulates ZGA and whether size thresholds for genome activation are conserved among vertebrate embryos. Our studies have shown that ZGA is firstly initiated in the small cells at the animal pole, which is the presumptive ectoderm, and gradually spread to the large cells at the vegetal pole, which is the presumptive endoderm. Our findings suggested a model that the three germ layers may be hierarchically orchestrated. To investigate whether manipulating the cell size gradient alters cell fate decision, we used temperature gradient controllers to reverse the cell size gradient within individual *Xenopus* embryos. Intriguingly, we discovered that reversing the cell size gradient reversed the spatial pattern of genome activation, supporting our previous observation that ZGA is dependent on cell size. To characterize which genes involved in germ layer specification are directly regulated by cell size, we have successfully developed a pipeline to simultaneously track nascent zygotic transcription and maternal degradation of each gene by RNA-seq. To achieve this, we metabolically labeled the all nascent RNA transcripts in embryos at different stages by using 5-ehtynyl uridine (EU) and click chemistry, followed by biotinylation and pulldown of nascent RNAs using streptavidin beads while leaving all maternal RNAs left in the flowthrough. By sequencing the nascent and maternal fractions of RNA, we provided a brand-new view on the dynamics of each gene. Surprisingly, we found that the behavior and rate of transcription of individual genes involved in the three-germ layer specification is dramatically different. Finally, to address whether a cell size threshold for genome activation is a conserved feature of early embryo development we are imaging ZGA in space and time in embryos from other model systems, including *X. tropicalis*, *D. rerio* and others. Preliminary results revealed a similar size-dependent spatial patterning of ZGA for *X. tropcalis* embryos. Taken together, our study provides a novel panoramic view of nascent zygotic transcription and maternal degradation, and our findings suggest cell size dependent ZGA and cell fate decision might be conserved in various embryonic systems.

87 Quantitative analysis of transcription factor binding and expression using calling cards reporter arrays *Jiayue Liu*[1,2], Christian Shively[1,2], Robi Mitra[1,2,3] 1) Department of Genetics, Washington University School of Medicine in St. Louis, St. Louis, MO,; 2) The Edison Family Center for Genome Sciences & Systems Biology, Washington University School of Medicine in St. Louis, St. Louis, MO ; 3) McDonnell Genome Institute, Washington University School of Medicine in St. Louis, St. Louis, MO.

Transcriptional changes control how cells divide, differentiate, and respond to their environments, yet we still do not understand much about gene regulation in eukaryotes. For example, the *in vivo* binding of transcription factors (TFs) remains difficult to predict solely from genomic sequence, and it is difficult to quantitatively explain levels of gene expression even when TF binding locations are known. Efforts to bridge this gap in our knowledge would be accelerated by novel experimental tools that can study TF binding and the resulting impact on gene expression in a quantitative fashion, allow for the rapid analysis of many user-specified regulatory sequences, and can detect cooperative interactions between TFs. Here, we report a tool, Calling Cards Reporter Arrays (CCRA) that is able to sensitively and accurately measure transcription factor binding and the consequences on gene expression for hundreds of synthetic promoters in yeast. We demonstrate that CCRA method is able to detect small changes in the free energy of TF binding with a sensitivity comparable to *in vitro* methods, enabling the measurement of binding landscapes *in vivo*. We use this method to characterize the binding landscapes of Cbf1p and MAX for all possible 1bp substitutions to their consensus motifs. We then use CCRA to study how cooperativity dictates TF binding *in vivo,* by analyzing the binding of the bHLH factor Cbf1p. We find that the cooperativity between Cbf1p dimers varies sinusoidally as the distance between two Cbf1p binding sites is changed, with an observed period of 10.65 base pairs. The helical phase of binding sites plays a major role in the cooperative binding of this factor, as “out of phase” sites incur an energetic cost of 3.40 kJ/mol (1.37 K_BT) relative to in-phase sites. Finally, we characterize the binding and expression of a group of TFs, Tye7p, Gcr1p, and Gcr2p, that are thought to act together as a “TF collective”, an important but poorly characterized model of TF cooperativity. We demonstrate that Tye7p is able to bind at promoters that do not encode its recognition site because it is recruited by other members of the collective, whereas the presence of recognition sites for Gcr1/2p are necessary and sufficient for their binding. This suggests a hierarchy where these factors can recruit Tye7p but not vice versa. Our experiments establish CCRA as a useful tool for quantitative investigations into TF binding and function.

88 Transcription rate modulation by network-level feedback promotes robust patterning outcomes Puttachai Ratchasanmuang[1], *Shawn Little*[1] 1) Univ. of Pennsylvania Perelman School of Medicine.

Embryogenesis can tolerate a range of genetic or environmental alterations; e.g., hemizygosity for critical transcription factors (TFs) usually generates normal phenotypes. It is often assumed that embryos tolerate a 50% reduction in mRNA production rate; tolerance can occur when a single locus generates sufficient gene product in the time available before that product is required. Alternatively, the remaining normal locus can undergo an adaptive increase in transcription rate. The prevalence and range of mechanisms of adaptation is not clear. Moreover, adaptation represents a challenge for factors that propagate gradients of patterning information at positions in the naïve tissue where, for example, 50% of the maximum production rate is the correct amount. It is unclear whether adaptive mechanisms exist for graded patterning factors, and if so how cells discern normal from aberrant changes in expression.

To address these questions, we compared expression of wild-type Drosophila embryos to embryos hemizygous for each of several TFs of the "gap gene" family. Gap genes are expressed in graded patterns across expression boundaries spanning several cell diameters. Whereas a textbook model presents gap gene activity as binary, recent work has suggested that multiple precise levels of gap gene expression are essential. However, heterozygotes are viable, raising questions of the importance of differential levels. Surprisingly, we found gap gene trajectories undergo complex changes in expression dynamics in hemizygotes. In regions of high expression, gap genes undergo partial adaptation through prolonged transcription. In contrast, in regions of low expression in boundaries, hemizygotes exhibit premature inhibition. Moreover, when a given gap gene is hemizygous, neighboring gap genes in overlapping domains become overexpressed. We show that these changes emerge from network-level dynamic interactions. We also find that a target of gap gene activity, the pair-rule gene even-skipped (eve), is minimally perturbed by the aberrant changes in gene expression. Quantitative modeling shows that eve regulatory elements collectively perform a joint interpretation of gap gene levels, so that near-normal expression is elicited despite apparently abnormal upstream regulators. These results show how network-level interactions prevent abnormal phenotypic outcomes in the presence of otherwise detrimental genetic changes.

89 Shadow enhancers can suppress input transcription factor noise through distinct regulatory logic *Rachel Waymack*[1], Alvaro Fletcher[1], German Enciso[1], Zeba Wunderlich[1] 1) University of California, Irvine.

Shadow enhancers are groups of two or more enhancers that drive overlapping spatiotemporal gene expression of the same target gene. Shadow enhancers have been identified across a wide range of organisms, particularly in association with developmental genes. Despite their apparent redundancy, shadow enhancers are critical for robust gene expression in conditions of environmental or genetic stress. To understand the mechanism by which shadow enhancers drive robust gene expression, we use the pair of shadow enhancers that control the developmental gene *Kruppel* in *Drosophila melanogaster* as a case study. The individual *Kruppel* enhancers are regulated by separate transcription factors (TF) and preliminary data suggests that this separation of TF inputs is a general feature of shadow enhancers in *D. melanogaster*. We test the hypothesis that shadow enhancers buffer transcriptional noise through a separation of TF inputs at the individual enhancers. By tracking transcription in living embryos using the MS2 system, we are able to compare enhancer activity in a large number of individual nuclei and quantify total levels and sources of noise. By measuring the transcriptional dynamics of several *Kruppel* shadow enhancer configurations, we show the individual member enhancers act largely independently. We find that TF fluctuations are an appreciable source of transcriptional noise and that the *Kruppel* shadow enhancer pair can better buffer this noise than single or duplicated enhancers. Stochastic modeling indicates that both the independent activity of the individual enhancers and the lower expression noise driven by the shadow enhancer pair are natural consequences of the separation of TF inputs. Further, we show the shadow enhancer pair is uniquely able to maintain low levels of expression noise across a wide range of temperatures. Our findings suggest that the separation of TF inputs is a key characteristic of shadow enhancers that enables them to buffer against a wide range of potential perturbations and that the widespread use of shadow enhancers may be due in part to this noise suppressing quality.

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Developmental Genetics: The Germline

90 Stem cell niche exit in *C. elegans* via orientation and segregation of daughter cells by a cryptic cell outside the niche *Kacy Gordon*[1], Jay Zussman[2], Xin Li[1], Camille Miller[1], David Sherwood[2] 1) The University of North Carolina at Chapel Hill; 2) Duke University .

Stem cells reside in and rely upon their niche to maintain stemness but must balance self-renewal with the production of daughters that leave the niche to differentiate. We discovered a mechanism of stem cell niche exit in the canonical *C. elegans* distal tip cell (DTC) germ stem cell niche mediated by previously unobserved, thin, membranous protrusions of the adjacent somatic gonad cell pair (Sh1). The presence of this cell type in the stem cell zone revolutionizes our understanding of the gonad structure. A disproportionate number of germ cell divisions occur at the DTC-Sh1 interface, and these divisions are asymmetric; stem-like and differentiating cell fates segregate across this boundary. Spindles polarize, pairs of daughter cells orient between the DTC and Sh1, and Sh1 grows over the Sh1-facing daughter, removing it from the niche. Impeding Sh1 growth by RNAi to cofilin and Arp2/3 perturbs the DTC-Sh1 interface and reduces the proliferating germ cell number. Because these membrane protrusions eluded detection for decades, it is possible that similar structures actively regulate niche exit in other systems.

91 Sex-specific ecdysone signaling is established by Doublesex to regulate gonad stem cell niche development *Lydia Grmai*[1], Erin Jimenez[1], Ellen Baxter[1], Mark Van Doren[1] 1) Johns Hopkins University.

Sexual dimorphism underpins development of nearly all metazoans and is executed by members of the conserved family of Doublesex (Dsx)/Mab-3 Related Transcription factors (DMRTs). Its founding member, *Drosophila* Dsx, translates chromosome inheritance into sexual differentiation programs. In mammals, sex differentiation also relies on the activity of sex-biased steroid hormones, estrogen and testosterone. While mammalian steroid hormone signaling is known to be dimorphic, the known roles for the insect steroid hormone ecdysone are monomorphic.

In the somatic gonad, sex-specific niche cells support germline stem cell (GSC) maintenance. In the testis, a tight cluster of post-mitotic cells termed the hub supports male GSCs, while the ovarian GSC niche includes both cap cells and the terminal filament (TF), a single stack of cells at the apex of each ovariole. We previously showed that *dsx* is required to ensure the correct niche forms in each sex: *dsx* mutant gonads initially specify a hub regardless of chromosomal sex, but during L3 approximately half of both XX and XY gonads "switch" to form TFs. Genomic analysis uncovered *Ecdysone receptor (EcR)* as a putative Dsx target, prompting us to examine its role in sex-specific niche formation. Interestingly, EcR protein and downstream transcriptional activity are normally present in the female, but not male, somatic gonad. Gonads lacking *dsx* express intermediate levels of EcR regardless of chromosomal sex. Depleting *EcR* impairs ovarian TF development, while ectopic EcR activation in the testis is deleterious to hub development. Because of this, we hypothesized that modulating ecdysone activity would skew the ratio of *dsx* mutant gonads that form a hub vs. TF. Indeed, depleting *EcR* in *dsx* mutants skewed gonads toward hub formation, while *EcR* over-expression decreased the percentage of *dsx* mutant gonads with a hub.

From this, we conclude that sex-specific steroid hormone signaling is a critical feature of gonad development in *Drosophila* and is regulated at the level of receptor expression rather than hormone abundance. Further, dimorphic EcR activity is required downstream of Dsx to secure the hub vs. TF decision. Future work will investigate the role Dsx plays in context-specific dimorphism of insect steroid hormone signaling. Importantly, our finding that ecdysone signaling is dimorphic in gonads raises the possibility that sexual dimorphism governs steroid hormone signaling in other *Drosophila* tissues.

92 Axon-like projections direct the self-renewal versus differentation cell fate decision in Follicle Stem Cells of the Drosophila ovary Eric Lee[1], Melissa Wang[1], Cindy Chau[1,2], Alberto Vargas[1], Jessica Reimach[1,2], Kelly Costa[1], *Alana O'Reilly*[1] 1) Fox Chase Cancer Center; 2) Immersion Science Program.

Stem cells cycle between periods of quiescence and proliferation, a process that is thought to promote healthy tissue aging. Proliferative stem cells compete more effectively for niche occupancy in some tissues, with drift toward clonality suppressed by synchronization of stem cell pools via periods of quiescence. The quiescence to proliferation transition is controlled, in part, by diet, such that periods of starvation or nutrient restriction promote quiescence, with rapid transition to proliferation upon feeding. *Drosophila* ovarian Follicle Stem Cells (FSCs), an epithelial stem cell population, arrest division after entering a nutrient-restricted state and initiate proliferation rapidly after feeding. The growth factor Hedgehog (Hh) is

an essential nutrient-dependent growth factor that mediates this transition, inducing multiple cellular responses in FSCs to re-start oogenesis after a period of quiescence. Here, we mapped the timecourse of cellular events that occur during the FSC quiescence to proliferation transition, finding dynamic interplay between proliferation, transcriptional activation of differentiation markers (e.g. Eya), autophagy, and morphological changes throughout a 24 hour timecourse after feeding. Of particular interest is the growth of axon-like projections that extend from FSCs to contact other cells in the stem cell compartment. We found that FSC projection growth followed proliferation induction, but correlated with rising expression of differentiation markers. Extension of FSC projections depended on *smoothened* (*smo*) and *Cubuitus Interruptus* (*Ci*), Hh effectors that promote transcriptional activation of Ci target genes. Utilizing a TU-tagging approach, we mapped Ci targets at critical timepoints during the quiescence to proliferation transition, identifying a key small G-protein regulator and its downstream signaling proteins as drivers of FSC projection growth, but not proliferation. Aberrant connections between FSC projections and germ cells, IGS/escort cells and other FSCs were observed when FSCs lacked sufficient expression of these projection regulators. Our work supports a model in which FSC projections mediate communication between distinct cell types to determine cell fate outcomes, with interfacing of FSC projections on germ cells driving a differentiation fate and connection to other FSCs promoting self-renewal.

93 Novel LOTUS-domain proteins recruit *C. elegans* Vasa to germ granules and are essential for developmental switches in the germline *Patricia Giselle Cipriani*[1,2], Olivia Bay[1], Vinay Mayya[3], John Zinno[1], Yu Guan[1], Hin Hark Gan[1], Michelle Gutwein[1], Hala Fahs[2], George Chung[1], Jiaxuan Chen[4], Paola Emhardt[1], Matthias Selbach[4], Thomas Duchaine[3], Fabio Piano[1,2], Kristin C Gunsalus[1,2] 1) Center for Genomics and Systems biology, New York University, New York, NY ; 2) Center for Genomics and Systems biology, New York University Abu Dhabi, UAE ; 3) McGill University, Montreal, Canada; 4) Max-Delbrück Center for Molecular Medicine (MDC), Berlin, Germany.

Germ granules are membraneless ribonucleoprotein (RNP) particles that form molecular condensates by liquid-liquid phase separation in germline cells. *C. elegans* germ granules, known as P granules, carry out many essential roles in germline development including the regulation of translation and small RNA pathways. Most known core P granule components belong to paralogous protein families, including the MEG intrinsically disordered proteins, the GLH DEAD-box Vasa helicase orthologs, and the PGL RNA-binding proteins. How these proteins assemble into granules and how the system is regulated throughout the organism's development is not completely understood. By *in vivo* co-immunoprecipitation of MEG-3, we have identified two novel paralogs that contain LOTUS domains and intrinsically disordered regions, which we have named MIP-1 and MIP-2 (**M**EG-3 **I**nteracting **P**roteins). MIP-1 and MIP-2 localize to P granules both in the embryo and in the germline, defining a new class of previously unidentified core germ granule components in *C. elegans*. MIP-1 localizes constitutively to P granules, whereas MIP-2 shows more dynamic expression at different stages of germline development. Depletion of both MIPs causes dissolution of GLH, PGL and MEG granules. It also produces temperature sensitive sterility and pleiotropic defects in germline development that affect progression through meiosis and gametogenesis. Individual loss of function of either protein affects the localization of its paralog and these produce opposing phenotypes: *mip-1* depletion results in larger granules and *mip-2* depletion gives rise to smaller granules. Biochemical and yeast-two hybrid analysis shows that the MIPs directly bind to each other and to GLH-1. We propose that the MIPs form a lattice-like network that helps recruit and organize RNP assemblies within P granules to promote key developmental transitions in the germline.

94 Courtship is a two-way conversation: Yeast mating as a model of cell-cell communication *Manuella Clark-Cotton*[1], Nicholas Henderson[1], Daniel Lew[1] 1) Duke University.

Cells track chemical signals during feeding, fertilization, development, and immune responses, but the mechanisms by which cells locate the source of a signal are elusive. The budding yeast, an established model of cell-cell communication, tracks a pheromone signal to locate a mating partner. Pheromone binding to cell surface receptors triggers the concentration of polarity proteins at a small zone of the cell cortex. This polarity site becomes oriented toward the partner, and through its effectors, directs polarized growth toward that mate. But how does a cell find its mate?

In classical models of polarization, a cell decodes the pheromone gradient and polarizes up-gradient. However, yeast in mating mixes first assemble transient polarity sites that move around the cell cortex, often orienting toward different partners in an apparent search process (the "indecisive period"), before forming stable polarity sites oriented toward the mate (the "committed period.") Previous work showed that receptors and G proteins are concentrated at stable polarity sites, suggesting that the transient polarity clusters might facilitate mating partner selection by promoting local sensing and secretion. This suggests an "exploratory polarization" model: when the patches of two potential mates are oriented away from each other, each cell senses a relatively low concentration of pheromone, and the patches continue to move, but when the patches of two partner cells become aligned, each cell senses a high concentration of pheromone, and both patches stabilize growth along their shared axis. To test this model, we performed live-cell fluorescence microscopy of mating mixes in which wildtype cells were paired with partners that either failed to make polarity clusters, or that made but could not stabilize clusters. Strikingly, we found that the wildtype cells displayed extended indecisive behavior and did not commit to such partners. We also confirmed that sensing, signaling, and secretion markers were enriched at transient polarity sites. Together, these data provide direct experimental evidence that local pheromone secretion promotes commitment to a partner. We conclude that reciprocal communication between two partners' polarity sites is essential for successful commitment to a mating partner, in support of the exploratory polarization model.

95 GCNA interacts with Spartan and Topoisomerase II to regulate genome stability Gregoriy Dokshin[1], Gregory Davis[2], Ashley Sawle[3], Matthew Eldridge[3], Peter Nicholls[4], Taylin Gourley[2], Katherine Romer[4,5], Luke Molesworth[2], Hannah Tatnell[2], Dirk de Rooij[4,6,7], Gregory Hannon[3,8], David Page[4,9], Craig Mello[1,10], *Michelle Carmell*[1,4,8,11] 1) RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA, USA; 2) Federation University, VIC 3841, Australia; 3) Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, UK; 4) Whitehead Institute, Cambridge, MA, USA; 5) Massachusetts Institute of Technology, Cambridge, MA, USA; 6) Utrecht University, The Netherlands; 7) University of Amsterdam, The Netherlands; 8) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA; 9) Howard Hughes Medical Institute, Whitehead Institute, Cambridge, MA, USA; 10) Howard Hughes Medical Institute, University of Massachusetts Medical School, Worcester, MA, USA; 11) Wellesley College, Wellesley, MA, USA.

DNA in all living systems is exposed to damage from both endogenous and exogenous sources. Mutations in germ cells are acutely harmful as these cells are uniquely tasked with passing their genomes to the next generation, a process critical for both short term reproductive success and long term fitness and survival of a species. We previously discovered the ancient GCNA protein family that is present across eukarya in cells

carrying a heritable genome, including pluripotent cells and germ cells of diverse multicellular animals. *Gcna* mutations in both *C. elegans* and mice significantly impact reproduction, suggesting that GCNA has functioned in the germline for at least 600 million years (**Carmell et al., 2016**). GCNA homologs Spartan/DVC-1 and Wss1 resolve DNA-protein crosslinks (DPCs), including Topoisomerase-DNA adducts, during DNA replication. We show that GCNA and Topoisomerase 2 (Top2) physically interact and colocalize on condensed chromosomes during mitosis, when Spartan is not present. We show that *C. elegans gcna-1* mutants are sensitive to Top2 poison and accumulate mutations consistent with low fidelity repair of DNA damage, leading to loss of fitness and fertility over generations. We also demonstrate that mouse GCNA interacts with TOP2, and *Gcna*-mutant mice exhibit abnormalities consistent with the inability to process DPCs, including chromatin condensation and crossover defects. Together, our findings provide evidence that GCNA maintains genomic integrity by supporting processing of Top2 DPCs in the germline and early embryo, where the genome is challenged with an increased DPC burden.

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Modeling Human Diseases in Diverse Systems

101 A muscle-to-oenocyte Pvf1 signaling axis protects against obesity *Arpan Ghosh*[1], Sudhir Tattikota[1], Yifang Liu[1], Claire Hu[1], Norbert Perrimon[1,2] 1) Harvard Medical School; 2) Harvard Hughes Medical Institute.

Obesity and associated cardio-metabolic diseases are one of the leading causes of mortality and morbidity world-wide. The mechanisms leading to obesity often involve multi-organ crosstalk that regulates energy homeostasis. However, our understanding of these inter-organ communication networks and their roles in metabolic homeostasis remain limited. As part of an ongoing effort in our lab to investigate organ crosstalk, we investigated tissue-specific roles of the *Drosophila* PDGF/VEGF pathway ligands Pvf-1, 2 and 3 in regulating lipid homeostasis. *Here we report a muscle-to-oenocyte Pvf1 signaling axis that protects the fly against obesity by activating mTOR in the fly oenocyte*. Knocking down *pvf1* (*mus>pvf1i*) specifically in the adult muscle leads to increases systemic lipid synthesis and obesity characterized by increased accumulation of lipids in the oenocyte and the adipose tissue (AT). Interestingly, loss of PvR signaling specifically in the adult oenocyte (*oeno>pvr^{DN}*), but not in the muscle, gut or AT, leads to similar phenotypes. Downstream of PvR, Pvf1 specifically activates the Pi3K/Akt1/mTOR signaling cascade in the oenocyte to suppress lipid synthesis and adiposity. However, expression levels of oenocyte-specific lipid synthetic genes such as *fasn2* and *fasn3* were rather downregulated in flies with oenocyte-specific loss of mTOR (*oeno>mTOR^{KD}*) indicating a more complex role for oenocyte mTOR signaling in lipid synthesis. To better understand how oenocyte-specific loss of mTOR signaling may lead to increased lipid synthesis we are investigating tissue specific changes in gene expression in the experimental animals using single nuclei sequencing. Our data reveals interesting AT/oenocyte/muscle specific changes in gene expression in *oeno>mTOR^{KD}* flies. Currently, we are investigating potential mechanisms that leads to obesity in *oeno>mTOR^{KD}* flies. These results along with any new mechanistic insight that explains the biological relevance of this pathway will be presented at the ATGC meeting.

102 Tell me how to go: The migration mechanisms of cell dissemination in vivo *Alejandra Cabrera*[1], Jiae Lee[1], Young Kwon[1] 1) University of Washington.

Metastasis, the spreading of tumor cells from primary site to distant organs, is a major contributor to the deaths of cancer patients. Cancer cells initiate metastasis by disseminating from the original tumor site. Thus, this initial step of metastasis would be an ideal target for preventing metastasis. Nevertheless, our knowledge on cancer cell dissemination is limited, in part because most of the studies concerning the molecular mechanism of dissemination have been done in cultured cells. We have established an in vivo model to study cell dissemination in the adult Drosophila intestine. Using this model, we discovered that extruding cells form actin-rich protrusions linked to invasive cell behavior exclusively at the basal side of the cells. These protrusions can reach the outer surface of the intestine by passing basement membrane and visceral muscle. Furthermore, they can even penetrate muscle segments, puncturing the tissue. Considering their structural features and invasive nature, these protrusions are reminiscent of invadopodia – the actin-rich protrusions playing a key role in cancer cell invasiveness. In addition, as the final step of cell dissemination, we found that cells could also disseminate from intestine using membrane blebs which lacked filamentous actin. These findings suggest that two distinct migration modes—the mesenchymal migration and the amoeboid migration— are subsequently used over the course of cell dissemination in the Drosophila intestine. Our findings elucidate the importance of both modes of cell migration on cell dissemination in vivo, enabling us to discover the molecular mechanisms underlying cell invasiveness using Drosophila genetics.

103 Multidisciplinary Analysis of patient-specific genetic interactions reveal a role for *Megalin / LRP2* in Hypoplastic Left Heart Syndrome *Georg Vogler*[1], Jeanne L Theis[2], Marco Tamayo[1], James Kezos[1], Bosco Trinh[1], Maria A Missinato[1], Karen Ocorr[1], Alexandre Colas[1], Timothy J Nelson[2], Timothy Olson[2], Rolf Bodmer[1] 1) Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA; 2) Mayo Clinic, Rochester, MN.

The etiology of diseases with complex genetic backgrounds is extremely difficult to understand due to the large number of potential interactors at play, and critical interactions are often masked by genetic redundancy in vertebrate models. Therefore, the study of complex disease requires approaches that satisfy both experimental throughput and absence of redundant pathways to uncover the mechanisms driving the disease. Here we present a multidisciplinary approach to study Hypoplastic Left Heart Syndrome (HLHS), which has an occurrence rate of 3 percent amongst all congenital heart defects (CHDs) and is often lethal. HLHS is characterized by severe underdevelopment of the left ventricle and other structures such as mitral and aortic valves and very little is known about the genetic underpinnings of this complex congenital disease. To identify potential candidate genes, we used whole genome sequencing and RNAseq on patient iPSC-derived cardiomyocyte (CM) cultures, followed by several bioinformatical prioritization filters. This created a shortlist of 10 candidates which we subsequently tested using functional assays in our established *Drosophila* heart model as well as iPSC-CMs. We found that knockdown of the LDL Receptor Related Protein 2 Megalin/LRP2 is necessary for Drosophila heart structure and function, as well for CM proliferation, which placed this gene at the center of an HLHS-disease gene network. Follow-up analysis revealed that the patient cells points to altered *(Sonic) Hedgehog*, *WNT/Wingless* and *P53* signaling, and we subsequently found genetic interaction between *Mgl/LRP2* and *SHH/hh* and *WNT/wg* in both of our assays: inhibition of GSK3beta and knockdown of the SHH receptor PTCH1 rescue LRP2 proliferation defects. In *Drosophila* hearts, mgl shows genetic interaction with hh loss-of function and a *Wingless/Wnt4* deficiency, indicating that it is *LRP2* that potentially interconnects these pathways in the heart. Based on these findings we broadened the analysis to a large cohort of > 150 patients with HLHS and systematically tested roughly 1000 candidate genes for their role during *Drosophila* heart function as well as iPSC-proliferation and recovered a large number of genes with distinct cardiac phenotypes and proliferation defects. With these data we are now building

patient-specific disease gene networks, which we are using to understand and close the genotype-to-phenotype gap found in the vast majority of HLHS patients.

104 Investigating stage dependent immune tolerance to heterologous cells for the purpose of creating humanized zebrafish *Anna Zdunek*[1], Shaila Mudambi[1], Mary Pasquale[1], Pooja Gajulapalli[1], Melissa Zakirova[1], Eric Glasgow[1] 1) Georgetown University.

Zebrafish (*Danio rerio*) serve as a key organism for cancer research. Our lab has developed a patient derived xenograft model in zebrafish embryos, Zevatars, in which we can identify the best therapy for a patient's tumor in a personalized medicine setting. A major limitation of this model is the inability to study immunotherapy, which relies on a species specific adaptive immune response. To overcome this we would greatly benefit from a humanized zebrafish model, in which the fish hematopoietic system is replaced with a human hematopoietic system, allowing for observation of single cell level adaptive immune interactions with human tumor xenografts. In zebrafish, definitive hematopoiesis is dependent on Cmyb, therefore the Cmyb null mutant line, *mybt*^{25127}, becomes bloodless although they survive up to 8 weeks, providing us an opportunity to restore the hematopoietic system of these mutants using stem cell xenografting. Transplanting hematopoietic stem and progenitor cells (HSPC) from zebrafish or goldfish whole kidney marrow into *mybt*$^{25127/t25127}$ mutants at 6 wpf rescues the hematopoietic system, while their heterozygous and wildtype (wt) siblings reject transplanted cells. We reasoned that transplantation at earlier stages, while the immune system is still developing, may provide increased tolerance. Hence we allografted HSPCs into 7dpf embryos obtained with *mybt*$^{25127/+}$ in-crosses. Multiple fish showed sustained engraftment for over 6 months. Surprisingly, upon genotyping, all fish with sustained engraftment were *mybt*$^{25127/+}$. No *mybt*$^{25127/t25127}$ were found, while only fish that had rejected the transplants were wt. The contrast of our results with the transplants at 6 weeks, indicated that the developmental stage at time of transplantation is critical for developing immune tolerance. Therefore, it is critical to understand how immune tolerance to heterologous cells changes depending on stage of development. As a first step, we injected human peripheral blood and its components into wt zebrafish 3-7 dpf, and observed short-term sustained engraftment over the course of the experiment for all groups. Based on these preliminary studies we will determine the optimal stage for immune tolerance of human HSPC transplants, followed by transplantation in cmyb mutants, in order to optimize conditions to create humanized zebrafish. If successful, this xenograft model would have profound consequences for basic studies of immunology and immunotherapy. This model may even enable personalized cancer diagnostics for predicting immunotherapy effectiveness by using a patient's HSPC coupled with their tumor biopsy samples.

105 The *C. elegans* model organism screening center for the NIH Undiagnosed Disease Network Huiyan Huang[1], Omar Itani[1], Anika Lindsey[1], Darian Turner[1], Zachary Dawson[1], Jian Chen[2], Ariz Mohammad[2], Gary Silverman[1], Neil Hanchard[3], Daryl Scott[3], David Spielberg[3], Jill Rosenfeld[3], Joel Krier[4], Elizabeth Fieg[4], Klaas Koop[5], Peter van Hasselt[5], Holger Rehmann[5], Matthew Osmond[6], Gail Graham[6], Ellen Macnamara[7], Cynthia J. Tifft[7], Dustin Baldridge[1], Stephen C. Pak[1], *Tim Schedl*[2] 1) Dept. of Pediatrics, Washington University School of Medicine; 2) Dept. of Genetics, Washington University School of Medicine; 3) Dept. of Molecular and Human Genetics, Baylor College of Medicine; 4) Brigham and Women's Hospital Genetics Division; 5) University Medical Center Utrecht, Utrecht University; 6) Children's Hospital of Eastern Ontario; 7) National Human Genome Research Institute, NIH.

Many patients with severe, chronic disease remain without a diagnosis despite extensive medical evaluation. The goal of the NIH Undiagnosed Diseases Network (UDN) is to provide a diagnosis for these challenging cases and to identify biological characteristics of newly discovered disease genes. The UDN uses a collaborative multidisciplinary approach that combines comprehensive medical workup, exome/genome sequencing, bioinformatics analysis, with functional studies in model organisms. *C. elegans* joined Phase II of the UDN in modeling nominated candidate disease gene-variants from patients. Functional studies in research organisms contribute to the diagnosis by assessing whether the gene-variant results in a phenotype, and thus is deleterious to protein function *in vivo*, and whether the genetic behavior in the model is consistent with the mode of inheritance in the patient. We are using CRISPR/Cas9 editing to knock-in evolutionarily conserved patient gene-variant(s) into the orthologous worm gene. There is a single ancestral *rab-5 C. elegans* ortholog relative to three *RAB5* human paralogs. We have examined a UDN case containing a *de novo* missense variant in the nucleotide-binding domain of *RAB5B*, presenting with interstitial lung disease and dysmorphic features. Modeling the *RAB5B* variant in the conserved *rab-5* residue we find that the variant is deleterious as it (i) behaves as a dominant negative in gene dosage studies, (ii) is defective in soluble protein endocytosis, and (iii) is defective in the formation of large early endosomes. By extension, the patient *RAB5B* variant is likely deleterious and the dominant negative behavior in *C. elegans* provides an explanation for the *de novo* dominant effect in the patient. We are also examining two UDN cases, and two GeneMatcher cases, containing *de novo* missense variants in *RAB5C*, presenting with macrocephaly as a common feature. Population studies indicate that *RAB5C* is a haploinsufficient gene in humans. Modeling one of the *RAB5C* missense variants, residing outside of the nucleotide-binding domain, indicates it is defective in soluble protein endocytosis. Modeling the other *RABC* variants is in progress. The distinct *RAB5B* and *RAB5C* patient presentation may involve differences in variant effects, differences in cell type expression, *RAB5A* redundancy, and paralog specific function. This work demonstrates the utility of *C. elegans* to define disease mechanisms in previously undiagnosed patients.

106 SCO-spondin defects and neuroinflammation identified as conserved mechanisms driving severe spine deformity across genetic models of idiopathic scoliosis *Chloe Rose*[1,2], David Pompili[1,2], Katrin Henke[3], Jenica Van Gennip[1,2], Anne Meyer-Miner[1,2], Rahul Rana[2], Stephane Gobron[4], Matthew Harris[3], Mark Nitz[2], Brian Ciruna[1,2] 1) The Hospital for Sick Children, Toronto, ON; 2) The University of Toronto, Toronto, ON; 3) Harvard Medical School, Boston, MA; 4) Alyz Biosciences, France.

Adolescent idiopathic scoliosis (AIS) affects 3-4% of children between the ages of 11 and 18. This disorder, characterized by abnormal three-dimensional spinal curvatures that typically develop during periods of rapid growth, occurs in the absence of congenital vertebral malformations or neuromuscular defects. Genetic heterogeneity and a historical lack of appropriate animal models have confounded basic understanding of AIS biology and, as a result, treatment options remain limited. Recently, genetic studies using zebrafish have linked idiopathic-like scoliosis to irregularities in motile cilia-mediated cerebrospinal fluid (CSF) flow. However, since loss of cilia motility in human primary ciliary dyskinesia patients is not fully associated with scoliosis, other pathogenic mechanisms remain to be determined. Here, we demonstrate that zebrafish *scospondin (sspo)* mutants develop late onset idiopathic-like spinal curvatures in the absence of obvious cilia motility defects. Sspo is a large secreted glycoprotein functionally associated with the subcommissural organ (SCO) and Reissner's fiber (RF) – ancient and enigmatic organs of the brain ventricular system reported to govern CSF homeostasis, neurogenesis and embryonic axis development. We demonstrate that irregular localization of Sspo around the SCO and RF is associated with acquired idiopathic scoliosis across diverse genetic models. Furthermore Sspo defects are sufficient to induce oxidative stress and neuroinflammatory responses implicated in AIS pathogenesis. Through screening for chemical suppressors of *sspo* mutant phenotypes, we also

identify potent agents capable of blocking severe juvenile spine deformity. Our work thus defines a new useful model for AIS and provides tools to realize novel therapeutic strategies.

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Visualizing Intracellular Dynamics

131 Visualizing the metazoan proliferation-differentiation decision *in vivo* *Rebecca Adikes*[1], Abraham Khorman[1], Jayson Smith[1], Michael Martinez[1], Taylor Medwig-Kinney[1], Tian-Ming Fu[3], Nicholas Palmisano[1], Ononnah Ahmed[1], Nicholas Weeks[1], Nuri Kim[1], Simeiyun Liu[1], Wan Zhang[1], Eric Betzig[3], Ariel Pani[2], Benjamin Martin[1], David Matus[1] 1) Stony Brook University, Stony Brook, NY; 2) University of Virginia, Charlottesville, VA ; 3) Janelia Research Campus, Ashburn, VA.

During organismal development, differential regulation of the cell cycle is critical to many cell biological processes, including cell fate specification and differentiation. While the mechanisms of cell cycle regulation are well studied, how control of the cell cycle is linked to differentiated cellular behavior remains poorly understood, mostly due to our inability to directly and precisely measure cell cycle state. In order to characterize cell cycle state live, we have adapted a cyclin dependent kinase (CDK) biosensor for *in vivo* use in the roundworm nematode, *Caenorhabditis elegans* and zebrafish. The biosensor uses the cytoplasmic/nuclear localization of a portion of Human DNA Helicase B (DHB) linked to a fluorescent protein to assess cell cycle state. The dynamic localization of DHB is the result of phosphorylation of the biosensor by CDKs. We have modified this sensor to allow for the algorithmic assessment of cell cycle state. We demonstrate here using a combination of spinning disk confocal and adaptive optics lattice light sheet microscopy the use of this biosensor to quantify lineage-specific differences between cycling cells, and to examine the link between quiescence and differentiation. Unlike other live cell imaging tools (e.g., FUCCI), we show that DHB can be used to distinguish between actively cycling cells in the G1 phase of the cell cycle and quiescent or terminally differentiated cells exited in G0. Additionally, we have used this tool to investigate cell cycle regulation during morphogenesis and regeneration. Thus, we provide here a new resource to study the control and timing of the metazoan cell cycle during cell fate specification and differentiation.

132 NudC phosphorylation silences dynein to promote anterograde cargo transport in axons Dane Kawano[1], Katherine Pinter[1], Alex Nechiporuk[2], *Katie Drerup*[1] 1) NICHD; 2) Oregon Health & Science University.

Cytoplasmic dynein is the primary microtubule minus end directed motor in cells. This motor controls a number of cellular processes, including cell division and the intracellular transport of protein and organelle cargos. Regulation of dynein is complex: evidence to date suggests that activation of the motor specifically requires that dynein be relieved of its autoinhibition (Phi conformation) and cargo must bind for efficient transport. Recent work points to the accessory protein Lis1 as a primary driver of confirmation switching and the use of the dynactin complex in association with Hook/Bicaudal adaptors as essential mediators of cargo attachment to the dynein motor. While we are beginning to understand the activation of dynein, how its inhibition is maintained to regulate cargo transport only at the proper time and place is still largely unknown. Importantly, most cargos are bound to both dynein and anterograde motors simultaneously, arguing for silencing as a critical mediator of long-distance transport. Using forward genetics and in vivo imaging of cargo motility, we have identified NudC as a mediator of dynein silencing in neurons. NudC is a known dynein-interacting protein essential for mitosis. We show that loss of NudC function leads to loss of anterograde (microtubule plus end directed) transport of vesicular cargos in post-mitotic neurons, including cargos bound by the dynein motor. This anterograde transport deficit leads to accumulation of cytosolic components in enlarged autophagosomes in axon terminals. Although the *nudc* allele in our mutant strain only mildly truncates the protein, it obliterates a Plk1 phosphorylation site known to be critical for regulation of NudC and Plk1 localization during cell division. Importantly, overexpression of a mutant form of NudC lacking this phosphorylation site causes morphological defects in neurons that phenocopy the *nudc* mutant phenotype, implicating NudC phosphorylation in dynein silencing. Together, our work reveals NudC as a critical regulator of dynein silencing and, consequently, proper anterograde cargo transport, which is essential for cargo distribution and utilization in neurons.

133 RNA nucleates phase separation of glycolysis enzymes in yeast in hypoxia *Gregory Fuller*[1], Mallory Freeberg[2], Ting Han[3], John Kim[1] 1) Johns Hopkins University, Baltimore, Md; 2) EMBL-EBI, Wellcome Genome Campus, Hinxton, Cambridgeshire, UK; 3) National Institute of Biological Sciences, Beijing, China.

When cells are deprived of oxygen, they rely solely on glycolysis for ATP production rather than respiration, which is far more efficient. We recently reported that glycolysis enzymes coalesce in single cytoplasmic granules in yeast termed Glycolytic (G) bodies in response to hypoxic stress. By tracking GFP tagged Pfk2, the beta subunit of the yeast phosphofructokinase, we were able to investigate G body biophysical properties *in vivo*. Recruitment to G bodies of Pfk2-GFP required interactions of both a disordered N terminal region and a structured C terminal region, revealing that Pfk2 recruitment requires multivalent interactions. G bodies were able to fuse in mating yeast cells on the order of minutes and decreased in size in response to 1,6 hexanediol, but did not fully dissolve. These properties are consistent with G bodies forming a more solid gel rather than liquid phase. Purification of G bodies and mass spectrometry revealed an enrichment of noncanonical RNA binding proteins in G bodies, including several glycolysis enzymes. RNAs purified from G bodies had substantial overlap with RNAs identified by PAR-CLIP that bind to glycolysis enzymes in normoxic conditions. To test how RNA affects G body formation, we tagged Pfk2 with a nonspecific RNase, MqsR. Induction of this fusion protein led to dose dependent loss of G bodies, suggesting that targeting an RNA nuclease to nascent G bodies inhibits their formation. Induction of this fusion protein in cells with preformed G bodies led to the accumulation of multiple foci, suggesting that RNA is required to maintain the structural integrity of G bodies. Thus, RNA is required to nucleate phase separation of glycolysis enzymes in G bodies as well as provide structural support to mature G bodies. Phase separation of protein and RNA in non-membrane bound condensates is an emerging theme in subcellular organization. However, we are only beginning to understand the functional implications of phase separation. Mutants with impaired G body formation are enriched for upstream glycolysis intermediates and depleted of downstream products. Furthermore, these mutants consume glucose at slower rates than wild-type cells with intact G bodies. Finally, targeted depletion of G bodies leads to decrease in competitive fitness in hypoxic conditions, suggesting that G bodies enhance glucose flux, thereby promoting cell division or survival in hypoxia.

134 Degron-tagged reporters probe membrane topology and enable the specific labelling of membrane-wrapped structures *Ann Wehman*[1], Katharina Beer[1], Gholamreza Fazeli[1], Kristyna Judasova[2], Linda Irmisch[1], Jona Causemann[1], Joerg Mansfeld[2] 1) Univ Wuerzburg; 2) TU Dresden.

Visualization of specific organelles in tissues over background fluorescence can be challenging, especially when reporters localize to multiple structures. Instead of trying to identify proteins enriched in specific membrane-wrapped structures, we use a selective degradation approach to remove reporters from the cytoplasm or nucleus of *C. elegans* embryos and mammalian cells using different degron-ubiquitin ligase pairs. Thereby, we demonstrate specific labelling of organelles using degron-tagged reporters, including extracellular vesicles, as well as individual neighboring membranes normally indistinguishable by light microscopy. We find that degron-tagged reporters facilitate long-term tracking of released cell debris and cell corpses, even during uptake and phagolysosomal degradation. We further show that degron protection assays can probe the topology of the nuclear envelope and plasma membrane during cell division, giving insight into protein and organelle dynamics. Furthermore, the ER membrane can protect degron-tagged reporters from cytosolic ubiquitin ligases, demonstrating the orientation of a protein in the secretory system. We also find that we can change the dynamics and location of degradation by altering the localization of the ubiquitin ligase. As endogenous and heterologous degrons are used in bacteria, yeast, plants, and animals, degron approaches can enable the specific labelling and tracking of proteins, vesicles, organelles, cell fragments, and cells in many model systems.

Publication:
Degron-tagged reporters probe membrane topology and enable the specific labelling of membrane-wrapped structures
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135 Cell competition regulates tissue growth and tumorigenesis via non-autonomous induction of autophagy *Rina Nagata*[1], Mai Nakamura[1], Yuya Sanaki[1], Nanami Akai[2], Shizue Ohsawa[2], Tatsushi Igaki[1] 1) Kyoto University; 2) Nagoya University.

Cell competition is a quality control process that selectively eliminates unfit cells from the growing tissue. For instance, cells with reduced protein synthesis ("losers") such as heterozygous mutants for a ribosomal protein gene (called *Minute* mutants) are eliminated from *Drosophila* imaginal epithelium when surrounded by wild-type cells ("winners"). We have recently established a new model of cell competition triggered by mutations in the RNA helicase gene *Hel25E*, which make cells to be losers when surrounded by wild-type cells. Like *Minute* mutants, *Hel25E* mutant cells showed reduced protein synthesis compared to wild-type. To identify factors that drive cell competition, we performed a genetic modifier screen in *Drosophila* imaginal discs and identified a series of mutations in *Vha* genes as dominant suppressors of *Hel25E*-induced cell competition. *Vha* genes encode components of the V-ATPase complex, an essential factor for autophagy. Strikingly, autophagy was specifically elevated in prospective loser cells nearby wild-type winner cells, and blocking autophagy in loser cells abolished their elimination. Mechanistically, elevated autophagy upregulated a proapoptotic gene *hid* via NFkB, and the elevated *hid* cooperated with JNK to effectively induce loser's death (Nagata et al., *Dev Cell*, 2019). Crucially, we found that this mechanism generally applied to cell competition caused by differences in protein synthesis between cells, such as *Minute*, Myc, *Mahjong*, or other mutation-induced cell competition. Furthermore, we found that autophagy-mediated cell death also promoted tumorigenesis caused by Hippo pathway mutants, which showed higher protein synthesis compared to wild-type neighbors. Our findings establish a common regulatory mechanism of tissue growth and tumorigenesis by cell competition whereby cells with higher protein synthesis induce autophagy-mediated cell death in their neighboring cells.

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New Technology and Systems Biology

118 Systematic humanization of yeast processes to understand human biology and disease Aashiq H. Kachroo[1], Mudabir Abdullah[1], Brittany M. Greco[1], Farhat Zafar[1], Courtney M. Gamache[1], Jon M. Laurent[2], Riddhiman K. Garge[3], Azat Akhmetov[3], Daniel R. Boutz[3], Edward M. Marcotte[3] 1) Centre for Applied Synthetic Biology, Department of Biology, Concordia University, Montreal, QC, Canada. ; 2) Institute of Systems Genetics, Department of Biochemistry and Molecular Pharmacology, NYU Langone Health, NY, USA ; 3) Center for Systems and Synthetic Biology, Department of Molecular Biosciences, The University of Texas at Austin, TX, USA.

The remarkable extent to which protein-coding genes are still functionally equivalent between humans and yeast emphasizes the power even of a distant organism for studying human gene function. Several human genome sequencing studies have discovered extensive genetic polymorphism in these genes, including many rare variants that cause or predispose to diseases. Exploring how this variation contributes to cellular function and overall human health remains a challenge and has not matched the rate at which variants are identified. Our laboratory has already created hundreds of humanized yeast strains (Science, 2015; eLife, 2017; BioRxiv, 2019). Humanized yeast act as physical reagents to characterize human genetic variation and define disease alleles, opening previously inaccessible chemical and genetic screens with the potential to ultimately treat diseases. We will discuss our progress towards making and applying humanized yeast. The humanization paradigm allows us to test if complete yeast and human systems are interchangeable in yeast. We will show our extensions of this work to humanize yeast biological processes in their entirety, focusing on the proteasome core, heme, and sterol biosynthesis pathways. The resulting strains carrying multiple human genes are more suitable for screening human genetic alleles. These engineered strains allow the simultaneous introduction of human genetic variants at various loci in an easily manipulated system for the study of human polygenic disease, providing a pathway-level activity measure for combinations of human variant alleles. If successful, it will lead to new insights into human genetic traits and epistatic interactions among genes belonging to the same genetic processes, which is still unachievable at this scale in mammalian cells.

References:
1. Kachroo, A.H., et al., *Science* (2015)
2. Kachroo, A.H., et al., *Elife* (2017)
3. Laurent, J.M., et al., *bioRxiv* (2019)

119 MAGIC: Mosaic Analysis by gRNA-Induced Crossing Over Sarah Allen*[1], Gabriel Koreman*[1], Ankita Sarkar*[1], Bei Wang[1], Mariana Wolfner**[1], Chun Han**[1] 1) Cornell University.

Analyses carried out in chimeric animals have contributed to many fundamental discoveries in developmental biology and cell biology. Common techniques to produce "mosaic" animals by mitotic recombination in *Drosophila melanogaster* rely on site-specific recombination systems such as Flp-FRT and require complicated genetic modification of chromosomes. Although useful in *Drosophila melanogaster*, these techniques are not generally available in other organisms. Here, we report the development of Mosaic Analysis by gRNA-Induced Crossing over (MAGIC), a new technique for the generation of mosaic animals using CRISPR/Cas9 and strategically placed gRNAs. This system produces double-strand breaks at specific sites in a chromosome, which can result in mitotic recombination between homologous chromosomes through homology-directed repair. We show that MAGIC efficiently produces homozygous clones in both somatic tissues and the germline in otherwise heterozygous animals. We further developed a toolkit to conveniently generate marked clones for genes located on chromosome arm 2L and demonstrated its application for gene function analysis in the wing imaginal disc and larval sensory neurons. MAGIC requires no genetic modifications of the chromosome of interest and thus can be used in unmarked, wild-type animals such as ones from the *Drosophila* Genetic Reference Panel (DGRP) collection. MAGIC can thus likely be applied to organisms beyond *Drosophila*, opening doors to novel studies in the broader field.

120 A Multiplexed CRISPR Screen for Essential microRNA-Target Interactions in C. elegans *Bing Yang*[1], Katherine McJunkin[1] 1) NIH/NIDDK.

MicroRNAs (miRNAs) regulate the expression of complementary target genes to ensure normal development and physiology. However, our understanding of miRNA gene regulatory networks is hampered by the difficulty of identifying miRNA target genes. Prediction algorithms can provide a long list of candidate genes, and empirical approaches can identify many of the targets bound by a miRNA. However, both approaches are plagued by false negatives and false positives. Furthermore, neither of these approaches delineates which of a miRNA's targets are the most biologically relevant, i.e. those that must be regulated by the miRNA to prevent deleterious phenotypes. Historically, forward suppressor screens in miRNA mutant backgrounds have identified such key targets of a few miRNAs. However, in the case of other miRNAs, such as the essential *mir-35-42* family, such suppressor screens were unsuccessful. Therefore, we devised a novel strategy to test the phenotypic impact of disrupting each predicted binding site for *mir-35-42* by CRISPR-Cas9 genome editing in *C. elegans*.

Our approach has multiple technical innovations. First, because of the large number of candidate genes, we developed a multiplexed screening approach that uses simultaneous injection of multiple pooled guide RNAs to increase throughput. Second, we read out the screen by deep sequencing of the edited alleles present in superficially wild type strains. Finally, because of the importance of the seed sequence of the miRNA (nucleotides 2-8) for target binding, we use the positional distribution of indels relative to the seed binding region as a metric to infer the phenotypic effects of mutating the miRNA binding site.

In our screen we tested 87 putative *mir-35-42* binding sites, reading out 1103 alleles from 570 strains. Of the top candidates from the screen, *egg laying defective 1* (*egl-1*) validated in single gene studies: disruption of *egl-1*'s *mir-35* binding site resulted in decreased embryonic viability and fecundity. Thus, relieving *egl-1* from *mir-35*-mediated repression partially phenocopies the *mir-35-42* mutant phenotypes, and this can be rescued by introducing compensatory mutations into *mir-35* that restore *egl-1* repression. This type of screen has the potential to accelerate the discovery of biologically-important miRNA targets. Moreover, any well-defined negative regulatory genomic element could be phenotypically screened by this method of multiplexed CRISPR followed by mutational profiling.

121 CRISPR-Cas13d induces efficient mRNA knock-down in animal embryos *Gopal Kushawah*[1], Joaquin Abugattas-Nuñez del Prado[2,3,4], Juan Martinez-Morales[2], Michelle DeVore[1], Alejandro Alvarado[1,6], Edward Málaga-Trillo[4], Carter M Takacs[5], Miguel A. Moreno-Mateos[2,3], Ariel A. Bazzini[1,7] 1) Stowers institute for medical research; 2) Andalusian Center for Developmental Biology (CABD), Pablo de Olavide University/CSIC/Junta de Andalucía, Ctra.; 3) Department of Molecular Biology and Biochemical Engineer, Pablo de Olavide University, Ctra. ; 4) Department of Biology, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado; 5) University of New Haven, West Haven, Connecticut 06516, USA.; 6) Howard Hughes Medical Institute, Stowers Institute for Medical Research, Kansas, MO, USA.; 7) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City.

Early embryonic development is driven exclusively by maternal gene products deposited into the oocyte. Although critical in establishing early developmental programs, maternal gene functions have remained elusive due to a paucity of techniques for their systematic disruption and assessment. CRISPR-Cas13 systems have recently been employed to induce RNA degradation in yeast, plants and mammalian cell lines. However, no systematic study of the potential of Cas13 has been carried out in an animal system. Here, we show that CRISPR-Cas13d is an effective and precise system to deplete specific mRNA transcripts in zebrafish embryos. We demonstrate that both zygotically-expressed and maternally-provided transcripts are efficiently targeted, resulting in an 80% average decrease in transcript level and the recapitulation of well-known embryonic phenotypes. Moreover, we show that this system can be used in medaka, killifish and mouse embryos. Altogether our results demonstrate that CRISPR-Cas13d is an efficient knock-down platform to interrogate gene function in animal embryos.

122 A Catalog of Polymorphic SINEC_Cf Insertions in the Dog Genome Jessica D. Choi[1], Sara E. Kalla[2], Allison Seebald[3], Max Tomlinson[3], Yun Seok Lee[1], Cassandra R. Ward[1], Nathan B. Sutter[1] 1) La Sierra University, Riverside, CA; 2) Baylor College of Medicine, Houston, TX; 3) Cornell University, Ithaca, NY.

Retrotransposons are ubiquitous in mammal genomes where they can account for nearly half of all sequence. The genome of the domestic dog, *Canis familiaris*, harbors the L1 LINE and several types of SINEs. In particular, the dog reference genome contains 171,386 annotations for SINEC_Cf, a SINE originating from tRNA. Many SINEC_Cf copies are very young, including some that have not yet gone to fixation. To discover polymorphic SINEs we constructed 62 sequencing libraries enriched with sequence flanking SINEC_Cf by hybridizing a primer to well-conserved sequence in SINEC_Cf's head that is usually absent from other SINE types. These libraries represent 59 breeds that broadly survey dog genome variation. We identified 81,747 putative polymorphic SINE insertions detected in at least one of our libraries but absent from the Boxer reference genome. Because SINEs are known to disrupt normal patterns of gene expression and splicing, we identified areas in dog genes where reference genome SINEs or LINEs are excluded or have a strand bias. For example, LINEs and SINEC_Cfs are excluded from introns near exons and both types of retrotransposons have a strand bias for intronic insertions. SINEC_Cf insertions in introns near exons can cause diseases such as narcolepsy; we find many insertions less than 30 bp from exons. Finally, we also find dozens of polymorphic SINE insertions in protein-coding exons (many in known pseudogenes) and hundreds of SINEs in UTRs and promoters. The high insertion rate of SINEC_Cf provides a natural mutagenesis screen in the dog genome.

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Undergraduate Session

126 Undergraduate Platform Keynote Address *Elaine Fuchs*[1] 1) Rockefeller University.

abstract is not available at the time of print

127 Characterizing protein aggregates in NUAK mutants using the *Drosophila* muscle tissue model *Marta Stetsiv*[1], David Brooks[1], Fawwaz Naeem[1,2], Sammantha Goetting[1], Erika Geisbrecht[1] 1) Kansas State University; 2) Ohio State University.

The inability to remove damaged cellular components or misfolded proteins is a hallmark of aggregate disorders. NUAK is a conserved serine/threonine protein kinase expressed in different tissues, including skeletal muscle, and has a known function in regulating cytoskeleton motors. Mammalian genomes contain two copies of the *NUAK* gene—*NUAK1 (ARK5)* and *NUAK2 (SNARK)*. Knockout of both genes in mice is embryonic lethal. However, mutation of the single *Drosophila NUAK* gene is viable through larval stages, allowing for the *in vivo* examination of proteins that may be involved in NUAK signaling. *NUAK* mutants produce thinning muscles, as well as dark regions that lack the myofibrillar proteins F-actin and Myosin heavy chain (MHC). Interestingly, these regions instead contain damaged organelles and the accumulation of select proteins, including Filamin (Fil) and CryAB. NUAK biochemically and genetically interacts with *Drosophila* Starvin (Stv), the ortholog of mammalian Bcl-2-associated athanogene 3 (BAG3). Consistent with a known role for the co-chaperone BAG3 and the Heat shock cognate 71 kDa (HSC70)/HSPA8 ATPase in the autophagic clearance of proteins, RNA interference (RNAi) of *Drosophila* Stv, Hsc70-4, or autophagy-related 8a (Atg8a) all exhibit muscle degeneration and muscle contraction defects that phenocopy *NUAK* mutants and are enhanced in a *NUAK* or *stv* mutant background. To elucidate where NUAK and Stv may be functioning in the autophagy signaling cascade, we tested whether Stv overexpression rescues NUAK-mediated muscle defects. Our results show that Stv overexpression alleviates defects associated with loss of NUAK, demonstrating that NUAK acts upstream of Stv. Identified using bioinformatics approaches, we also uncovered *jaguar* (*jar*) as a gene that interacts with *NUAK* and *stv*. *Jar* encodes for the Myosin VI (Myo VI) protein, a molecular motor that transports cargo towards the minus-end of actin filaments. Thus, we speculate that MyoVI may be important for transport of proteins destined for degradation in muscles. Collectively, our results demonstrate a novel role for NUAK and the Stv-Hsc70-4 complex in the autophagic clearance of proteins that may eventually lead to treatment options for protein aggregate myopathies and protein misfolding found in neurodegenerative diseases.

128 Alternative *mec-2* isoforms exhibit neuron type-specific expression and function *Canyon Calovich-Benne*[1], Ryan Bixby[1], Trey Dalton[1], Elizabeth Miller[1], Alexa Vandenburg[1], Adam Norris[1] 1) Department of Biological Sciences, Southern Methodist University, Dallas, TX.

Alternative splicing can contribute to functional diversity by creating multiple isoforms of a single gene. Alternative splicing has been widely observed but it is often unknown whether specific isoforms are expressed in unique cell types, and whether they are functionally relevant. Neuron-specific RNA sequencing (RNA-seq) of *C. elegans* in our lab showed that the conserved membrane protein *mec-2* exhibits unique splicing patterns in specific neurons. The canonical *mec-2A*isoform is expressed in mechanosensory neurons, but the non-canonical *mec-2B* isoform was detected in neurons other than in mechanosensory neurons. We have recently validated our RNA-Seq results using isoform-specific endogenous fluorescent tags, and determined that the *mec-2B* isoform is present in chemosensory neurons as well as mechanosensory neurons. We then tested whether there was a functional role for the *mec-2B* isoform in chemosensory neurons. Chemotaxis assays show that *mec-2* mutants have a significant deficiency in chemotaxis to various organic volatile odorants. Using the CRISPR-Cas9 system we forced the expression of one or the other isoform of *mec-2* to see whether there is a difference in behaviors. We found worms expressing only *mec-2A* cannot smell but do sense soft touch, and worms only expressing *mec-2B* cannot sense soft touch but can smell various odorants. We therefore believe that we are uncovering novel expression and functional differences of *mec-2A* and *mec-2B* in a cell-specific manner.

129 Distinguishing Between Self and Foreign siRNA in the *C. elegans* Germline *Diljeet Kaur*[1], Zoran Gajic[2], Sam Gu[1] 1) Rutgers University; 2) New York University .

Small interfering RNA (siRNA) – mediated transcriptional gene regulation, or nuclear RNAi, plays an evolutionarily conserved function in the silencing of transposons and foreign DNA to protect genomic integrity, especially in the developing germline. *C. elegans* provides a highly tractable system to study this pathway and its role in transgenerational epigenetic inheritance. Despite recent progresses, the biogenesis and regulation of endogenous siRNAs remain elusive. To address this question, we used CRISPR-mediated genome engineering to develop various *C. elegans* strains containing a “siRNA generator,” by inserting different elements from “self” genes, which produce low levels of endo-siRNAs, into a “foreign” LTR retrotransposon, which produces high levels of endo-siRNAs. The objective of this approach was two-fold: 1) we wanted to see how this insertion would affect the siRNA profile at the LTR retrotransposon and 2) we wanted to determine whether the inserted sequence would produce endo-siRNAs that could silence the target loci in *trans*. To date, we have created 20 strains containing siRNA generators. Analysis of these strains indicates varied effects on the LTR retrotransposon's siRNA profile, and the ability of the siRNA generator insert-derived siRNAs to act in *trans* remains ambiguous. Further characterization will provide insight into the regulation of siRNA homeostasis and how eukaryotic genomes distinguish between self and foreign nucleic acids.

130 PRISM-stop targeted integration in *aquaporin1a1* and *1a2* reveals a requirement during vascular morphogenesis *Jacklyn Levey*[1], Grant Stefanik[1], Ying Wang[1], Jordan Welker[1], Natalie Arnold[1], Kenna McKeegan[1], Melanie Torrie[1], Darius Balciunas[3], Colette Abbey[4], Steve Ekker[2], Karl Clark[2], Maura McGrail[1], Kayla Bayless[4], Jeffery Essner[1] 1) Iowa State University, Ames, IA; 2) Mayo Clinic, Rochester, MN; 3) Temple University, Philadelphia, PA; 4) Texas A&M, College Station, TX.

Aquaporin1a1 (*aqp1a1*) is an integral membrane protein that facilitates the transport of water molecules between cells. *Aquaporin1a2* (*aqp1a2*) is a tandem duplicate of *aqp1a1* and could be genetically redundant. Previous in situ localization experiments demonstrated that *aqp1a1 and 1a2* are expressed primarily in endothelial cells during embryonic development in zebrafish. Targeted integration of a Gal4VP16 pGTag cassette into the *aqp1a1* locus also showed expression of the RFP from a UAS-RFP transgene in endothelial cells throughout the embryo. Using a transgenic approach with *Tol2*, a UAS-*aqp1a1-egfp* transgenic with an endothelial specific Gal4VP16 driver from the *fli1b* locus showed localization to the luminal membrane during vascular tube formation. To test the genetic requirements of *aqp1a1* and *1a2* during vascular development in zebrafish, indel and knock-in alleles were generated using CRISPR-Cas9. For the knock-in alleles, an insertional mutagenesis approach which relied on

short homology in the targeting cassette was used to integrate a PRISM-stop cassette containing stop codons in all three frames followed by a poly adenylation sequence. The PRISM-stop insertional mutants also contained reporter genes to help visualize the presence of the targeted allele using either RFP or BFP expression in the lens. Two knock-in mutant lines were isolated for the *aqp1a1* gene with precise integrations at both ends of the cassette, expressing RFP in the lens. Each were used to create homozygous mutants that showed collapsed intersegmental vessels and abnormal endothelial branching. In contrast, no phenotype was observed in homozygous mutant embryos with indel mutations in *aqp1a1*, suggesting genetic compensation. Consistent with the phenotype in the knock-in alleles, knockdown of *aqp1a1* in HUVECs resulted in reduced endothelial sprouting. A double mutant was made by inserting a PRISM stop cassette that expressed BFP in the lens into the *aqp1a1* and *1a2* genes. The cassette integrated precisely into the*aqp1a2* gene but only the 5' side of the cassette in *aqp1a1* was precise. When the double mutant line was used to generate homozygous mutants, collapsed intersegmental vessels and abnormal endothelial branching was observed like the *aqp1a1* mutant alone, indicating that the *aqp1a1* gene is primarily required for vascular development. Together, these results demonstrate a role for *aqp1a1* in endothelial migration and morphogenesis in both zebrafish and humans.

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Crow Award Talks (PEQG)

112 Introduction to James Crow *Bret Payseur*

abstract is not available at the time of print

113 Natural selection on the *Arabidopsis thaliana* genome in present and future climates *Moises Exposito-Alonso*[1,4,5], Hernan Burbano[2,5], Oliver Bossdorf[3], Rasmus Nielsen[4], Detlef Weigel[5] 1) Department of Plant Biology, Carnegie Institution for Science, Stanford, CA, USA; 2) Research Group of Ancient Genomics and Evolution, Max Planck Institute for Developmental Biology, Tübingen, Germany; 3) Institute of Evolution and Ecology, University of Tübingen, Tübingen, Germany; 4) Department of Integrative Biology, University of California Berkeley, Berkeley, CA, USA; 5) Department of Molecular Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany.

Through the lens of evolution, climate change is an agent of natural selection that forces populations to change and adapt, or face extinction. However, current assessments of the risk of biodiversity associated with climate change do not typically take into account how natural selection influences populations differently depending on their genetic makeup. Here we make use of the extensive genome information that is available for *Arabidopsis thaliana* and measure how manipulation of the amount of rainfall affected the fitness of 517 natural *Arabidopsis* lines that were grown in Spain and Germany. This allowed us to directly infer selection along the genome. Natural selection was particularly strong in the hot-dry location in Spain, where 63% of lines were killed and where natural selection substantially changed the frequency of approximately 5% of all genome-wide variants. A significant portion of this climate-driven natural selection of variants was predictable from signatures of local adaptation (R^2 = 29–52%), as genetic variants that were found in geographical areas with climates more similar to the experimental sites were positively selected. Field-validated predictions across the species range indicated that Mediterranean and western Siberian populations—at the edges of the environmental limits of this species—currently experience the strongest climate-driven selection. With more frequent droughts and rising temperatures in Europe, we forecast an increase in directional natural selection moving northwards from the southern end of Europe, putting many native *A. thaliana* populations at evolutionary risk.

114 Quantifying selection on heritable variation in human complex traits *Yuval Simons*[1], Hakhamanesh Mostafavi[1], Guy Sella[2] 1) Stanford University, CA; 2) Columbia University, NY.

Many traits of interest are highly heritable and genetically complex, meaning that much of the variation they exhibit arises from differences at numerous loci in the genome. While it has long been thought such traits are often subject to stabilizing selection, the strength of selection acting on variants has remained unknown. Here we rely on theory we previously developed and on findings from human GWAS to infer the distribution of these selection coefficients in humans. We show that the frequency of a genome-wide significant association provides an upper bound on the selection coefficient of the variant it tags and that the association's estimated effect size provides a lower bound on the selection coefficient. This allows us to use a maximum-likelihood framework to infer the distribution of selection coefficients in the range between 10^{-2} and 10^{-5}. We applied our method to various quantitative traits, including anthropometric traits like height and BMI, life history traits like age at menarche, and biomarkers, like IGF1, LDL and urea levels. To the best of our knowledge, these are the first estimates of the distribution of selection coefficients acting on heritable variation in human complex traits. Our results suggest that heritable variation in most of these traits is predominated by variants under weak selection (with selection coefficients around 10^{-4}). The veracity of our inferences is supported by genealogy-based estimates of the allelic ages of genome-wide significant associations: the median estimated allelic age for these traits is ~10k generations compared to a median age of ~15k generations for frequency-matched random SNPs, consistent with our predictions based on the estimated distribution of selection coefficients. Our inferences allow us to predict the results of future, larger GWAS in Europeans, and inform the design of GWAS in other human populations.

115 Recombination, variance in genetic relatedness, and selection against introgressed DNA *Carl Veller*[1], Nathaniel Edelman[1], Pavitra Muralidhar[1], Martin Nowak[1] 1) Harvard University.

The genomic proportion that two relatives share identically by descent—their genetic relatedness—can vary depending on the patterns of recombination and segregation in their pedigree. We calculate the precise connection between genome-wide genetic shuffling and variance in genetic relatedness. For the relationships of grandparent-grandoffspring and siblings, the variance in genetic relatedness is a simple decreasing function of *r*-bar, the average proportion of locus pairs that recombine in gametogenesis. These calculations allow us to characterize how recombination affects the rate at which selection eliminates deleterious introgressed DNA after hybridization—by modulating the variance of introgressed ancestry across individuals. Thus, species with low aggregate recombination rates, such as *Drosophila*, purge introgressed DNA more rapidly and more completely than species with high aggregate recombination rates, such as humans. These conclusions hold for different genomic regions as well. Within the genomes of several species, positive correlations have been observed between local recombination rate and introgressed ancestry. Our results imply that these correlations will often be driven more by recombination's effect on the purging of deleterious introgressed alleles than its effect in unlinking neutral introgressed alleles from deleterious alleles. In general, our results demonstrate that the aggregate recombination process—as

quantified by *r*-bar and analogs—acts as a variable barrier to gene flow between species.

116 Predicted shifts in dominance increase the likelihood of soft selective sweeps. *Pavitra Muralidhar*[1], Carl Veller[1], Nate Edelman[1] 1) Harvard University.

Understanding the mechanics of adaptation is a central goal of evolutionary biology. A major current question is whether adaptation in the face of new environmental challenges typically occurs via hard sweeps, which originate from a single copy of a beneficial mutation, or soft sweeps, which originate from multiple independent instances of a beneficial mutation. Previous work on this topic has generally assumed that the fitness dominance of the focal allele remains constant across the changing selective environments. However, our molecular and empirical understanding of dominance suggests that this should, in fact, seldom be the case: alleles are typically recessive when deleterious and dominant when beneficial. Here, we show that this empirically supported pattern of changing dominance dramatically increases the probability that selective sweeps will be soft. As a case study, we examine the evolution of pesticide resistance at the *Ace* locus in *Drosophila,* a classic example of a soft selective sweep. Our modelling demonstrates that a soft sweep was the expected outcome, even for smaller population sizes than have previously been assumed to be required for explaining the haplotype diversity observed at this locus. Our work provides a new perspective on how dominance influences the process of adaptation, and offers strong predictions about the relative prevalence of soft versus hard sweeps in different genomic regions.

117 Adaptive evolution at a meiosis gene mediates species differences in the rate and patterning of recombination *Cara Brand*[1,2] 1) University of Pennsylvania; 2) University of Rochester.

Crossing over between homologous chromosomes during meiosis repairs programmed DNA double-strand breaks, ensures proper segregation, enhances the efficacy of natural selection among genetically linked sites, and determines the genomic distribution of nucleotide variability in populations. Little however is known about the molecular genetic changes or population genetic forces involved in the evolution of recombination rates between species. We show that a dicistronic meiosis gene, *mei217/mei-218*, with a history of rapid evolution acts as a global, trans-acting modifier of the rate and chromosomal distribution of crossing over between two closely related *Drosophila* species. Using transgenic flies, we find that species differences in crossing over are attributable to changes in the strengths of crossover assurance, crossover interference, and centromeric suppression of crossing over. We speculate that rates of crossing over evolved in part to mitigate fluctuating, species-specific risks of ectopic recombination between non-homologous transposon insertions. Regardless of its causes, the evolution of *mei-217/mei-218*-mediated changes in recombination landscapes may contribute to downstream species differences such as the chromosomal distribution of nucleotide variability and rates of nondisjunction.

Thursday, April 23 1:30 PM - 2:30 PM

The Ins and Outs of NIH Peer Review

123 Overview *Mary Mullins*[1] 1) University of Pennsylvania.

abstract is not available at the time of print

124 Title to come *Sharon Buganich*[1] 1) NIH/CSR.

abstract is not available at the time of print

125 Title to come *Lystranne Maynard-Smith*[1] 1) NIH.

abstract is not available at the time of print

Thursday, April 23 2:30 PM - 3:30 PM

COVID - 19 response by the NSF and NIH

137 NSF BIO's COVID-19 response and funding opportunities *Mattthew Olson*[1] 1) NSF.

abstract is not available at the time of print

139 Overview of NIH Extramural Response to COVID-19 Pandemic *Michael Lauer*[1] 1) NIH.

abstract is not available at the time of print

Thursday, April 23 3:45 PM - 5:45 PM

Genomics and Systems Biology (Mammal)

162 Modeling gene x treatment effects in the Collaborative Cross and other replicable multiparent populations *William Valdar*[1], Yanwei Cai[1], Merrie Mosedale[2,3] 1) Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 2) Institute for Drug Safety Sciences, University of North Carolina at Chapel Hill, Research Triangle Park, North Carolina; 3) Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, Chapel Hill, North Carolina.

At the heart of precision medicine is the notion that the effect of a drug treatment or other therapeutic intervention may vary between individuals but will often do so in a way that is in part attributable to--and potentially predictable by--genetics. Yet genetic effects on treatment response, also known as gene-by-treatment interaction or GxT, are challenging to study in outbred populations: doing so ideally requires comparison of genetically identical individuals under matched placebo/unexposed and treatment/exposed conditions; otherwise, the genetic effect on the treatment response is inevitably confounded with the genetic effect on the baseline phenotype. An unconfounded assessment is possible, however, in repli-

cable populations--given a suitable population and study design. Specifically, when it comes to identifying and localizing genetic sources of a GxT effect via quantitative trait locus (QTL) mapping, an ideal experimental population should be one that encompasses broad genetic diversity while also providing biological replicates: the replicates allow unconfounded estimation of treatment response while the diversity allows that treatment response to be evaluated under many alternate genetic configurations. I will describe how we are using Collaborative Cross, a multiparent parent population (MMP) of recombinant inbred strains derived from eight diverse inbred founder strains, to characterize GxT in a set of three studies on drug-induced liver injury arising from the use of three now-recalled therapeutic drugs (Mosedale et al 2017 PMID:28115652; Mosedale et al 2019 PMID:31501888). In discussing these, I will emphasize how the population and study design allows the use of relatively simple statistical models, including assessment of GxT heritability, and how these relate to more complicated models that would be used in design-population combinations that are less apt. Time permitting, I will also briefly relate these studies build on principles developed in earlier work on GxT in diallels of the CC founders (Crowley et al 2014 PMID:24240528; Maurizio et al 2018 PMID:29187420).

163 Genetic dissection of initial cocaine sensitivity and behavioral sensitization using the Collaborative Cross and Diversity Outbred mouse populations *Sarah Schoenrock*[1,4], Ashley Olson[3,4], Michael Leonardo[3,4], Leona Gagnon[3,4], Vivek Philip[3,4], Elissa Chesler[3,4], Lisa Tarantino[1,2,4] 1) Department of Genetics, School of Medicine, University of North Carolina, Chapel Hill, NC; 2) Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC; 3) The Jackson Laboratory, Bar Harbor, ME; 4) Center for Systems Neurogenetics of Addiction.

Recent data suggests that cocaine use and overdose deaths due to cocaine have risen appreciably in the last several years in the US. However, effective treatment options for cocaine use disorders remain extremely limited. Human twin studies support a high heritability for the development of cocaine dependence (h2~0.65) indicating a significant role for genetics.
The Center for Systems Neurogenetics of Addiction (CSNA) was formed with the goal of identifying genetic and biological relationships between stages and patterns of cocaine addiction and behaviors that predict drug abuse. The CSNA uses a systems genetics approach that takes advantage of genetically diverse yet tractable rodent populations, the Collaborative Cross (CC) and Diversity Outbred (DO) and their eight inbred founder strains (129S1/SvImJ, A/J, CAST/EiJ, C57BL/6J, NOD/ShiLtJ, NZO/HlLtJ, PWK/PhJ and WSB/EiJ). The CC and DO were designed to more accurately model the heterogeneous genetic background present in the human population. Genetic diversity is expanded in these populations compared to traditional mouse resources, thereby enhancing the ability to capture a broader range of phenotypes and identify causal genetic variants.
As part of the CSNA, we are examining initial locomotor sensitivity and behavioral sensitization to cocaine. We will present data from the eight CC/DO founders showing significant strain, sex and strain by sex differences for many of our behavioral phenotypes with heritabilities ranging from 0.15 to 0.5. We will also present preliminary QTL mapping data generated from the DO population in which we identified regions on Chrs 2, 6, 14 and 18 that are associated with initial sensitivity and behavioral sensitization to cocaine. We will also highlight several CC strains with extreme behavioral phenotypes and discuss work we are doing to examine underlying mechanisms, including pharmacokinetics and the monoaminergic systems, that may contribute to the unique cocaine behavioral phenotypes in these strains.

164 the genetic architecture of insulin secretion Mark P. Keller[1], Mary E. Rabaglia[1], Kathryn L. Schueler[1], Donnie S. Stapleton[1], Daniel M. Gatti[2], Matthew Vincent[2], Kelly A. Mitok[1], Ziyue Wang[3], Takanao Ishimura[2], Shane P. Simonett[1], Christopher H. Emfinger[1], Rahul Das[1], Tim Beck[3], Christina Kendziorski[4], Karl W. Broman[4], Brian S. Yandell[5], Gary A. Churchill[2], Alan D. Attie[1] 1) University of Wisconsin-Madison, Biochemistry Department; 2) The Jackson Laboratory; 3) University of Leicester, Department of Genetics; 4) University of Wisconsin-Madison, Department of Biostatistics and Medical Informatics; 5) University of Wisconsin-Madison, Department of Horticulture.

Genetic susceptibility to type 2 diabetes primarily affects β-cell function. However, a genetic study to directly interrogate β-cell function *ex vivo*has previously never been done. We isolated 233,447 islets from 483 Diversity Outbred (DO) mice maintained on a Western-style diet, and measured insulin secretion in response to a variety of secretagogues. Insulin secretion from DO islets ranged >1,000-fold even though none of the mice were diabetic. The insulin secretory response to each secretagogue had a unique genetic architecture; some of the loci were specific for one condition, whereas others overlapped. In parallel, we performed RNA-seq on the DO islets, enabling us to identify gene regulatory loci and nominate gene candidates that mediate insulin secretion differences among the mice. Human loci that are syntenic to the insulin secretion QTL from mouse are also associated with diabetes-related SNPs in human GWAS. We report on three genes, *Ptpn18*, *Hunk*and *Zfp148*, where the phenotype predictions from the genetic screen were fulfilled in transgenic mouse models. Our study provides a picture of the interplay between genetic variation, insulin secretion, and gene regulation in pancreatic islets. By merging our studies in mouse with diabetes GWAS in human, we can enhance the identification of gene drivers, and connect genetic variants to target genes and their influence on β-cell function.

165 Controlling phenotypic variability and reproducibility through characterization and stable control of the microbiome in mouse models. *James Amos-Landgraf*[1], Aaron Ericsson[1], Elizabeth Bryda[1], Craig Franklin[1] 1) University of Missouri.

Many mammalian models exhibit highly variable phenotypes between investigators and institutions, often resulting in irreproducible results between laboratories. While great strides have been made to control genetic variation in inbred and genetically modified lines, the transfer of animal models to alternate laboratories and institutions around the globe with variable levels of biosecurity has created animals that have highly variable microbiomes that can dramatically influence the phenotype. The Mutant Mouse Resource and Research Center at the University of Missouri has investigated the microbiome and its role in established mammalian genetic models of disease. The MU MMRRC characterized fecal samples from all of the donated mice over the last 5 years and found highly variable microbiomes with dramatically variable presence and relative abundances of microbial taxa. To aid in our understanding of the impact of variable microbiomes on various genetic models we have developed four colonies of surrogate dams that each harbor distinct complex microbiomes resembling those found in four major mouse distributors. We have used these populations to rederive six mouse strains and models to characterized the impact on the previous published phenotypes. The models include the *IL10* knockout on two different genetic backgrounds, *Apc*^{Min}, NOD, the BTBR behavior model, and the DSS induced model of colitis. Each of these models show phenotypic variability associated with changes of the microbiome. To begin to resolve the causative bacteria that may be driving the phenotypic variability we have developed methods for the anaerobic growth of several bacterial species and administration and colonization of mouse populations that harbor existing complex microbiomes, and show changes in the phenotypic outcome. Additionally, we have explored the use of metabolomics to identify bacterially and host derived metabolites that reflect differences in the microbiome. This has helped identify new host genetic variants that reflect changes in both the bacterial populations and the phenotype. The ultimate goal of the MMRRC is to provide researchers with reproducible models that with stable phenotypes, through cryoarchiving, genetic and metagenomic characterization, reanimation

and distribution of mouse models with appropriate microbiomes.

Thursday, April 23 3:45 PM - 5:45 PM

New Technologies and Resources (Zebrafish)

179 Optimization of a high-throughput platform for the morphological and behavioral characterization of zebrafish larvae Alexandra Colón-Rodríguez[1], KaeChandra B. Weyenberg[1], José M. Uribe Salazar[1], Alejandra Quezada[1,2], Brittany Radke[1], Aditya Sriram[1], Emily Jao[1], Pamela Lein[3,4], Megan Y. Dennis[1,4] 1) Genome Center and Department of Biochemistry & Molecular Medicine, School of Medicine, University of California, Davis, CA; 2) MIND Institute, School of Medicine, University of California, Davis, CA; 3) Sacramento State RISE Program, California State University, Sacramento, CA; 4) Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA.

In recent years, zebrafish have become commonly used as a model for studying human traits and disorders. Their high fecundity and rapid development allow for more robust, high-throughput experiments compared to other vertebrate models. Given that zebrafish share >70% gene homologs with humans and benefit from highly efficient CRISPR editing, we are now able to rapidly generate mutations impacting practically any gene of interest. Unfortunately, our ability to phenotype mutant larvae has not kept pace. To address this challenge, we seek to develop a protocol that obtains multiple phenotypic measurements of individual zebrafish larvae in a vastly parallel fashion. We propose measuring morphological features (e.g., body length, eye area) using the Vertebrate Automated Screening Technology (VAST) system and movement/behavior using the DanioVision Observation Chamber. By assaying thousands of wildtype zebrafish and testing a variety of conditions, we determined optimal parameters that allow morphological imaging of larvae at two time points (3 days post fertilization (dpf) and 5 dpf) coupled with motion tracking of behavior at 5 dpf that avoids developmental defects or physical damage. As a proof-of-principle, we tested our approach on a novel CRISPR-generated mutant zebrafish line carrying a null-allele of *syngap1b*, ortholog to human *SYNGAP1* implicated in autism spectrum disorder, intellectual disability, and epilepsy. Previously published results show that morpholino knockdown of *syngap1b* leads to seizure-like traits and neurodevelopmental defects in larvae. Using our optimized high-throughput phenotyping protocol, we recapitulated these results. In particular, we observed significantly increased seizure-like activity in both heterozygous and homozygous mutant larvae (5 dpf) compared to wildtype siblings in the presences of GABA-antagonist pentylenetetrazol. Additionally, larvae exhibited significantly increased eye size (5 dpf) suggesting neurodevelopmental defects, though this effect was not previously reported in morphants. Ongoing work includes improving the automated scoring of VAST images and incorporating transgenic reporter lines to expand our repertoire of phenotypic measures. In summary, we have developed an effective rapid parallel phenotyping pipeline to characterize morphological and behavioral features of individual larvae in a robust and consistent fashion, thereby improving our ability to better pinpoint genes important in human traits and disorders.

180 NTR 2.0: an improved nitroreductase targeted cell ablation system Abby Sharrock[1], Tim Mulligan[2], Kelsi Hall[1], David White[2], Frazer Matthews[2], Makeila Williams[2], Elsie Williams[1], Selena Washington[2], Katherina Le[2], Danielle Meir-Levi[2], Meera Saxena[2], David Ackerley[1], *Jeff Mumm*[2] 1) Johns Hopkins University, Baltimore, MD; 2) Victoria University, Wellington, New Zealand.

The bacterial prodrug-converting enzyme nitroreductase (NTR) enables genetically directed, cell-specific ablation. In regenerative species such as zebrafish, the NTR/prodrug system has facilitated an expansion in cellular regeneration paradigms. The first enzyme adapted to model systems for targeted cell ablation is encoded by the *E. coli NfsB* gene (*NfsB_Ec*). Unfortunately, high prodrug concentrations, approaching general toxicity (e.g. 10 mM metronidazole), are required to achieve effective cell death with the original “NTR 1.0”. This limits the time window available to evaluate cell function and precludes chronic degenerative disease modeling. Moreover, NTR-resistant cell types have been reported which fail to ablate even after prolonged high concentration prodrug exposures. To overcome these limitations, we cloned NTR homologs from different bacterial species and screened for improved prodrug activity in *E. coli* and human cell lines. Top performers underwent directed evolution and structure-guided mutagenesis to identify variants with further enhancements in prodrug conversion efficiency. A mutant of the *NfsB* gene from *Vibrio vulnificus* (*NfsB_Vv*) was identified that exhibits a ≥100-fold improvement in prodrug activity. Transgenic zebrafish expressing the *NfsB_Vv* double mutant, aka “NTR 2.0”, confirmed improved activity, undergoing robust cell ablation after a 24 hr, 100 μM Mtz exposure. Tests across different cell types showed effective cell loss at Mtz concentrations ranging from 10 to 200 μM, including formerly resistant cell types. As zebrafish showed no deleterious effects from Mtz exposures of ≤1 mM over more than one month, NTR 2.0 supports sustained cell loss paradigms useful for interrogating cell function and mimicking degenerative disease states. Intriguingly, preliminary evidence suggests that NTR-mediated ablation proceeds through DNA damage related cell death pathways relevant to neurodegenerative disease. The potential for creating inducible, titratable, cell-specific degenerative disease models, in addition to enhanced targeted ablation efficacy, suggests NTR 2.0 will be a broadly useful tool for studies of cell function, cellular regeneration, and for physiologically modeling degenerative disease states.

181 A Comparison of CRISPR/Cas9-Based Methods for Creating Amino Acid Substitutions in Zebrafish. *Yvonne Rosario*[1], Chon-Hwa Tsai-Morris[1], James Iben[1], William Belden[2], Joe Zoeller[1], Steven Coon[1], Benjamin Feldman[1] 1) NIH/NICHD; 2) Dep. of Animal Sciences, RutgersUniversity.

We have combined a comparison of efficiencies of CRISPR-based strategies for generating single amino-acid (AA) changes with the goal of developing zebrafish disease models at several loci with orthology to human genes under investigation at NIH labs. Precise genome edits are generated at relatively lower rates than standard disruptions and it has been established that using gRNAs that cut with high efficiency positively impacts incorporation of sequence from co-injected DNA templates. Nineteen gRNAs were custom-ordered from IDT, microinjected along with commercially available Cas9 or Cpf1 proteins and evaluated for their ability to induce double-strand breaks (DSBs) within close proximity of our targeted codons. Seven of the 19 gRNAs had cutting scores higher than 95%. An eighth gRNA had a lower, but acceptable, cutting score. We next tested several precise editing strategies in different combinations with these eight gRNAs. These precise editing strategies included: basic homology directed repair (HDR) using Cas9 or Cpf1 and single-stranded oligo templates, chemically enhanced HDR with Cas9 and single-stranded oligo templates, Cas9 with double-stranded DNA templates[1], and a Cas9-streptavidin-biotin-oligo complex[2]. All method-locus pairs were microinjected into embryos in four biological reps and harvested for genomic DNA. These DNAs were then PCR-amplified per locus, barcoded per method or control, and pooled again for NextGen sequencing analysis of precise edit frequencies. Increased rates were observed for incorporation of oligo template-specific information in certain interventions, but this was complicated by the fact that most reads also included undesired alterations.

[1]A Wesley *et al.*, bioRxiv 431627

[2]M. Ma *et al.*, *Cell Res* **27,** 578–581 (2017)

182 pGTAG and pPRISM: Two expanded tool sets for using short regions of homology for precise DNA integration at CRISPR/Cas9 cut sites *Jeffrey Essner*[1], Sekhar Kambakam[1], Jordan Welker[1], Cassandra Bullard[2], Wesley Wierson[1], Maira Almeida[1], Caroline Sabotta[1], Darius Balciunas[3], Stephen Ekker[2], Karl Clark[2], Maura McGrail[1] 1) Iowa State University, Ames IA; 2) Mayo Clinic, Rochester MN; 3) Temple University, Philadelphia PA.

We present a methodology for targeted integration into the zebrafish genome with diverse targeting cassettes for generation of fluorescence-selectable alleles. Prior work has utilized homology directed repair for creating knock-ins in zebrafish at sites of directed double-strand DNA breaks. However, the frequencies of precise integrations using these methods are relatively low, which makes recovery of desired events difficult. Here, we build on the method first published by Hisano et al., 2015 using short regions of homology for precise DNA integration by introducing two new suites of donor vectors for creating knock-in alleles: pGTAG, plasmids for precise Gene TAGing for vector-free, multicolor tagging; and pPRISM, plasmids for PRecise Integration with Secondary Markers to drive precise integration with multiple options for secondary fluorescent reporters at the tagged site. These new suites of vectors contain gRNA sites designed for liberation of the reporter gene cassette and type IIS restriction enzyme sites for ease of cloning homology arms. The pGTAG cassette series enables integration of 2A-fusion tags of RFP, GFP, or Gal4/VP16 to disrupt gene function and provide a fluorescent report. The pPRISM cassette contains cargo for integration of a 2A-RFP or a premature stop codon and a secondary marker in one of six colors to track the targeted allele, even when the locus of interest is expressed at a low level. Based on fluorescence expression, integration efficiencies for both vector suite series were between 20 to 70% of the injected F0s. Moreover, 27% of F0s selected by fluorescence transmit a precise integration event of the cassette in the targeted gene. We anticipate these expanded vector suites and our methods for in vivo functional studies will expand and diversify the utility of homology directed gene editing.

NIH R24 OD020166

183 Chemoptogenetic Induction of Neuronal Mitochondrial Damage *in vivo* *Binxuan Jiao*[1,2], Wenting Xie[1,2], Qing Bai[1], Vladimir Ilin[1], Claudette St. Croix[1], Simon Watkins[1], Bennet Van Houten[1,4], Marcel Bruchez[3], Edward A. Burton[1,5] 1) University of Pittsburgh, Pittsburgh, PA, USA; 2) Tsinghua University, Beijing, China; 3) Carnegie Mellon University, Pittsburgh, PA, USA; 4) UPMC Hillman Cancer Center, Pittsburgh, PA, USA; 5) Pittsburgh VA Healthcare System, Pittsburgh, PA, USA.

Mitochondrial dysfunction has been observed in several neurodegenerative diseases including Parkinson's disease (PD). In order to investigate the biochemical and cellular consequences of mitochondrial damage in neurons, we have developed transgenic zebrafish that produce singlet oxygen in neuronal mitochondria on exposure to far-red light. Since the effective range of highly-reactive and short-lived $^1O_2$ is extremely small, this results in selective mitochondrial oxidative damage with remarkable spatial resolution, the severity of which is dictated by light dose. Following exposure to far-red light, we observed neuronal mitochondrial swelling and loss of cristae, and decreased whole-animal respiration and ATP levels. Loss of mitochondrial function and bioenergetic collapse resulted in neuronal depolarization, acute neurobehavioral deficits and eventually cell death. In addition to providing a new tool for ablating neuronal circuitry *in vivo*, this approach will enable us to elucidate the mechanisms whereby less severe oxidative mitochondrial damage initiates and mediates neurodegeneration. We are currently deploying this novel technology in dopaminergic neurons to investigate the pathophysiology of PD.

184 Defining zebrafish oogonial stem cells and their somatic cell niche at single-cell resolution Yulong Liu[1], Matthew E. McFaul[1], Michelle E. Kossack[1], Stefan Siebert[1], Samuel Horst[1], Jack F. Cazet[1], Celina E. Juliano[1], Bruce W. Draper[1] 1) University of California, Davis.

Nanos2 is a conserved RNA-binding protein that has been shown to be expressed in the germline stem cells (GSCs) of many vertebrates. Previous studies have shown the Nanos2 function is crucial for GSC maintenance in male mice. Unlike mice, were GSCs are only present in males, there is ample evidence in teleost, such as zebrafish and medaka, that GSCs are present in both adult males and females. Importantly, our lab has previously identified Nanos2 as a probable GSC-specific gene in zebrafish. Furthermore, *nanos2* mutant larval fish have germ cells but mutant adults are sterile, consistent with the hypothesis that Nanos2 is required for GSC specification and/or maintenance. To further this analysis, we have performed single-cell RNA sequencing (scRNA-seq) on germ cells purified from 40-day post-fertilization ovaries to comprehensively characterize the gene expression profile of all stages of early ovarian germ cells- from *nanos2+* oogonial stem cells to early differentiating oocytes. In addition, we have also profiled the expression of somatic ovary cells with one goal to identify the cells that constitute the oogonial stem cell niche. To date we have profiled 10,658 germ cells and 13,965 somatic cells. Our germ cell data set has identified oogonial stem cells, mitotic oocyte progenitor cells, cells that are progressing thought meiotic prophase I and cells that have arrested in diakinesis and initiated oogenesis (Stage 1A oocytes). For each stage we have identified stage-specific markers, which include new markers for oogonial stem cells as well as their immediate descendants, the oocyte progenitor cells. Our somatic cell data set has identified the three major cell types of the ovary, the follicle cells, the steroidogenic theca cells and the ovarian stomal cells. To our surprise we have discovered that the follicle and stromal cells are not composed of uniform cell populations but instead can be divided into several subpopulations based on their gene expression profiles and their unique localization within the ovary. We are particularly interested in the stomal cell population, as it is the most heterogeneous and the least well characterized of the major somatic cell populations. We have so far determined that stromal cells can be divided into at least five distinct cell populations and have identified marker genes for each. Interestingly, we have found that one stromal cell subpopulation localizes to a discrete domain on the lateral surface of the ovary that we have previously hypothesized was the location of the oogonial stem cell niche. Thus, our cell-type resolution analysis has identified candidate specialized somatic cell populations that likely play important roles in maintaining and regulating oogonial stem cells in a vertebrate.

185 scRNAseq developmental trajectories to investigate differentiation *Jeffrey Farrell*[1], Yiqun Wang[2,3], Stefan Siebert[4], Bushra Raj[2], Celina Juliano[4], Alexander Schier[2,3] 1) National Institute of Child and Human Development, NIH, Bethesda, MD; 2) Harvard University, Cambridge, MA; 3) Biozentrum, Basel, Switzerland; 4) University of California Davis, Davis, CA.

A crucial quest in developmental biology is to understand the sequence of gene expression in each cell type as it is specified and differentiates and to understand how those genes function to instruct each cell's role and its morphological characteristics. To uncover such cascades of gene expression from single-cell RNA sequencing data, we previously developed the computational approach URD. URD is a semi-supervised approach that uses biased random walks through transcriptomic data to build a branching tree of the paths cells take in gene expression as they develop. I will discuss how we have applied this approach to identify the developmental trajectories and gene expression cascades during early zebrafish embryogenesis (from zygotic genome activation to early somitogenesis), retinal and hypothalamic differentiation in zebrafish, and homeostatic differentiation

in adult *Hydra*. We are now focused on using these data to identify gene expression programs that prepare for and execute cellular remodeling during differentiation. Our initial efforts have focused on understanding the organization of the gene expression cascades within the notochord and prechordal plate, two cell types that share a common progenitor. Both cell types become highly secretory during differentiation and prepare themselves for this cellular stress in advance. We find that these cell types employ a core transcriptional program (the unfolded protein response), but each cell type uses specific regulators to customize this response for its particular secretory needs.

186 Zebrafish CRISPR screening validates and classifies a set of novel candidate genes for human congenital heart defects Kylie Kerker[1,2], Gist H. Farr III[1], Whitaker Reed[1,2], David R. Beier[1,2], *Lisa Maves*[1,2] 1) Seattle Children's Research Institute; 2) University of Washington.

Congenital heart defects (CHDs) are the leading cause of infant death due to birth defects. In spite of exceptional efforts, exome sequencing studies of CHD patients have thus far accounted for only about 20% of the genetic contribution to CHDs. A major hurdle that remains for understanding the causes of CHDs is the identification and validation of the human CHD genes that are as yet unknown. To address this hurdle, we are taking a novel approach to identify new CHD-candidate genes. We identified human haploinsufficient genes from the ExAC database and used single-cell sequencing data to determine which of these genes are expressed during early mammalian heart development. This process revealed a novel set of 233 human genes that are outstanding candidates for roles in human heart development and CHDs. We are undertaking a CRISPR screening approach in zebrafish to validate and characterize the functions of this novel set of CHD-candidate genes in heart development in a high-throughput manner. In a pilot screen of 22 genes analyzed thus far, we found that 8/22 genes exhibited defects in heart development in G0 CRISPR zebrafish embryos. The heart defects observed fall into 3 classes: cardia bifida (severe class, 2 genes), heart looping defects (intermediate class, 5 genes), and blood pooling (1 gene). Our results show that we can efficiently generate heart-defect-related phenotypes by CRISPR in G0 zebrafish embryos. Our results also show that we can identify novel CHD genes and support our hypothesis that CHD genes will fall within phenotypic classes. We predict that these approaches will allow classification and mechanistic understanding of novel human CHD genes. Through an effort that integrates human, mouse, and zebrafish analysis, we may potentially characterize a significant fraction of the remaining unknown genetic contribution to CHDs.

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Cell Fate and Patterning (*Xenopus*)

172 Deep cytoplasmic sorting during *Xenopus* oocyte-to-embryo transition Hyojeong Hwang[1], Jia Fu[1], Wenyan Mei[1], *Jing Yang*[1] 1) University of Illinois.

In many species, the zygotic genome is quiescent following fertilization. The genetic program responsible for early embryonic development is executed by RNAs and proteins that are maternally deposited in the oocyte, completely independent of transcriptional regulation. It is currently unclear how maternal factors are stored in the oocyte in an inactive state, but become remodeled and activated during the oocyte-to-embryo transition (OET). We recently observed that in *Xenopus* oocyte, mRNAs encoding proteasome components are present in a gradient, with highest levels in the animal pole and the trailing end extending into the vegetal hemisphere. During the OET, proteasome mRNAs undergo a vegetal-to-animal translocation. As a result, the proteasome system, previously thought to be ubiquitiously expressed and serve “house-keeping” roles inside the cell, becomes highly enriched in the animal hemisphere of ovulated egg and early embryos. Our results further reveal that relocalization of the proteasome system during the OET is a prerequisite for embryonic germline development.
Here we report that the proteasome dynamics during the OET relies on several novel cellular and molecular mechanisms, which we collectively refer to it as “deep cytoplasmic sorting”. We show that in the oocyte, a significant amount of proteasome mRNAs are associated with the endoplasmic reticulum (ER) that is located around the germinal vesicle and in the animal cortex. ER-asociation of proteasome mRNAs is mediated by RNA-binding proteins such as TIS11B, which has the ability to form large reticular meshwork that is interwinded with the ER. Intriguingly, during oocyte maturation, proteasome mRNAs detach from the ER and are actively transported toward the animal hemisphere. Meanwhile, ER is remodeled into a fine tubular network distributed in the entire animal hemisphere, forming a “maze”-like structure that prevents vegetal diffusion of proteasome mRNAs. Interfering with the ER tubular network formation by enucleation or treatment of F-actin inhibitors impairs the proteasome dynamics during the OET. Based on these observations, we conclude that deep cytoplasmic sorting during the OET is critically important for proper localization of maternal factors and the remodeling of the proteasome, ER, and perhaps other organelles/cellular machineries in the oocyte. Our work thus provides novel mechanistic insights into the storage and remodeling/activation of maternal factors during the OET.

173 A Spatial Gradient of Cell Size Controls Genome Activation and Contributes to Vertebrate Early Development *Wenchao Qian*[1], Hui Chen[1], Matthew Good[1, 2] 1) Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2) Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA.

Zygotic genome activation (ZGA) is a process in embryonic cells that awakens and begins transcribing thousands of zygotic transcripts. ZGA marks the maternal-to-zygotic transition in which development switches from maternal to zygotic control. ZGA is essential for subsequent gastrulation and germ-layer specification, but how ZGA is triggered in vertebrate embryogenesis is poorly understood. Through metabolically labeling the nascent transcripts and wholemount imaging, our lab has found that in *Xenopus* blastula embryos, genome activation correlates with cells achieving a threshold size, due to blastomere division in the absence of growth. We identified a highly stereotyped spatial and temporal pattern of ZGA that occurs initially in small cells at the animal pole and is delayed by two hours in cells of the vegetal pole. This spatiotemporal pattern tightly correlates with the gradient of cell sizes present in the blastula embryo, which induced by the difference in cell division timings. Although the gradient of cell sizes is evolutionally conserved in lamprey and amphibian species, less is known about its role in regulating the early vertebrate embryogenesis. To understand the biological significance of the gradient of cell sizes and the resulting pattern of transcriptional onset, we have developed an embryo temperature controller to eliminate or reverse the gradients. It works by slowing down the division rate of fast-dividing cells in the animal pole and speeding up the division of slow dividing vegetal cells. By generating *Xenopus* blastula embryos with reversed cell size gradients, we found that the spatial pattern of ZGA was also reversed; genome activation occurred first in the vegetal pole and was delayed in the animal pole. Those ‘reversed’ embryos were arrested in gastrulation when they failed to close the blastopore. Intriguingly, those arrested embryos still try to closure neural fold, and the conflict of the direction of cell movement in these two events may explain the developmental arrest observed in those embryos. Moreover, with the treatment of a less extreme temperature gradient, portions of embryos showed defects on head induction and gut formation at the

tadpole stage, perhaps the animal pole with transcriptional delay missed an important developmental window for responding to inductive signals which are originated in the vegetal pole. This work suggests that regulated spatial patterns of ZGA are important for coordinated embryonic development, particularly in blastula embryos that contain gradients of cell sizes.

174 Functional analysis of Noggin-like genes *Prashath Karunaraj*[1,2], Peter Dearden[2], Andrew Cridge[2], Caroline Beck[1] 1) Dept. of Zoology, University of Otago, Dunedin, New Zealand; 2) Dept. of Biochemistry, University of Otago, Dunedin, New Zealand.

TGFb is one of seven key signalling pathways that determine the cell fate in metazoan organisms. A number of extracellular antagonists are known to regulate the TGFb signalling pathway. One of these antagonists is Noggin, a cysteine knot family protein, well-known for its role in dorsal-ventral patterning in vertebrates. Earlier studies have shown that a conserved "CLIP" domain at the N terminus of this protein is crucial for binding to and inhibit BMPs. Orthologs of noggins (Noggin-like) have also been identified in the polychaete *Capitella*, planarian *Schmidtea mediterranea*, and aphid *Acyrothosiphon pisum*. When expressed in *Xenopus* embryos, planarian noggins have the expected dorsalizing function, but planarian noggin-like proteins generate opposite phenotypes, ventralized embryos[1]. Interestingly, sequence analysis revealed that noggin-like genes share a common origin with arthropod trunk and PTTH, both of which activate RTK pathway[2]. The question arises here that how these similar and related proteins have coupled themselves to different pathways. To understand this, we focused on the functional analysis of *L. gigantea* noggins, *A. pisum* noggin-like (ApNL) genes and *D. melanogaster* trunk using overexpression in *Xenopus* embryos and animal cap assays. Our findings suggest that ApNL1 has an expected dorsalizing function when ectopically expressed, but surprisingly also induces endoderm and mesoderm in animal caps. Deletion of the CLIP domain in ApNL1 also results in dorsalized embryos and endoderm induction in animal caps. However, ectopic expression of ApNL2 produces lower percentage of ventralized embryos, but fails to induce endoderm or mesoderm in animal caps. Introduction of the CLIP domain into ApNL2 results in a low percentage of dorsalized embryos. Both *L. gigantea* noggins develop dorsalized embryos and show endoderm induction. Fusion of CLIP domain to DmTrk results in dorsalized embryos, but surprisingly wild type DmTrk also induces mesoderm and endoderm. Gastrulation defects are commonly observed with ectopic expression of these genes. Our results suggest ApNL1 and DmTrk are closely related to each other, and also have a dual role: inducing both mesodermal and endodermal tissues.

Keywords: Transforming Growth Factor, antagonists, Noggin, dorsal-ventral patterning

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Genomics/Systems Biology and Gene Regulation (*Drosophila*)

155 OVO-B, but not OVO-A, is required for female germ cell viability and has downstream targets in addition to *otu* in the female germline *Leif Benner*[1,2], Brian Oliver[1] 1) Section of Developmental Genomics, Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; 2) Department of Biology, Johns Hopkins University, Baltimore, MD.

Sexual reproduction has evolved as a fundamental mechanism in biology and its implementation is essential to the propagation of life. Each sex must initiate a genetic program instructing the germline to differentiate into its sex-specific gamete. Even though this is an important process, we have little insight into how these sex-specific genetic programs are established. One candidate for the establishment of sex-specific genetic programs is the transcription factor OVO. OVO encodes two antagonistic isoforms, the repressor OVO-A and activator OVO-B and is required for female germline viability in *Drosophila*. OVO-B activates the expression of *otu* and in turn ensures female specific splicing of *Sxl*, which is necessary for female germline differentiation. We tested if supplementing OTU or SXL in the absence of OVO-B would rescue female germline defects. In order to accomplish this, we placed a GAL4-3xSTOP coding sequence into the OVO-B reading frame which should express GAL4 under *ovo-B* regulatory sequences and result in an *ovo*$^{-}$ allele (*ovo*$^{ovo\text{-}B\text{-}Gal4}$). We surprisingly found that homozygous *ovo*$^{ovo\text{-}B\text{-}Gal4}$ female germ cells survived but had an arrested oogenesis phenotype. Furthermore, the expression of OTU or SXL failed to relieve the arrested oogenesis phenotype in *ovo*$^{ovo\text{-}B\text{-}Gal4}$ females. Therefore, OVO-B must activate the transcription of other genes outside of *otu* and *Sxl* that are necessary for female germline differentiation. Female germ cell survival in *ovo*$^{ovo\text{-}B\text{-}Gal4}$ flies was unexpected since classical *ovo*$^{-}$ alleles result in female germ cell death. We decided to delete the promoters of both *ovo-A* and *ovo-B* and determine the germ cell phenotype. Female germ cells lacking OVO-A had no discernible phenotype, however, germ cells lacking OVO-B failed to survive to adulthood. This indicates that female germ cells do need OVO-B, but not OVO-A, for survival. Also, the current annotation of *ovo* isoforms is incomplete since *ovo*$^{ovo\text{-}B\text{-}Gal4}$ germ cells were viable instead of the expected death phenotype observed in germ cells lacking *ovo-B*. We have also tagged OVO-A and OVO-B and find that both isoforms localize to germ cell nuclei in females. OVO-A localization is restricted to specific regions of the germarium while OVO-B localizes to all germ cell nuclei. ChIP-seq will be performed on these tagged versions in order to measure OVO-A and OVO-B occupancy genome-wide and determine which genes OVO regulates for female germ cell survival and differentiation.

156 Genome activation and transcriptome diversity: A dual sex-specific role for the *Drosophila* Clamp protein in splicing and transcription during early embryonic development *Mukulika Ray*[1], Ashley Conard[1], Erica Larschan[1] 1) Molecular Biology Cell Biology and Biochemistry Department, Brown University, Providence, RI, USA.

Chromatin **l**inked **a**dapter for **M**SL **p**rotein (CLAMP) is a DNA binding protein associated mainly with an open chromatin environment. First discovered as recruiter of **M**ale **s**ex **l**ethal (MSL) dosage compensation complex to the male X-chromosome in *Drosophila*, CLAMP is unlike most other members of this complex in being abundantly present on all chromosomes in both the sexes. Evolutionarily, it is more ancient than MSL complex and binds to both promoters and introns. Therefore, it is likely that CLAMP has an ancient role and was co-opted by MSL complex upon the evolution of dosage compensation. Also, many MSL binding sites evolved from intron-exon boundaries. To determine this ancient role for CLAMP, we identified CLAMP interacting proteins in both the sexes. We found that CLAMP binds to a large number of proteins, on and off chromatin, in a sex-specific manner, the largest class being that of RNA binding proteins. In this study, we found CLAMP binding to many RNA spliceosome components such as Hrb27C, MLE (also an MSL component), and Squid. Interestingly, the interaction between CLAMP and Squid occurs specifically in females and that between CLAMP and MLE occurs in males as part of MSL complex. Since both Squid and MLE are known to influence sex-specific alternative splicing, we hypothesized that CLAMP specifically recruits these two proteins to regulate sex-specific splicing that drives sex determina-

tion. Indeed, our analysis of RNA splicing in early developing embryo showed that CLAMP regulates sex-specific splicing including that of *Sex lethal*, initiator of sex determination pathway. Next, we measured how CLAMP regulates Squid and MLE recruitment genome-wide on chromatin before and after zygotic genome activation in both the sexes using Cut-and-Run. We have integrated this data with iCLIP for the CLAMP, Squid and MLE to identify RNA transcripts that are interacting with one or multiple proteins. By integrating iCLIP and Cut-and-Run, we determined genes where CLAMP binds to the chromatin as well as transcripts they produce to decipher mechanism of transcription coupled with RNA processing during very early development to drive sex determination. Overall, our data suggest the ancient role of CLAMP in sex-specific splicing was co-opted by the dosage compensation machinery to couple dosage compensation with sex-determination as MSL binding sites evolved from intron-exon junctions.

157 Tissue-specific chromatin occupancy by the pioneer factor Zelda in *Drosophila melanogaster* *Elizabeth Larson*[1], Hideyuki Komori[2], Tyler Gibson[1], Danielle Hamm[1], Cheng-Yu Lee[2], Melissa Harrison[1] 1) Department of Biocmolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, WI; 2) Department of Cell and Developmental Biology and Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Cellular reprogramming is controlled by a set of specialized transcription factors. These reprogramming factors possess specific qualities that define them as pioneer transcription factors: the ability to bind nucleosomal DNA, establish accessible chromatin domains and facilitate the binding of other transcription factors. During early *Drosophila* embryogenesis, Zelda functions as a pioneer factor to drive the dramatic reprogramming that is required immediately following fertilization to remodel the zygotic transcriptome. In addition to its essential role during the initial stages of embryogenesis, Zelda is also required later in development. To determine how development shapes the ability of a pioneer factor to drive reprogramming, we identified a role for Zelda in neural stem cells. Like other stem cells, the neural stem cells divide asymmetrically, creating an identical daughter cell and a partially differentiated progeny. We demonstrated that misexpression of Zelda in the partially differentiated progeny can reprogram these cells to a stem cell fate. Thus, Zelda is able to access the genome and drive transcription of factors required for stem cell fate self-renewal. Given the pioneering qualities of Zelda in the early embryo and the ability of Zelda to reprogram cell fate in both the embryo and larval brain, we hypothesized that Zelda would occupy the same set of genomic loci in both the totipotent cells of the early embryo and in the pluripotent neural stem cells. As expected, ChIP-seq identified thousands of loci that are bound by Zelda both in the embryo and in the stem cells, including the regulatory region of the stem cell self-renewal factor Deadpan. Contrary to expectations, we identified many more loci that were uniquely bound in either the embryo or the neural stem cells. Zelda binding in the early embryo is driven largely by DNA sequence, but Zelda-bound regions specific to the neural stem cells were not enriched for the canonical Zelda-binding motif. Instead, sites uniquely bound by Zelda in the neural stem cells were enriched for promoter regions, which are generally accessible. Together these data demonstrate that binding of the pioneer factor Zelda is dependent on the tissue in which it binds and suggests that additional chromatin features or tissue-specific cofactors are instrumental in defining DNA binding by this pioneer factor.

158 Sex-dependent and sex-independent controls of size variation in natural populations *Hirokazu Okada*[1], Ryohei Yagi[1], Vincent Gardeux[2], Bart Deplancke [2], Ernst Hafen[1] 1) ETH Zurich; 2) Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.

Size of organs/organisms is a polygenic trait. Many of the growth-regulatory genes constitute conserved growth signaling pathways. However, how these multiple genes are orchestrated at the systems level to attain the natural variation in size including sexual size dimorphism is mostly unknown. Here we take a multi-layered systems omics approach to study size variation in the *Drosophila* wing. We show that expression levels of many critical growth regulators such as Wnt and TGFβ pathway components significantly differ between sexes but not between lines exhibiting size differences within each sex, suggesting a primary role of canonical growth regulators in sexual size dimorphism. We also show that cell size in the wing significantly differs between sexes but not between lines within the same sex, supporting the sex-dependent cell size regulation. In contrast, we find that between-line size variation is largely regulated by genes with a diverse range of cellular functions, most of which have never been implicated in growth. Only a few canonical growth genes including a receptor of steroid hormone ecdysone exhibit association with between-line size differences. Tissue compartment-specific RNAi knockdown of the novel genes validates their size-regulatory functions. In addition, we show that expression quantitative trait loci (eQTLs) linked to these novel growth regulators accurately predict population-wide, between-line wing size variation. In summary, our study unveils differential gene regulatory systems that control wing size variation between and within sexes.

159 A functional investigation of conserved cryptic peptides encoded by smORFs identifies two novel mitochondrial components *Justin Bosch*[1], Berrak Ugur[3], Israel Pichardo-Casas[1], Jorden Rabasco[1], Felipe Escobedo[1], Hugo Bellen[2,3], Ben Brown[4], Susan Celniker[4], Norbert Perrimon[1,2] 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute; 3) Baylor College of Medicine, Houston, TX; 4) Lawrence Berkeley National Laboratory, Berkeley, CA.

produced small peptides (<100 amino acids) are important regulators of physiology, development, and metabolism in diverse organisms. Recent studies have predicted that thousands of novel peptides may be translated from genes containing small open reading frames (smORFs). Yet this class of genes has been largely overlooked and uncharacterized due to their small size and questionable functional relevance. To address this knowledge gap, we identified a set of 184 small peptides with sequence conservation among diverse metazoan species. Based on this conservation, we predict that these peptides have a high likelihood of functional significance in humans. Using a combination of bioinformatic and genetic screening in *Drosophila melanogaster*, we identified two previously uncharacterized peptides in this dataset that are important for development and neuronal activity. Furthermore, analysis of the subcellular localization of these peptides, and their loss of function phenotypes, suggests they play role in mitochondrial function. We expect our ongoing phenotypic analysis of smORF genes in *Drosophila* to provide a wealth of information on the biological functions of this poorly characterized class of genes in humans.

160 Evolutionary conservation and divergence of 3D genome organization in *Drosophila* *Nicole Torosin*[1], Aparna Anand[1], Tirupathi Rao Golla[1], Weihuan Cao[1], Christopher Ellison[1] 1) Rutgers University, Piscataway, NJ.

Topologically associating domains, or TADs, are functional units that organize chromosomes into 3D structures of interacting chromatin in species ranging from Drosophila to humans. TADs play an important role in regulating gene expression by constraining enhancer-promoter contacts; there is evidence that deletion of TAD boundaries leads to aberrant expression of neighboring genes. While the mechanisms of TAD formation have been well-studied, current knowledge on the extent of TAD conservation across species is inconclusive. Due to the integral role TADs play in gene regulation, their structure and organization is expected to be conserved during evolution. One study reported conservation of TAD boundaries in rhesus macaque, dog, rabbit, and mouse genomes. Additionally, when comparing the human genome to twelve other vertebrate species, researchers found that breakpoints for evolutionary rearrangements are strongly clustered at TAD boundaries. However, more recent research suggests that

TAD structures diverge relatively rapidly. For example, only 43% of TADs are shared between humans and chimpanzees. We use Hi-C chromosome conformation capture to measure evolutionary conservation of whole TADs and TAD boundary elements between D. melanogaster and D. triauraria, two species which diverged ~28 million years ago. We found that significantly more boundary elements are conserved than domains between these two species. Our results show that TADs have been reorganized since the common ancestor of D. melanogaster and D. triauraria, yet the sequence elements that specify TAD boundaries remain highly conserved. We propose that evolutionary divergence in 3D genome organization results from shuffling of conserved boundary elements across chromosomes, breaking old TADs and creating new TAD architectures. This conclusion will be further evaluated by expanding the analysis to other Drosophila species in the future.

161 Transcribing loci in close proximity do not share a Pol II hub *Shao-Kuei Huang*[1], Chrus Rushlow[1] 1) New York University.

Pinpointing 3D distributions of RNA polymerase II (Pol-II) and its target genes is crucial for understanding transcriptional mechanisms in living cells. This is made possible with recent advancements in microscopy technologies that have acquired the resolution to detect protein-DNA and protein-protein interactions in the ~100 nm range. Genome activation in Drosophila begins at about one hour post fertilization when the pioneer factor Zelda strongly activates about 100 genes. Using Pol-II immunohistochemistry (IHC), we observed at least 50 speckles of strong staining at this stage. By combining IHC and RNA FISH, we observed co-localization of these speckles with early-expressed genes, prompting us to conclude that Pol-II speckles are transcriptional hubs at the early gene loci. This presents a unique opportunity to study transcriptional mechanisms in vivo because transcription happens sparsely in these embryos compared to later embryos even one hour older. A possible mechanism to modulate transcription could be for genes to share Pol-II machinery, therefore we asked whether genes in close proximity share Pol II speckles. With dual color FISH labeling active genes, we found the distance between two transcribing foci in nuclear cycle 12 is on average proportional to the chromosomal distance between them. In nuclear cycle 14 when thousands of genes are activated, the foci distances remain the same. However, occasionally, we observed distant loci come into close proximity, as previously seen in Hi-C data (Hug et al, 2017). Using Pol-II IHC and dual-color FISH, we observed separable Pol-ll speckles even when two transcribing loci are within 200 nm. These data suggest that regardless of the 3D distance between two active genes, they do not share Pol-II pools as a single hub at this stage. Future work will study the dynamics of this behavior and its biological effect on transcriptional output by live imaging Pol-II-GFP and endogenous genes labeled with MS2 or PP7.

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Disease Models and Aging (*Drosophila*)

147 Epithelial homeostatic mechanisms prevent tumorous overgrowth by causing the extrusion of RasV12 expressing clones Jamie L. Lahvic[1], Erika A. Kumar[1], Victor K. Yu[1], Iswar K. Hariharan[1] 1) University of California, Berkeley.

Epithelia have diverse mechanisms for eliminating unfit cell types, including tissue-inappropriate cells and over- or under-proliferating cells. These endogenous homeostatic processes may also eliminate pre-cancerous cells. We have observed that entire RasV12 clones extrude basally from the *Drosophila* wing epithelium, which matches observations seen in cell culture and mammalian models. This clonal extrusion is preceded by a constriction of the apical cytoskeleton, which may cause the loss of apical attachment of the clone. Specific molecular mechanisms in wild-type neighbor cells likely regulate this extrusion process. Clones more frequently extrude from the pouch and hinge of the wing disc, regions known to experience high rates of cell competition, than from the notum. Additionally, JNK signaling is activated surrounding some RasV12 clones, as indicated by an AP-1 reporter line and staining for the JNK target, MMP1. We have developed two large-scale genetic techniques to identify additional molecular mechanisms which regulate RasV12 clone extrusion in a cell non-autonomous manner. First, we are performing a functional RNAi screen to knock down genes in wild-type cells and assessing the effect on RasV12 clones and the epithelium as a whole. Second, we are using the synthetic-Notch Q system to label the immediate wild-type neighbors of RasV12 clones with the ultimate goal of assessing gene-expression changes in those cells. These experiments will help define the molecular mechanisms of a little-studied form of cell competition, RasV12 clonal extrusion. Importantly, many dangerous cancers begin as small clones of Ras overactive cells, and these experiments have the potential to identify novel methods of targeting these cancers, by enabling wild-type cells to directly eliminate tumor cells.

148 Polyploidy in the adult *Drosophila* brain *Shyama Nandakumar*[1], Olga Grushko[1], Laura Buttitta[1] 1) University of Michigan.

Recent discoveries suggest that neuronal aneuploidy and hyperploidy are more common than previously thought, and prevalent in many organisms. Increased ploidy in postmitotic neurons has also been associated with age-related cognitive decline and neurodegeneration. We recently discovered that under normal physiological conditions, cells in the ageing *Drosophila* brain exhibit cell cycle re-entry and polyploidy, making it an excellent system to study this process. We developed a sensitive flow-cytometric assay to quantify ploidy changes in individual brains and different cell types. Our work indicates that cell cycle re-entry occurs in multiple cell types in the brain, and increases with age. Polyploidy levels can be manipulated through genetic modulation of DNA replication licensing factors and increasing DNA damage leads to an earlier onset of polyploidy in the brain. We observe a constant rate of cell death with age with very little to no neurogenesis or mitoses, suggesting that the fly brain must cope with a significant loss of cells over the lifespan of the organism. Studies in other postmitotic tissues have demonstrated that cells can become polyploid in response to wounding and damage and perform a compensatory or beneficial function in tissues without a resident stem cell population. We are developing genetic tools that will serve as 'polyploidy sensors' to study this in various fly tissues. We hypothesize that neuronal polyploidy plays a protective role and compensates for the loss of cells in the fly brain. We are currently testing this in fly models of neurodegeneration.

149 A Drosophila model of Kras/Lkb1 tumorigenesis uncovers oncogenic Kras levels as a key determinate in malignant transformation Briana Rackley[1], Chang-Soo Seong[1], Evan Kiely[1], Manali Rupji[2], Bhakti Dwivedi[2], John M. Heddleston[3], William Giang[1,2], Neil Anthony[1,2], Teng-Leong Chew[3], *Melissa Gilbert-Ross*[1, 2] 1) Emory University School of Medicine, Atlanta, GA; 2) Winship Cancer Institute of Emory University, Atlanta, GA; 3) HHMI, Janelia Research Campus, Ashburn, VA.

The genetic and metabolic heterogeneity of RAS-driven cancers has confounded therapeutic strategies in the clinic. Emerging evidence suggests that co-occurring alterations in tumor suppressor genes can add to this complexity, due to both autonomous and non-autonomous effects on tumor

progression. To address this, rapid and genetically tractable animal models are needed that recapitulate the heterogeneity of RAS-driven cancers in vivo. Here, we generate a whole animal model of *Kras*-driven carcinoma that mimics oncogenic Kras copy number amplifications, along with concomitant loss of the *Lkb1* tumor suppressor. We show that low-level expression of oncogenic Kras (Ras^{Lo}) promotes the survival of *Lkb1*-mutant tissue, results in autonomous cell cycle arrest, and promotes non-autonomous overgrowth of surrounding wild-type tissue. In contrast, high-level expression of oncogenic Kras (Ras^{Hi}) transforms *Lkb1*-mutant tissue resulting in lethal malignant tumors. Our molecular analysis reveals for the first-time concurrent activation of the mTOR and AMPK pathways in mosaic malignant *Kras/Lkb1* tumors in vivo, and demonstrate the genetic and pharmacologic dependence of these tumors on CaMK-activated AMPK. To demonstrate the translational relevance of our findings, we show that *LKB1*-mutant human lung adenocarcinoma patients with KRAS copy number amplifications exhibit worse overall survival and increased AMPK activation, a phenotype not seen in *KRAS* mutant patients with loss of TP53. Our results suggest that high levels of oncogenic KRAS is a driving event in the malignant transformation of LKB1-mutant tissue, and uncover a novel vulnerability that may be used to target this aggressive genetic subset of RAS-driven tumors.

150 Resolving the contribution of the microbiome in aging *Arvind Shukla*[1], Kory Johnson[1], Joy Gu[1], Irina Kuzina[1], Edward Giniger[1] 1) National Institute of Neurological Disorders and Stroke.

Carriage of indigenous microbes brings along both biological benefits and biological costs, which influence lifespan. In order to understand this relationship, certain questions need to be answered:
How do we quantify aging itself, apart from lifespan analysis?
Does the presence of a microbiome accelerate the rate of aging or suppress it or both, depending on context?
What are the molecular events that change during the aging process and which ones are dependent on the microbiome?
Here we answer these questions in a Drosophila model using global gene expression profiling of normally-raised vs axenic flies.
First, we developed a rigorous quantitative metric for physiological age based on gene expression profiling. This analysis identified 1628 genes as aging classifiers, which is nearly 10% of the Drosophila genome, and thus acts as a suitable sample to quantify aging. We find that, whereas the expression profile is constantly evolving in flies raised in normal, microbe-rich conditions, applying this metric to flies raised in axenic condition reveals that newly-eclosed flies jump almost immediately to a “middle-aged” (30day) pattern of gene expression, which then remains largely unchanged through to nearly the end of the adult lifespan. Thus, while in our survival analysis flies raised in axenic conditions live ~20% longer than normally-grown flies, the more profound effect is on the pattern of aging itself.
Comparing the normally-grown to axenic flies we find that ~70% of the aged-dependent gene expression changes observed in normal conditions fail to occur under axenic conditions. Most notable among these changes are the failure to observe high-level expression of stress-response genes in young (10d) flies, or high-level expression of innate immune genes in old (45d) flies. This demonstrates that the commonly-observed, age-correlated changes in expression of stress and immune genes are not an intrinsic part of the aging program, but rather are selective responses to the state of the microbiome at different stages of the lifecycle. In between, at middle age (30d), the fly is essentially insensitive to the microbiome: only 64 genes (out of 14,688 assayed) show significant differences in expression between normal and axenic conditions.
These data provide compelling evidence that the presence of the microbiome acts as a major regulator of aging in Drosophila. In normal conditions, the fly transiently increases expression of stress response genes to cope with the presence of the microbiome when it is young, and selectively induces high-level immune activation at old age to control the microbiome. These observations are apparently not limited to Drosophila, as the human orthologs of our normal aging classifier genes are associated with many human pathologies of aging, whereas most of these processes are absent in a similar analysis of the aging classifiers from axenic flies.

151 YAP/TAZ transcription co-activators create therapeutic vulnerability in EGFR mutant glioblastoma *Renee Read*[1], Krishanthan Vigneswaran[1], Boyd Nathaniel[1], Lallani Shoeb[1], Oh Se Yeong[1], Boucher Andrew[1], Neill Stewart[1], Olson Jeffrey[1] 1) Emory University School of Medicine.

Glioblastoma (GBM), neoplasms derived from glia and neuro-glial progenitor cells, are the most common and lethal malignant primary brain tumors diagnosed in adults, with a median survival of 14 months even with current treatments. GBM tumorigenicity is often driven by genetic aberrations in receptor tyrosine kinases (RTKs), such as EGFR, and the Pi-3 kinase (PI3K) signaling pathway. Using a *Drosophila* glioma model and human patient-derived GBM cells, we identified the YAP and TAZ transcription co-activators, effectors of the Hippo pathway which promote gene expression via the TEAD transcription factors, as key drivers of GBM tumorigenicity downstream of oncogenic EGFR signaling. YAP and TAZ are highly expressed in EGFR mutant human GBMs, and their knockdown in EGFR mutant GBM cells inhibits proliferation and elicits apoptosis. Our results indicate that YAP/TAZ-TEAD directly regulate transcription of SOX2, C-MYC, and EGFR itself to create a feedforward loop to drive survival and proliferation of human GBM cells. Moreover, the benzoporphyrin derivative verteporfin, a disruptor of YAP/TAZ mediated transcription, specifically induced apoptosis of cultured patient-derived GBM cells, suppressed expression of YAP/TAZ transcriptional targets, including EGFR, and conferred significant survival benefit in an orthotopic xenograft GBM model. Our efforts led us to design and initiate a phase 0 clinical trial of Visudyne, an FDA-approved liposomal formulation of verteporfin, where we were able to use intraoperative fluorescence to observe verteporfin uptake into tumor cells in GBMs in human patients. Together, our data indicate that Verteporfin is a promising therapeutic agent for EGFR mutant GBM.

152 Tumors kill hosts through inflammatory disruption of the blood-brain barrier in Drosophila *Jung Kim*[1], David Bilder[1] 1) University of California, Berkeley.

Tumors kill hosts through disruption of local environment and systemic physiological perturbations, but the mechanisms underlying the latter are not well understood. Using an adult Drosophila model, we found that malignant tumors produced cytokines which induce widespread host activation of Jak/STAT signaling and caused premature lethality. STAT activity was particularly high in the cells that comprise the fly blood-brain barrier (BBB). Strikingly, tumor-bearing hosts displayed disruption of BBB structure and function, whereas flies dying from other causes retained an intact BBB. STAT signaling in BBB cells was both necessary and sufficient for tumor-induced BBB defects and early mortality. Importantly, preventing STAT activation in the BBB not only rescued barrier function but also extended the lifespan of tumor-bearing hosts. We observed BBB damage in other pathological conditions that exhibit elevated inflammatory signaling, including obesity and infection, and blocking Jak/STAT signaling in the BBB extended the lifespan. Taken together, BBB dysfunction is a novel paraneoplastic syndrome that also underlies multiple physiological morbidities. I will also present data about this new paradigm in the mouse model system.

153 Occluding Junction Modulation in Aging and Disease *Anna Salazar*[1,2], Martin Resnik-Docampo[2], Matthew Ulgherait[3], Rebecca Clark[4], Mimi Shirasu-Hiza[3], D. Leanne Jones[2], David Walker[2] 1) Christopher Newport University, Newport News; 2) UCLA, Los Angeles, Ca; 3) Columbia University, New York, New York; 4) Durham University, Durham, UK.

We have shown that altered expression of occluding junctions in the guts of *Drosophila melanogaster* can lead to various hallmarks of aging, including modulation of intestinal homeostasis, modulation of microbial dynamics, modulation in immune activity, and lifespan modulation. Loss of a specific occluding junction, Snakeskin (Ssk), leads to rapid and reversible intestinal barrier dysfunction, altered gut morphology, dysbiosis, and a dramatically reduced lifespan. Remarkably, restoration of Ssk expression in flies showing intestinal barrier dysfunction rescues each of these phenotypes previously linked to aging. Intestinal up-regulation of Ssk protects against microbial translocation following oral infection with pathogenic bacteria. Furthermore, intestinal up-regulation of Ssk improves intestinal barrier function during aging, limits dysbiosis, and extends lifespan. Furthermore, perturbing barrier function in the gut has non-cell-autonomous impacts. These findings indicate that intestinal occluding junctions may represent prolongevity targets in mammals, in addition to their possible roles in intestinal dysfunction and disease.

154 The insulin-like peptide Dilp6 is a key factor to inhibit growth in *Drosophila* in response to Toll signaling *Miyuki Suzawa*[1], Bradley Joseph[1], Nigel Muhammad[1], Michelle Bland[1] 1) University of Virginia, Department of Pharmacology, Charlottesville, VA.

Gram-positive bacteria or fungal infection activates the Toll signaling pathway and drives synthesis of antimicrobial peptides that carry out the humoral immune response in *Drosophila*. Toll signaling in the larval stage suppresses whole-animal growth, leading to a 12–15% decrease in adult wing size in animals that expressed constitutively-active Toll^{10b} in larval fat body. To determine which molecules control peripheral growth in response to fat body Toll signaling, we performed RNA sequencing to identify genes that are regulated by acute expression of Toll^{10b} in fat body. Using this approach, we identified *Drosophila insulin-like peptide 6 (Dilp6)* as a selective target of Toll signaling in fat body. *Dilp6* transcripts were significantly reduced by 45% following transgenic activation of Toll^{10b} or its target Dif in third-instar larvae. *Dilp6* expression was similarly decreased after an immune challenge with the Gram-positive bacteria *Enterococcus faecalis* in *Drosophila* mid-third instar larvae. To measure endogenous circulating Dilp6, we used CRISPR/Cas9 to insert HA and FLAG epitope tags into exons encoding the B- and A-chains in the X-linked *Dilp6* locus. We used this allele, Dilp6^{HF}, to measure hemolymph Dilp6 levels by ELISA. Circulating Dilp6^{HF} levels were reduced by 75-80% in larvae expressing Toll^{10b} in fat body and were rescued by knockdown of Dif. Increased expression of *Dilp6* in control fat bodies led to a 2.6% increase in wing size and significantly rescued reduced wing growth in animals expressing Toll^{10b} in fat body. This correlated with wing disc Akt phosphorylation; elevated Dilp6 expression in fat body drove insulin signaling in wing imaginal discs, while knockdown of Dilp6 in fat body led to significant reductions in wing disc Akt activation. Interestingly, innate immune signaling lowers circulating Dilp6 levels while starvation does not. This suggests that immune activity poses a unique metabolic challenge that must be met with a systemic change to suppress growth. Our data fit into a model of impaired growth and reduced IGF1 levels resulting from chronic infection and/or inflammation in childhood, and they suggest that growth suppression is a conserved response to innate immune signaling.

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Genomics, Gene Regulation, and Systems Biology (*C. elegans*)

141 The Argonaut NRDE-3 and MET-2 redundantly target SET-25 to full length transposable elements *Susan Gasser*

Establishing, maintaining and erasing chromatin domains are fundamental processes that shape the epigenetic memory of a cell. Lysine 9 methylation on histone H3 (H3K9me) is one of the defining histone modifications of heterochromatin. Silencing of satellite repeats, transposable elements and developmentally regulated genes, through H3K9me is essential to ensure genome stability (Zeller and Padeken et al., 2016). To understand how H3K9me2/me3 domains are established and maintained we investigated the dependencies of the 2 H3K9 specific histone methyl transferases (HMTs) in *C. elegans*. SET-25 (SUV39h1/h2) catalyzes H3K9me3, while MET-2 (SetDB1) deposits H3K9me1/me2 (Towbin et al., 2012). ChIPseq experiments in *met-2* and *set-25* mutant embryos showed that while the majority of H3K9me3 domains are completely dependent on MET-2 for the recruitment of SET-25, however ~10% of H3K9me3 domains, particularly evolutionary young transposons, can be methylated by SET-25 independently from MET-2. A RNAi screen showed that SET-25 targeting to these sites is dependent on the somatic Argonaut NRDE-3 and the MBT domain protein LIN-61. Analysis of the *nrde-3, lin-61*, *met-2* and *set-25* single and double mutants showed that LIN-61 functions by reinforcing SET-25 binding to existing H3K9me2/me3 regions. In contrast NRDE-3 is redundant with MET-2 for the targeting of SET-25 to full length transposable elements. Loss of *nrde-3* and *met-2* results in the synergistic upregulation of transposable elements and embryonic lethality.

142 Regulation of alternative splicing in tissues and distinct neuronal subtypes in *C. elegans* Bina Sugumar[1], Pallavi Pilaka[1], Xico Gracida[2], Yun Zhang[2], *John Calarco*[1] 1) University of Toronto; 2) Harvard University.

The central nervous system is a network of multiple neuronal subtypes, each with unique functional roles in the organism that collectively regulate behaviour. The heterogeneity of neuronal subtypes is thought to necessitate the high levels of alternative splicing in the nervous system. While many documented examples of splicing differences between broad tissue-types exist, there remains much to be understood about the functionally relevant splicing differences between neuronal subtypes, and the splicing factors and the *cis*-sequence elements involved in establishing tissue and neuron subtype-specific splicing patterns. Using the Translating Ribosome Affinity Purification coupled with deep-sequencing (TRAP-seq) method in *C. elegans*, we have obtained high coverage transcriptome snapshots for three broad tissue classes (nervous system, muscle, and intestine) and two neuronal subtypes (dopaminergic and serotonergic neurons). We have identified hundreds of isoforms that exhibit distinct splicing patterns across broad tissue types, and also between neuronal subtypes and the rest of the nervous system. Tissue- and neuronal subtype-regulated alternative exons are generally shorter, more likely to be frame-preserving, are enriched in specific cis-regulatory motifs when compared with constitutively spliced exons, and a subset overlap with protein domains. Intriguingly, our analysis has also identified examples of micro-exons less than 15 nucleotides in length, initially thought to exist primarily in vertebrate genomes. Collectively, our results indicate an important and rich layer of tissue-specific gene regulation at the level of alternative splicing.

143 A temporally regulated switch from non-canonical to canonical Wnt signaling stops QR descendant migration through a Slt/Robo and RGA-9/RhoGAP dependent mechanism *Erik Schild*[1], Lorenzo Rella[1], Shivam Gupta[2], Euclides Pòvoa[1], Annabel Ebbing[1], Marco Betist[1], Andrew Mugler[2], Hen-

drik Korswagen[1] 1) Hubrecht Institute, Utrecht, the Netherlands; 2) Purdue University, West Lafayette, IN.

Members of the Wnt family of secreted signaling proteins are key regulators of cell migration, but the underlying signaling mechanisms that determine whether the cell moves, changes direction, or stops remain poorly understood. The *C. elegans* QR lineage provides a powerful model system to study Wnt-dependent cell migration at single cell resolution. We have previously shown that the migration of the QR descendants (QR.d) relies on the non-canonical CAM-1/Ror and MOM-5/Frizzled pathways, which separately control polarity and migration.

Additionally, we found that once the QR.d reach their final position, they stop their migration by switching on canonical, β-catenin dependent Wnt signaling. This switch depends on cell intrinsic regulation of *mig-1*/Frizzled. We have found that the regulation relies on a cellular clock, which is independent of the cell cycle. Mathematical modeling of QR.d migration suggests that an accumulating activator or a decaying repressor can account for the highly precise temporal regulation in this system.
Here, we show that canonical Wnt signaling counteracts the MOM-5/Fz-dependent migration pathway to stop QR.d migration. To gain insight into this cross-talk mechanism, we performed mRNA sequencing on isolated QR.d and found that canonical Wnt signaling induces a specific transcriptional program. Among the upregulated targets are two genes that are necessary and sufficient for migration termination: *eva-1*, which encodes a component of the Slt/Robo pathway, and *rga-9*, which encodes a conserved Rho GTPase activating protein (GAP). Consistent with the role of EVA-1 in Slt/Robo signaling, slt-1/Slt, sax-3/Robo and the putative downstream effector srgp-1/srGAP are also required.

Rho GTPases are important regulators of cell polarity and migration, and several family members function partially redundantly in QR.d migration. Our results indicate that these Rho GTPases are at the center of the interaction between the MOM-5/Fz and canonical Wnt pathways. Epistasis analysis suggests that MOM-5/Fz acts through PIX-1, a guanine nucleotide exchange factor (GEF) that promotes Rho activity. Conversely, canonical Wnt signaling induces RGA-9 expression and may activate SRGP-1 through Slt/Robo signaling, both of which are predicted to inhibit Rho activity. Based on these results, we propose that crosstalk between canonical and non-canonical Wnt signaling at the level of Rho activators and inhibitors controls the stereotypic migration of QR descendants.

144 Repressive H3K9me2 protects lifespan against the transgenerational burden of germline transcription in *C. elegans* *Teresa Lee*[1], Heidi David[1], Amanda Engstrom[1], Brandon Carpenter[1], David Katz[1] 1) Emory University, Atlanta GA.

In *Caenorhabditis elegans*, mutations in WDR-5 and other components of the COMPASS H3K4 methyltransferase complex extend lifespan and enable its inheritance. We show that *wdr-5* mutant longevity is itself a transgenerational trait that corresponds with a global enrichment of the heterochromatin factor H3K9me2 over twenty generations. In addition, we find that the transgenerational aspects of *wdr-5* mutant longevity require the H3K9me2 methyltransferase MET-2, and can be recapitulated by removal of the putative H3K9me2 demethylase JHDM-1. Finally, we show that the transgenerational acquisition of longevity in *jhdm-1* mutants is associated with accumulating genomic H3K9me2 that is inherited by their long-lived wild-type descendants at a subset of loci. These results suggest that the accumulation of heterochromatin in the germline facilitates the transgenerational establishment and inheritance of a complex trait. Based on these results, we propose that transcription-coupled H3K4me via COMPASS limits lifespan by encroaching upon domains of heterochromatin in the genome.

145 *In vivo* regulation of an X-specific condensin's binding dynamics in *C. elegans* Laura Breimann[1,2], Ana Morao[1], Nina Maryn[1], Krishna Bikkasani[1], Vic-Fabienne Schumann[2], Kustrim Cerimi[2], Parvathy Manoj[1], Michael Carrozza[1], Jun Kim[1], Andrew Woehler[2], Stephan Preibisch[2], *Sevinc Ercan*[1] 1) Department of Biology, New York University, NY; 2) Berlin Institute for Medical Systems Biology, MDC, Germany.

Condensins are evolutionarily conserved molecular motors that bind to DNA and form loops to compact chromosomes. Recent studies of condensins and related SMC complexes have demonstrated their ability to use ATPase activity to move along DNA while progressively enlarging loops *in vitro*. Yet, regulators of condensin movement *in vivo* are unclear. We hypothesize that histone modifications may regulate the dynamics of condensin association with chromatin fiber *in vivo*. Here, we provide evidence supporting this hypothesis through analysis of a conserved mitotic histone modification regulating the binding of an X-specific condensin in *C. elegans*. In *C. elegans*, a modified condensin forms the core of the dosage compensation complex (DCC). DCC binds specifically and increases the level of H4K20me1 on the X. We and others showed that mutations that reduce or increase H4K20me1 affect dosage compensation. Here we performed ChIP-seq and FRAP (fluorescence recovery after photobleaching) experiments to analyze DCC spreading and binding dynamics in mutants that affect H4K20 methylation. Our experimental set up involves induced expression of a GFP tagged SMC subunit in the presence of endogenous protein. We validated the set up by showing that the GFP tagged protein1) incorporates into condensin complex (IP-western) 2) binds to the X with the same pattern as endogenous complex (ChIP-seq) 3) and requires the ATPase activity (EQ null mutation). Our preliminary data suggest that H4K20me1 regulates the range of DCC spreading and FRAP dynamics of DCC binding *in vivo*. We also found that a DCC subunit's HEAT domain interacts with histone tails *in vitro* and this interaction is sensitive to H4K20 methylation. Analysis of published Hi-C data from others indicate changes in DNA contacts upon reduced H4K20me1 on the X. Together, these results are consistent with a model in which HEAT domain interaction with histone tails affecting the dynamics of condensin binding and range of DNA interactions *in vivo*.

146 A neuronal thermostat controls membrane fluidity in *C. elegans* Olivia Casanueva[1], *Laetitia Chauve*[1], Sharlene Murdoch[1], Francesca Hodge[1], Fatemah Masoudzadeh[1], Hanneke Okkenhaug[1], Greg West[1], Anne Segonds-Pichon[1], Steven Wingett[1], Michael Wakelam[1], Andrea Lopez[1] 1) Babraham Institute.

An organisms' ability to adapt to heat can be key to its survival. This is a particularly relevant adaptive process to understand in view of anthropogenic climate change. Cells adapt to temperature shifts by adjusting lipid desaturation levels and the fluidity of membranes in a process that is thought to be controlled cell autonomously. We have discovered that subtle, step-wise increments in ambient temperature can lead to the conserved heat shock response being activated in head neurons of *C.elegans*. This response is exactly opposite to the expression of the lipid desaturase FAT-7 in the worm's fat store cells. We find that the over-expression of the master regulator of this response, Hsf-1, in head neurons, causes extensive fat remodeling to occur across tissues. These changes include a decrease in FAT-7 expression and a shift in the levels of unsaturated fatty acids in the plasma membrane. These shifts are in line with membrane fluidity requirements optimised to survive in warmer temperatures. We have identified that the cGMP receptor, TAX-2/TAX-4, as well as TGF-β/BMP signaling, as key players involved in the transmission of neuronal stress to peripheral tissues. This is the first study to suggest that a thermostat-based mechanism can centrally coordinate membrane fluidity in response to

warm temperatures across tissues in multicellular animals.

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New Technologies and their Impact (Yeast)

175 Introduction of Michael Snyder for the Winge-Lindegren Address *Michael Knop*[1] 1) University of Heidelberg.

abstract is not available at the time of print

176 Winge-Lindegren Address given by Mike Snyder, Stanford Univeristy *Michael Snyder*[1] 1) Stanford Univeristy.

abstract is not available at the time of print

177 A high throughput method to assay mutation rate: Determining the pathogenicity of Msh2 variants associated with Lynch syndrome Anja R. Ollodart[1], Chiann-Ling C. Yeh[1], Adam S. Gordon[2], Aaron W. Miller[1], Brian H. Shirts[3], Maitreya J. Dunham[1] 1) Genome Sciences, University of Washington, Seattle, WA ; 2) Department of Pharmacology, Northwestern University, Evanston, IL; 3) Department of Laboratory Medicine, University of Washington, Seattle, WA.

The reasons to study mutation rate are endless, but the reasons not to are simple: it is time consuming and tedious. Traditional methods such as fluctuation tests and mutation accumulation experiments are low throughput and often require significant tuning to get accurate results. We established a new method to determine the mutation rate of many strains simultaneously using continuous culture coupled with deep sequencing. We have applied this method to assay the mutation rate of *Saccharomyces cerevisiae* strains carrying mutations in the gene encoding Msh2, a DNA repair enzyme in the mismatch repair pathway (MMR). Loss of function (LOF) mutations in *MSH2* are associated with an increase in cancer risk classified as Lynch syndrome, and many mutations in this gene remain uncharacterized with respect to their contribution to disease risk. Our new method takes advantage of continuous culture in a chemostat, in which an increase in the frequency of resistance to canavanine is due to neutral *de novo* LOF mutations within the *CAN1* gene. This allows resistance to accumulate linearly at the mutation rate. We can track the frequency of each *MSH2* allele in the canavanine resistant pool using deep sequencing to determine the rate at which strains carrying each allele produce *can1* mutations. Chemostats inoculated with a pool of published *MSH2* variants were used to bench mark the new method against Luria-Delbrück fluctuation tests. Alleles found to be non-functional in previous work were high mutators in our pooled screen. While WT-like alleles were naturally at low frequency in the canavanine resistance pool, they were still accurately assayed. We have now curated an additional 244 variants of hMsh2 from ClinVar and other clinical testing data which mapped onto orthologous sites in yMsh2. Each allele is marked by ~10 independent barcodes that have been associated with the linked variant using long read sequencing, allowing us to collect internal replicates and control for background mutation accumulation. We subjected these additional alleles to the same assay in both the deletion and wild type background. Analysis of these data sets is in progress and will allow us to determine the function and dominance of each allele. This method is capable of studying the mutation rate of many microbial species and can be applied to problems ranging from the generation of high-fidelity polymerases to assaying antibiotic resistance.

178 Onyx: A benchtop platform for massively parallel editing of the yeast genome *Nandini Krishnamurthy*[1], Bryan Leland[1], Katherine Krouse[1], Krishna Yerramsetty[1], Richard Fox[1], Michael Clay[1], Eric Abbate[1], Jacqueline Rosains[1], Daniel Held[1], Steve Federowicz[1], Charlie Johnson[1], Tian Tian[1], Miles Gander[1], Eileen Spindler[1], Andrea Halweg-Edwards[1], Jim Bierle[1], Kara Juneau[1], Tom Rogers[1], Timothy Shaver[1], Andrew Garst[1], Deanna Church[1], Ian Fiddes[1], Craig Struble[1], Michael Graige[1], Paul Hardenbol[1], Yiming Kang[2], Michael Brent[2], Leslie Mitchell[3], Jef Boeke[3] 1) Inscripta Inc, Boulder, CO; 2) Washington University, St. Louis, MO; 3) NYU Langone Medical Center, New York, NY.

CRISPR-based genome engineering has the potential to accelerate discovery. Unfortunately, current approaches suffer from limitations in scalability, efficiency, diversity of edit types and accessibility. The Onyx platform, developed by Inscripta, enables high-throughput delivery of diverse edit types, allowing rapid engineering of gene, pathway, and genome-wide targets in *S. cerevisiae* and *E. coli*. The platform simplifies the complex editing workflow by offering an end-to-end solution from design to engineered cell library, including software, reagents, benchtop instrument and analytics. We present 3 applications including metabolic engineering, strain optimization and expression engineering. (1) Using the Onyx platform, libraries totaling >130,000 single edits across the entire yeast genome were generated to increase tyrosine production. We engineered both known enzymes and unbiased genome-wide targets. All 7 enzymes in the yeast tyrosine biosynthesis pathway were modified through scanning saturation mutagenesis. For the unbiased approach, edits across the yeast genome included insertion of 5 different terminators at the 3' end of genes, deletion of transcription factor (TF) binding sites, and truncation of every gene in the yeast genome. This campaign covered 93% of genes in the yeast genome with at least one design. Screening with high-throughput mass spectrometry identified novel hits in known enzymes as well as >25 new hits throughout the genome. (2) With collaborators from Sc2.0, we designed 936 edits in a yeast strain containing a synthetic version of chromosome 6 (syn6) to detect potential "bugs" that contribute to a poor-growth phenotype. We recapitulated several previously identified bugs in syn6 and rapidly identified 48 novel potential bugs. (3) For the expression engineering application, we swapped putative TF binding sites for neutral sequence to help validate models predicting biologically significant TF regulatory networks. We designed libraries ranging from 570 to 9700 edits, covering TF binding sites regulating a total of 3000 genes. Across these three projects, we generated libraries of cells that covered >90% of the designed edits, with 30-60% of the cells containing a complete intended edit. Ultimately, the Onyx platform enables comprehensive testing of genomic changes and rapid identification of effective genotypes. This will have far-reaching benefits for biology, bio-industrial science, agriculture, healthcare, and alternative energy.

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Adaptation in Natural Populations (PEQG)

166 A chromosomal inversion underlies forest adaptations in deer mice *Olivia Meyerson*[1], Emily Hager[1], Brock Wooldridge[1], Hopi Hoekstra[1] 1) Department of Organismic and Evolutionary Biology, Department of Molecular and Cellular Biology, Museum of Comparative Zoology, Howard Hughes Medical Institute, Harvard University, Cambridge, MA.

When organisms colonize novel environments, often several traits are involved in adaptation, raising the question of how these adaptive traits coevolve and may be co-inherited. To examine this question at the genetic level, we focused on two ecomorphs of the North American deer mouse, *Peromyscus maniculatus*, a forest and a prairie form, thought to have recently diverged (~10,000 ya) when the ancestral prairie form colonized forested habitat following the last glacial retreat. Among many morphological differences, forest mice have substantially longer tails and darker coat colors than their prairie counterparts—two traits that likely arose as adaptations to the new forest environment. Using quantitative-trait locus (QTL) mapping, we found that variation in both tail length and coat color map to the same genomic region on chromosome 15. This genomic region shows both suppressed recombination and elevated F_{ST}, indicating that it is a likely chromosomal inversion. Using PacBio long-read sequencing, we resolved the inversion to be a complex 40Mb chromosomal rearrangement. We found that the inversion acts additively for both traits and explains >10% and >45% of the variation in tail length and coat color respectively. We next isolated the inversion in a congenic line to directly measure its effect, and to measure gene-expression differences of candidate pigment and tail development genes. To characterize the evolutionary history of the inversion, we measured its frequency in wild populations and found that the inversion is nearly fixed in the forest population, but is completely absent from the prairie population. Finally, we sampled mice along a 50-km environmental transect between the forest and prairie populations and resequenced their genomes. Across the transect, we found that genome-wide ancestry tracks with natural barriers to gene flow, whereas the change in inversion frequency aligns with changes in forest-type and soil-color, suggesting that the inversion has been subjected to natural selection associated with environmental differences. Together, our findings suggest that major chromosomal rearrangements can facilitate rapid adaptive events through linkage of multiple beneficial alleles affecting multiple phenotypic traits.

167 RNAi pathways repress reprogramming of *C. elegans* germ cells during heat stress *Alicia Rogers*[1], Carolyn Phillips[1] 1) University of Southern California.

Repression of cellular reprogramming in germ cells is critical to maintaining cell fate and fertility. When germ cells mis-express somatic genes they can be directly converted into other cell types, resulting in loss of totipotency and reproductive potential. Identifying the molecular mechanisms that coordinate these cell fate decisions is an active area of investigation. RNAi pathways perform transcriptional and post-transcriptional regulation to maintain appropriate gene expression, silence foreign genetic elements, and play key roles in development and fertility. Here we show that RNAi pathways play a key role in maintaining germline gene expression and totipotency after heat stress. By examining transcriptional changes that occur in *mut-16* mutants, lacking a key protein in the RNAi pathway, at elevated temperatures we found that genes normally expressed in somatic tissues are mis-expressed in germ cells while transcript levels of germline-specific genes are unchanged. This trend was also observed in *hrde-1* mutants, which fail to direct transcriptional silencing of RNAi-targeted genes. By RT-qPCR, we showed the upregulation of soma-specific genes in *mut-16* mutants at 25°C occurs in the gonad. In addition, we used fluorescent microscopy to visualize the expression of a soma-specific marker in the germline tissue of *mut-16* mutants at 25°C. These findings indicate that the RNAi pathway plays a key role in preventing aberrant expression of somatic genes in the germline during heat stress. We propose that this regulation likely occurs in part through the maintenance of germline chromatin by the nuclear RNAi pathway. We are currently performing experiments to address this possibility. Identification of new pathways governing germ cell reprogramming is critical to understanding how cells maintain proper gene expression and may provide key insights into how cell identity is lost in some germ cell tumors.

168 The making of the monarch: A constrained adaptive path to toxin resistance *Marianthi Karageorgi*[1], Simon C. Groen[2], Fidan Sumbul[3], Julianne N. Pelaez [1], Kirsten I. Verster[1], Jessica M. Aguilar[1], Amy P. Hastings[4], Susan L. Bernstein[1], Teruyuki Matsunaga[1], Michael Astourian[1], Geno Guerra[5], Felix Rico[3], Susanne Dobler[6], Anurag A. Agrawal[4,7], Noah K. Whiteman[1] 1) Department of Integrative Biology, University of California, Berkeley, USA; 2) Department of Biology, Center for Genomics and Systems Biology, New York University, New York, USA; 3) LAI, U1067 Aix-Marseille Université, Inserm, CNRS, Marseille, France; 4) Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, USA; 5) Department of Statistics, University of California, Berkeley, USA; 6) Molecular Evolutionary Biology, Zoological Institute, Biocenter Grindel, Universität Hamburg, Germany; 7) Department of Entomology, Cornell University, Ithaca, USA.

Identifying the genetic mechanisms of adaptation requires the elucidation of links between the evolution of DNA sequence, phenotype, and fitness. Convergent evolution can be used as a guide to identify candidate mutations that underlie adaptive traits, and new genome editing technology is facilitating functional validation of these mutations in whole organisms. We combined these approaches to study a classic case of convergence in insects from six orders, including the monarch butterfly (*Danaus plexippus*), that have independently evolved to colonize plants that produce cardiac glycoside toxins. Many of these insects have evolved parallel amino acid substitutions in the α subunit (ATPα) of the Na^+/K^+ ATPase, the physiological target of cardiac glycosides. We found substitutional paths involving three ATPα amino acids (111, 119 and 122) that are associated with cardiac glycoside specialization. Using CRISPR/Cas9 genome engineering, we edited the native ATPα gene in *Drosophila melanogaster* and retraced the substitutional path taken across the monarch butterfly lineage. We could show *in vivo*, *in vitro* and *in silico* that the path confers resistance and target site insensitivity to cardiac glycosides, culminating in triple mutant "monarch flies" as resistant as the monarch butterflies. We also demonstrated that the order in which the substitutions evolved ameliorated, but did not eliminate, antagonistic pleiotropy through epistasis. In our current work, studying natural populations of *Drosophila* carrying the "monarch" genotype in the three ATPα amino acids (111,119 and 122), we aim to elucidate whether convergent compensatory substitutions have evolved in the monarch and other insect specialists to eliminate the fitness costs of the resistance substitutions. Overall, our work illuminates how the monarch butterfly evolved resistance to cardiac glycoside toxins and reveals rules that govern the evolution of novel adaptive traits.

169 Human isolates of *S. cerevisiae*: colonization, pathogenicity, and in-host microevolution viewed through domestication history Alexandra Imre[1,2], Hanna Rácz[1,3], Péter Oláh[4,5], Zsuzsa Antunovics[6], Ilona Dóczi[7], Renátó Kovács[8,9], László Majoros[8], István Pócsi[1], Ksenija Lopandic[10], Devin Bendixsen[11], Rike Stelkens[11], *Walter Pfliegler*[1] 1) Department of Molecular Biotechnology and Microbiology, University of Debrecen, Debrecen, Hungary; 2) Kálmán Laki Doctoral School of Biomedical and Clinical Sciences, University of Debrecen, Debrecen, Hungary; 3) Doctoral School of Nutrition and Food Sciences, University of Debrecen, Debrecen, Hungary; 4) Department of Dermatology, Venereology and Oncodermatology, University of Pécs, Pécs, Hungary; 5) Department of Dermatology, University Hospital of Düsseldorf, Düsseldorf, Germany; 6) Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary; 7) Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary; 8) Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary; 9) Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary; 10) Institute of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria; 11) Zoology Department, Stockholm University, Stockholm, Sweden.

The status and origin of the yeast *Saccharomyces cerevisiae* as a member of the human microbiome and an emerging human pathogen are not fully understood. Although recent works have shed light on the genomic features of human isolates, lack of detailed data on hosts complicates the evaluation of the role of the yeast in the human body. To understand *S. cerevisiae* colonization and infection origins and mechanisms, we sampled 62 isolates (both pathogenic and commensal) from 58 patients at Hungarian clinics over the course of three years. Isolates were subjected to detailed genetic characterization, karyotyping, and short-read genome sequencing. We compared these human isolates to i) 30 locally available commercial probiotic and baking yeasts, ii) previously published infectious isolates, and iii) to yeasts found in the microbiomes of indigenous cultures with a non-Westernized lifestyle. Using comparative phylogenomics, we found evidence that commercially available yeasts, especially probiotics and baking strains, both colonize and infect humans frequently. We also show that the domestication-driven genome evolution of commercial yeast has resulted in different adaptation strategies during colonization and infection of the human host. Baking yeast derived human isolates preferably colonize the female genital tract and display large-scale and frequent chromosome copy number changes and rearrangements in their mosaic genomes. Probiotic-derived human isolates lack anatomic niche preference and show lower levels of genome structure variation. To trace the ancestry and structural genomic variation of all human isolates, we applied a novel phylogenomic network analysis using alignment and assembly free methods. Strikingly, this analysis suggests that the remarkable genomic plasticity of the baking-derived strains has not arisen naturally, but instead results from artificial strain crossing and improvement strategies applied in the mid-20th century. Tetrad analysis showed that this plasticity can not be attributed to meiotic processes but arises in clonal populations, leading to high geno- and phenotypic clonal heterogeneity. The genomic adaptations we observe in human isolates derived from commercial strains are in striking contrast with the indigenous gut-colonizing yeasts. Together, these data show that the pathogenic and colonization potential of *S. cerevisiae* has been drastically altered by domestication and directed strain improvement in the recent past.

170 Learning the properties of adaptive regions with functional data analysis *Mehreen Mughal*[1], Hillary Koch[1], Jinguo Huang[1], Francesca Chiaromonte[1], Michael DeGiorgio[2] 1) Pennsylvania State University, University Park, PA; 2) Florida Atlantic University, Boca Raton , FL.

Identifying regions of positive selection in genomic data remains a challenge in population genetics. Most current approaches rely on comparing values of summary statistics calculated in windows. We present an approach termed *SURFDAWave*, which translates measures of genetic diversity calculated in genomic windows to functional data. By transforming our discrete data points to be outputs of continuous functions defined over genomic space, we are able to learn the features of these functions that signify selection. This enables us to confidently identify complex modes of natural selection, including adaptive introgression. We are also able to predict important selection parameters that are responsible for shaping the inferred selection events. By applying our model to human population-genomic data, we recapitulate previously identified regions of selective sweeps, such as *OCA2* in Europeans, and predict that its beneficial mutation reached a frequency of 0.02 before it swept 1,802 generations ago, a time when humans were relatively new to Europe. In addition, we identify *BNC2* in Europeans as a target of adaptive introgression, and predict that it harbors a beneficial mutation that arose in an archaic human population that split from modern humans within the hypothesized modern human-Neanderthal divergence range.

171 Genome-wide association study (GWAS) of bleaching tolerance in a Great Barrier Reef coral *Zach Fuller*[1], Joseph Pickrell[2], Peter Andolfatto[1], Mikhail Matz[3], Line Bay[4], Molly Przeworski[1] 1) Columbia University; 2) Gencove; 3) University of Texas at Austin; 4) Australia Institute of Marine Science.

Although reef-building corals are rapidly declining worldwide, there is considerable variation in bleaching response and heat tolerance within populations, which is in part heritable. To map the genetic basis of this variation and develop individual predictors of bleaching in the wild, we conducted a genome-wide association study (GWAS) of bleaching in *Acropora millepora* from the Great Barrier Reef. We first generated a chromosome-scale genome assembly and obtained whole genome sequences for 190 phenotyped samples collected at 12 reefs, across which we found little population structure. We show that we can reliably impute genotypes in low-coverage sequencing data with a modestly sized reference haplotype panel to obtain millions of high confidence single nucleotide polymorphism (SNP) calls. We find evidence of long-term balancing selection in a heat-shock co-chaperone, *sacsin*, and test 6.8 million SNPs for associations with variation in bleaching. Moreover, we show a polygenic score constructed from the GWAS estimates to be a significant predictor of bleaching and can distinguish the most tolerant individuals from the most susceptible. We then demonstrate the feasibility of such an approach by scaling up our GWAS to an increased sample size of more than 1000 whole-genome sequenced and phenotyped individuals. These results thus set the stage for the use of genomic-based prediction in coral conservation strategies.

Friday, April 24 10:00 AM - 10:15 AM

GSA Award Presentations

187 2019 Thomas Hunt Morgan Medal *Dan Hartl*[1] 1) Harvard University.

abstract is not available at the time of print

188 2020 Edward Novitski Prize *Welcome Bender*[1] 1) Harvard Medical School.

abstract is not available at the time of print

189 2020 Elizabeth Jones Award for Excellence in Education *Seth Bordenstein*[1] 1) Vanderbilt University.

abstract is not available at the time of print

Friday, April 24 10:30 AM - 11:15 AM

Gruber Genetics Prize Presentation

190 GSA Welcome *Denise Montell*[1] 1) University of California Santa Barbara.

abstract is not available at the time of print

191 Gruber Foundation Welcome *A. Sarah Hreha*[1] 1) The Gruber Foundation.

abstract is not available at the time of print

192 Presentation of 2020 Gruber Foundation Genetics Prize *Allan Spradling*[1] 1) Carnegie Institution/HHMI.

abstract is not available at the time of print

193 Quorum-sensing communication: from viruses to bacteria to eukaryotes *Bonnie Bassler*[1] 1) HHMI/Princeton University.

abstract is not available at the time of print

Friday, April 24 11:30 AM - 1:00 PM

Developmental Genetics: Cell Specification and Competition

194 Dynamic self-generation of FGF morphogen gradients by cytonemes during *Drosophila* tracheal patterning *Lijuan Du*[1], Sougata Roy[1] 1) University of Maryland.

Gradients of morphogen signaling proteins are essential for tissue patterning and morphogenesis. However, the mechanism of gradient formation is controversial and remains unexplored in most morphogenetic contexts. To investigate how signals disperse and how the dispersion mechanism is dynamically modulated to shape gradients in three-dimensional tissue structures, we focused on *Drosophila* trachea. In *Drosophila* embryo, FGF expressed in six different clusters surrounding a tracheal placode, which expresses its receptor FGFR, induces branching morphogenesis of the primary branches from the placode. By checking native FGF:GFP from genomic knock-in allele, we uncovered that FGF:GFP doesn't form a pre-patterned extracellular gradient. Rather, FGF gradient is formed within each recipient tracheal branch, and the shape of the gradient is scaled dynamically with branch growth. Further, formation of branch-specific gradient depends on cytoneme-mediated FGF transport. In a growing tracheal branch, the cells close to the FGF-source contain many polarized cytonemes to contact it and receive FGF. The number of such cytonemes gradually reduces with increasing distance from FGF-source. Although FGF was thought to induce a chemotactic response in trachea, we discovered it also acts as a morphogen to activate concentration-dependent gene expression, inducing pointed-P1 at distal tip with high FGF and cut at proximal stalk with low FGF-levels. The transcription factors Pointed-P1 positively while Cut negatively regulate formation of FGFR-containing cytonemes. This positive and negative feedbacks of FGF signaling thereby set up the gradient of polarized cytonemes along the recipient branch. Consequently, FGF gradient is self-generated/sustained, adopting precise branch-specific shapes. These results reveal a robust mechanism where morphogens self-generate precise tissue-specific gradient shapes through feedback regulation of cytoneme-mediated dispersion.

195 The BMP signaling gradient is interpreted as concentration thresholds during dorsal-ventral patterning of the embryonic axis *Hannah Greenfeld*[1], Mary Mullins[1] 1) University of Pennsylvania, Philadelphia, PA.

Bone Morphogenetic Protein (BMP) acts as a morphogen to pattern the dorsoventral (DV) embryonic axis in all vertebrates. In zebrafish, a gradient of BMP signaling activity specifies multiple ventral cell fates whereas suppression of BMP signaling results in dorsal cell fates. However, it is unknown how cells along the DV axis interpret and translate distinct levels of BMP signaling into differential gene activation to specify multiple cell fates. Morphogen gradients have been shown to induce differential gene activation by multiple mechanisms, including by either the steady-state amount of signaling, differences in signal duration, or the steepness of the graded signal across cells. Here, we investigate the mechanism by which BMP signaling provides positional information to cells across the DV embryonic axis. To identify the genes that are directly regulated by BMP signaling, we performed RNA-seq on *bmp7* mutant embryos treated with a translation inhibitor and rescued by BMP2/7 protein injection. The BMP target genes were found to be expressed in three distinct domains of the embryo. We used quantitative measurements of the BMP transcriptional effector phosphorylated Smad5 (pSmad5) to examine the spatial relationship between BMP signaling and activation of different target genes in cells across the entire embryo. Embryonic cells respond to distinct levels of pSmad5 to activate three different target genes. We find that specific pSmad5 levels precisely position target gene expression boundaries in both wild-type embryos and in BMP antagonist mutant embryos where the shape of the gradient is altered. The pSmad5 gradient contains multiple threshold levels that position the expression of different target genes. To address the role of signal duration to pattern ventral cell fates, we tested the requirement of prolonged BMP ligand exposure to activate target gene expression and found that all three genes are activated rapidly following BMP ligand exposure. Together, our data support a model whereby the BMP gradient is interpreted as a classic concentration-dependent morphogen providing positional information to pattern gene expression along the embryonic DV axis.

196 Localized and tissue-wide gene expression changes during regeneration of *Drosophila* imaginal discs revealed by single-cell analysis *Melanie Worley*[1], Nicholas Everetts[1], Riku Yasutomi[1], Nir Yosef[1], Iswar Hariharan[1] 1) Univ California, Berkeley.

Regeneration of damaged tissues is a complex process that requires the surviving cells to respond, proliferate, and repattern to replace what was lost. *Drosophila* imaginal discs are capable of regenerating following blastema formation, which is marked by local cell proliferation and increased cellular plasticity. It is not well understood what occurs within individual cells during regeneration, including what genetic programs promote cellular reprogramming and whether repatterning recapitulates early development. Genetic methods in *Drosophila* have enabled the study of imaginal disc regeneration *in situ* following the ablation of a defined portion of the disc by the spatially-restricted expression of a pro-apoptotic gene during a short period of larval development. Until now, most studies of regeneration have used either bulk RNA sequencing or *in situ* hybridization of small numbers of genes to study this process. The method of single-cell RNA sequencing has allowed us to look for gene expression changes, with spatial

resolution, on a genome-wide basis. We used single-cell transcriptomics to profile cells collected from two time-points during normal development and cells from regenerating wing discs. We harmonized these distinct data sets to generate a combined cell atlas, allowing us to assign cells to different domains in the tissue including the regeneration blastema. By investigating gene expression changes at single-cell resolution, we observed diverse cellular responses at different distances from the site of tissue damage. We characterized unique transcriptional profiles of an inner and outer blastema, as marked by the gene expression of *upd3* and *Ilp8.* In addition, we observed a global response to tissue damage where cells from the regenerating discs express many marker genes that are normally expressed only earlier in development. In contrast, many genes expressed locally around the site of damage were regeneration specific. Our analysis of the single-cell data has uncovered a number of genes that are important for promoting proper regeneration including predicted transcription factors and secreted proteins. Functional investigation of the roles of these genes in regeneration will be presented. These studies will lead to a better understanding of the cellular and molecular basis of regeneration.

197 Epithelial integrity monitoring via ligand-receptor segregation ensures malignant cell elimination David Bilder[1], *Geert de Vreede*[1] 1) Univ California, Berkeley.

Cell competition is a tumor-suppressive mechanism that can remove dangerous cells from a tissue. Tumor Necrosis Factor (TNF)-stimulated c-Jun N-terminal kinase (JNK) activation eliminates clonal malignancies from Drosophila imaginal epithelia, but why this signaling axis is specifically activated in tumor cells is not known. Here we show how receptor-mediated JNK signaling is triggered to drive competitive elimination of cells mutant for Scribble-class tumor suppressors. The TNF ligand driving cell competition derives not from local sources but instead is constantly present in circulation. This systemic ligand is latent because it is spatially segregated from its receptor, which is apically localized in the lumen of normal tissue. Polarity defects associated with malignant transformation cause the TNF receptor to mislocalize basolaterally, allowing access to ligand and autonomous activation of apoptotic signaling. Related phenomena are seen at the wound site of physically damaged epithelia and are required for repair. These findings reveal a mechanism of polarized separation of ligand and receptor that can generally monitor epithelial integrity to promote tissue homeostasis.

198 Cell competition as a selection against aneuploid cells Zhejun Ji[1], Jacky chuen[1], *Nicholas Baker*[1] 1) Albert Einstein Col Med.

Aneuploidy and other large scale genetic changes cause human birth defects and spontaneous abortions, occur in nearly all cancers, and are hallmarks of aging. There is evidence that cells with such large scale genetic changes can be eliminated from otherwise normal tissues. Cell competition provides a plausible mechanism for recognizing such cells. In *Drosophila*, cell competition leads to the elimination from mosaics of cells heterozygous for mutations in any of most ribosomal protein genes. Because 80 ribosomal protein genes are spread across the genome, their copy number might be a marker for cells with large-scale genetic changes. Accordingly, we report that cells heterozygous for large deletions can be eliminated when they include ribosomal protein genes, and that elimination depends on the cell competition pathway that we have characterized genetically in *Drosophila*, in which one particular ribosomal protein, RpS12, induces expression of a transcription factor, Xrp1, which enables elimination of *Rp* mutant cells. Without cell competition, cells carrying large deletions can remain and contribute to adult structures, even when they include *Rp* genes. Cell competition is also responsible for removing the majority of cells eliminated by the genetic damage they receive after irradiation, indicating that *Rp* copy number changes must be a general feature of most kinds of significant genome damage. Based on the cancer predisposition associated with Diamond Blackfan Anemia, the ribosomopathy caused by human *Rp* mutations, we suggest that cell competition normally removes ~80% of preneoplastic cells in humans.

199 Genetic basis and evolutionary context for structural color shift in the Buckeye butterfly (*Junonia coenia*) *Rachel Thayer*[1,2], Frances Allen[1], Nipam Patel[1,2] 1) University of California Berkeley, Berkeley, CA; 2) Marine Biological Laboratory, Woods Hole, MA.

Structural color is a pervasive natural phenomenon, caused by photonic nanostructures that scatter light. Diverse organisms use structural color to mediate ecological interactions and create specific optical effects such as iridescence. Despite its importance for living systems, the biological processes that generate structural color largely remain elusive. We used *Junonia coenia*, a lab-tractable butterfly with extreme intraspecific structural color variation, to investigate the evolution and genetic regulation of a simple photonic nanostructure. We show that *J. coenia* responded to artificial selection on wing color by a 71% thickness increase of each cover scale lamina, thus shifting wing color from brown to blue. There was no associated change in cover scale pigmentation, providing an opportunity to genetically isolate the loci that specifically regulate the structural component of wing color. We interbred wild-type and artificially selected butterflies to map quantitative trait loci that control 1) nanostructural dimensions and 2) the patterning of blue structural color over the wing surface. Butterflies with CRISPR/Cas9 induced mutations in the *optix* wing patterning gene gain blue scales, due to a thickness increase similar to that observed after artificial selection. Lastly, we show that lamina thickness variation underlies the color diversity that distinguishes seasonal variants, sexes, and species throughout the genus *Junonia*. Thus, quantitatively tuning a single dimension of the existing scale architecture allows butterflies to evolve a broad spectrum of hues over both micro and macroevolutionary time frames. Because the lower lamina is an intrinsic component of typical butterfly scales, our findings imply that lamina structural color influences wing color in many butterfly taxa.

Friday, April 24 11:30 AM - 1:00 PM

Models of Neurological Diseases

207 Probing the Mechanism of ROS-induced Lipid Droplet formation and Implications for Alzheimer's disease *Matthew Moulton*[1,2], Scott Barish[1,2], Jake Harland[1,2], Paul Marcogliese[1,2], Hugo Bellen[1,2,3] 1) Baylor College of Medicine; 2) Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital; 3) Howard Hughes Medical Institute at Baylor College of Medicine.

The apolipoprotein APOE4 is the highest known genetic risk factor for Alzheimer's disease (AD), implicating lipid dysregulation in AD pathogenesis. We have developed an animal model of reactive oxygen species (ROS) induced lipid droplet (LD) formation and age-dependent, progressive neurodegeneration. We previously demonstrated that elevated ROS in neurons triggers the production of lipids that become peroxidated by ROS. These peroxidated lipids are shuttled out of neurons and taken up by glia where they accumulate in LDs. While initially protective, the prolonged sequestration of peroxidized lipids in glia eventually leads to the demise of both glia and neurons. Glial LD formation requires expression of the apolipoprotein, *Glial Lazarillo* (*GLaz*), and the expression of human ApoE4 in fly retinal glia reduces glial LD formation and promotes neuronal demise.

However, ApoE3 and, particularly the AD resistant isoform, ApoE2, both facilitate LD formation and offer protection against neurodegeneration. We now seek to understand the mechanism of lipid transport between neurons and glia using our fly model. We have identified genes required for LD formation that overlap with AD risk associated alleles identified in genome wide association studies (GWAS) suggesting that lipid homeostasis is critical in the development and pathogenesis of AD.
Lipid dysregulation may also affect Aβ42-induced neurotoxicity. APOE4 carriers have higher rates of amyloid deposition and lower rates of amyloid clearance than individuals harboring APOE3 or APOE2, suggesting an interplay between lipid transport and Aβ42 clearance. Aβ42 is a lipophillic molecule that binds to APOE and the APOE receptor, LRP1. The major human isoforms of APOE have different lipid and amyloid binding properties, with APOE3 having a higher affinity for lipids and Aβ42 when compared with APOE4. Thus, we hypothesize that lipid uptake in glia may contribute to the clearance of Aβ42 by promoting its uptake in glia. In the presence of ROS, however, the neurotoxic effects of Aβ42 may be enhanced due to the breakdown in LD stability we have documented. Here, we demonstrate that elevated ROS exacerbates Aβ42-induced neurodegeneration in both fly and mouse models. Altogether, our data implicate a link between lipid uptake, ROS, Aβ production, and neurodegeneration and suggest that ROS mitigation could be an important therapeutic strategy for AD.

208 Single Cell Transcriptomics Reveals Misregulated Cellular and Molecular Networks in a Mouse Model of Fragile X Syndrome *Elisa Donnard*[1], Huan Shu[2], Thuyvan Luu[2], Paul Greer[2], Manuel Garber[1,2] 1) Bioinformatics and Integrative Biology, University of Massachusetts Medical School; 2) Program in Molecular Medicine, University of Massachusetts Medical School.

Fragile X syndrome (FXS) is the most common form of inherited intellectual disability and results from the inactivation of a single gene, *Fmr1*. FXS shows striking phenotypic differences in synaptic morphology and function, with severe impact to the cognitive abilities of patients which are replicated in animal models. Despite decades of intensive research, we still lack an overview of the molecular and biological consequences of the loss of the encoded protein FMRP in the brain. One of the better established roles of FMRP is that of a translational repressor of specific mRNAs in neurons, and its absence has also been shown to correlate with a mild increase in basal protein synthesis in the hippocampus. Previous attempts to detect transcriptional changes using bulk RNA sequencing (RNA-Seq) of the brain have likely been hindered by the complexity and heterogeneity of the tissue. Furthermore, in cell culture approaches, even though the profiled cells are homogeneous, the transcriptional program may not accurately reflect the *in-vivo* expression resulting from the interaction between multiple cell types. To obtain a first systematic transcriptional landscape of the FXS brain, we applied single cell RNA-Seq (scRNA-Seq) to profile over 21,000 cells from the dissociated cortex of wild type (FVB.129P2-*Pde6b*$^{+}$ *Tyr*$^{c\text{-}ch}$/AntJ) and FVB.129P2-*Pde6b*$^{+}$ *Tyr*$^{c\text{-}ch}$ *Fmr1*^{tm1Cgr}/J *Fmr1* knockout (KO) mice. We find that loss of FMRP resulted in changes across a broad spectrum of processes including translation, ion homeostasis and synaptic functions, with neurons being the most highly impacted cell type. Furthermore, many of these processes show opposite effects in neurons and astrocytes, revealing a drastically different impact of FMRP loss in these two cell types. We find that mRNAs annotated as FMRP targets are specifically downregulated in neurons, and that excitatory and inhibitory neurons show unique sets of downregulated genes, which supports the excitatory-inhibitory imbalance theory of autism. Analysis of microglia subtypes revealed a depleted *Ms4a7*+ population in FXS, which suggests a cell differentiation deficit during this critical period. Our data is the first attempt to dissect the cell-type specific contributions to FXS using the power of scRNA-Seq. Our results show how the loss of FMRP affects the intricate interactions between brain cell types, which could potentially open new doors to therapeutic interventions. It showcases how a systems approach can guide future studies on the mechanisms behind complex diseases like autism.

209 Downregulation of innate immunity suppresses seizures in *prickle* mutants Krishna M. Nukala[1], Anthony J. Lilienthal[1], Shu Hui Lye[2], Alexander G. Bassuk[3], Stanislava Chtarbanova[2], J. Robert Manak[1,3] 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Department of Biological Sciences, University of Alabama, Tuscaloosa, AL; 3) Department of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, IA.

Epilepsy is a neurological disorder characterized by seizures and affects ~1% of the population. Previous work in our laboratory has shown that mutations in *prickle* (*pk*), a planar cell polarity gene, cause myoclonic-like seizures and ataxia in *Drosophila*, similar to what is observed in humans carrying mutations in orthologous *PRICKLE* genes. To begin to explore what transcriptional changes in seizure-prone *pk* flies might underlie the seizure phenotype, we performed a transcriptome and gene ontology (GO) analysis of *pk* mutant brains compared to controls. This analysis revealed 'Immunity' to be the most statistically significant GO category, including genes that encode anti-microbial peptides (AMPs) which are direct targets of the NF-kB transcription factor Relish (Rel). Although AMPs are strongly induced in response to bacterial and/or fungal pathogens, their expression is also upregulated in various mutants exhibiting neurological defects and Rel-dependent neurodegeneration. Using genetic, histological and immunohistochemical approaches, we chose to investigate whether *pk* mutant brains also undergo immune-mediated neurodegeneration, and if so, whether reducing innate immune activation might suppress the neurodegeneration and seizure phenotypes. Here, we report that *pk* mutants exhibit a continual accumulation of degenerating neurons throughout adulthood that correlates with a progressive, age-dependent increase in seizure activity when compared to controls. Notably, genetic removal of *Relish* in *pk* null mutants leads to both a reduction in neurodegeneration as well as a robust (but not complete) reduction in seizure activity, demonstrating that the innate immune response plays a significant role in the neurodegeneration and, ultimately, seizure phenotype in *pk* mutants. To the best of our knowledge, this is the first connection between an innate immune response and epilepsy, and suggests a potential novel treatment modality for epilepsy patients carrying *PRICKLE* mutations. This work is supported by NIH/NINDS R01NS098590.

210 Creating and Understanding Next-Generation Mouse Models of Alzheimer's Disease *Gregory Carter*[1], Gareth Howell[1], Michael Sasner[1], Adrian Oblak[2], Stacey Rizzo[3], Paul Territo[2], Bruce Lamb[2] 1) The Jackson Laboratory; 2) Indiana University; 3) Univeristy of Pittsburgh.

Alzheimer's disease (AD) is the most common form of dementia, with no effective prevention strategies or treatments. Therapeutic developments have been limited by incomplete understanding of the disease and a lack of translatable animal models. Current models are based on early-onset, familial variants whereas most diagnosed cases are late onset with multiple causal factors. Now that genome-wide association studies have identified dozens of candidate loci with unknown functional roles, we are able to engineer a new generation of late-onset mouse models.

To this end, the Model Organism Development and Evaluation for Late-onset AD (MODEL-AD) Program has been established to create animal models of late-onset AD for functional studies and preclinical testing. To date, we have created over 20 new mouse models based on human genetic risk factors. Example candidates include variants in *APOE*, *TREM2*, and *ABCA7*. Primary screening for disease relevance has been performed using gene expression and frailty assays, with additional deep phenotyping including *in vivo* imaging, neuropathology, proteomics, and metabolomics on select strains staged at multiple ages.

All data are systematically aligned to orthologous data from human studies to identify specific AD-related alterations in each mouse model, thereby linking genetic variants to potential functional roles in disease origin and progression. Extensive preclinical studies have been performed to identify target engagement and disease modification for multiple candidate therapeutics. We will highlight multiple results with potential disease relevance and report on initial preclinical outcomes. These findings include modifications in brain metabolism for the specific disease alleles observed by *in vivo* imaging and transcriptomics, vascular deficits in *APOE4* models, a catalog of multi-omic outcomes from multiple strains, and modifications of genetic effects across different inbred strain backgrounds. We will also describe our extensive data and resource sharing mechanisms to broadly disseminate all models and associated data without restrictions.

211 Genome-wide discovery of human-gene toxicity modifiers of α-synuclein *Ishita Haider*[1], Yali Chi[1], Shuzhen Chen[1], Elliott Hayden[1], Shulin Ju[1], Quan Zhong[1] 1) Wright State University, Dayton, OH.

Lewy bodies, mainly composed of abnormal aggregations of a small lipid-binding protein primarily expressed in the brain, α-synuclein, is the pathological hallmark of Parkinson's disease (PD) and Lewy body dementia. Both missense mutations and increased copy numbers of the *SNCA* gene encoding α-synuclein lead to PD. Although the membrane-associated function of α-synuclein and its toxicity to neurons have been suggested, the mechanisms still remain unclear. Interestingly, both the membrane association and the cellular toxicity of α-synuclein can be recapitulated in yeast. Using yeast genetic screens, hundreds of suppressor and enhancer genes have been discovered, revealing complex cellular processes involved in α-synuclein toxicity. Mammalian homologs of several yeast modifier genes were found to have a similar effect in neurons, supporting the existence of conserved mechanisms pertinent to α-synuclein toxicity in yeast. We reasoned that human genes without yeast homologs cannot be identified in genetic screens using only yeast genes. Furthermore, genetic screens using yeast models containing toxic levels of α-synuclein might not be effective in uncovering factors that trigger the initial molecular events promoting α-synuclein aggregation and toxicity. To address these limitations, we expressed *SNCA* at a much lower level that does not lead to aggregation or toxicity in yeast. We found unique plasma membrane localization of α-synuclein in this non-toxic model, consistent with its lipid and protein binding capacity. We next constructed an overexpression library containing ~15,000 human-gene clones. Using this library, we identified human-gene suppressors and enhancers of α-synuclein toxicity. Interestingly, many of the identified human modifier genes have membrane-associated functions, including vesicle-mediated cellular trafficking, cell polarity regulation, lipid modification, etc. Characterization of some of the modifier genes reveals that the reduced or enhanced toxicity of α-synuclein does not always correlate with changes in its ability to form cytoplasmic foci, suggesting distinct modifier mechanisms. Ongoing research focuses on understanding cellular changes induced by α-synuclein and the different groups of human modifier genes. Such modifiers may provide insights into the genetic buffering in neurodegeneration.

368 TRPV4 disrupts mitochondrial transport and causes axonal degeneration via a CaMKII-dependent elevation of intracellular Ca2+ Brian Woolums[1], Brett McCray[1], Hyun Sung[1], Masashi Tabuchi[1], Jeremy Sullivan[1], Will Aisenberg[1], Mark Wu[1], Charlotee Sumner[1], *Thomas Lloyd*[1] 1) Johns Hopkins School of Medicine, Baltimore, MD.

Finely tuned calcium dynamics are essential for normal neuronal function, and excessive calcium flux is implicated in the pathogenesis of neurodegenerative diseases. The mechanisms that lead to neuronal dysfunction and degeneration downstream of calcium entry remain poorly defined. Mutations in the non-selective cation channel TRPV4 cause inherited neurodegeneration syndromes, including a motor-predominant peripheral neuropathy called Charcot Marie Tooth disease subtype 2C (CMT2C), but the molecular mechanisms are unknown. Here, we show that expression of a neuropathy-causing TRPV4 mutant (TRPV4[R269C]) in Drosophila causes dose-dependent neuronal dysfunction and axonal degeneration, which are rescued by genetic or pharmacological blockade of TRPV4 channel activity. To investigate signaling mechanisms involved in mutant TRPV4 mediated toxicity, we performed a genetic modifier screen in the fly wing (using the CCAP-GAL4 driver), and identified CaMKII as a potent genetic modifier of mutant TRPV4. TRPV4[R269C]triggers increased intracellular calcium through a calcium/calmodulin-dependent protein kinase II (CaMKII)-mediated mechanism, and CaMKII inhibition prevents both increased intracellular calcium and neurotoxicity in *Drosophila*and cultured primary mouse neurons. Importantly, TRPV4 activity impairs axonal mitochondrial transport in both Drosophila larval motor axons and in cultured mouse trigeminal neurons. We further show that TRPV4-mediated neurotoxicity is modulated by the calcium-binding mitochondrial GTPase Miro. Our data support a model whereby increased intraneuronal calcium downstream of TRPV4-induced CAMKII activation causes neurodegeneration by inhibiting miro-mediated axonal transport of mitochondria. Our findings highlight an integral role for CaMKII in neuronal TRPV4-associated calcium responses, the importance of tightly regulated calcium dynamics for mitochondrial axonal transport, and the therapeutic promise of TRPV4 antagonists for patients with TRPV4-related neurodegenerative diseases.

Friday, April 24 11:30 AM - 1:00 PM

Through a Population Genetics Lens (PEQG)

218 Attacks on genetic privacy via uploads to genealogical databases *Michael Edge*[1], Graham Coop[1] 1) University of California, Davis.

Direct-to-consumer (DTC) genetics services are popular for genetic genealogy, with tens of millions of customers as of 2019. Several DTC genealogy services allow users to upload their own genetic datasets in order to search for genetic relatives. A user and a target person in the database are identified as genetic relatives if the user's uploaded genome shares one or more segments in common with that of the target person---that is, if the two genomes share one or more long regions identical by state (IBS). IBS matches reveal some information about the genotypes of the target person. We describe several methods by which an adversary who wants to learn the genotypes of people in the database can do so by uploading multiple datasets. For example, using a method we call IBS tiling, we estimate that an adversary who uploads approximately 900 publicly available genomes could recover at least one allele at SNP sites across the majority of the genome of a median person of European ancestries. In databases that detect IBS segments using unphased genotypes, approximately 100 uploads of falsified datasets can reveal enough genetic information to allow accurate genome-wide imputation of every person in the database. We provide simple-to-implement suggestions that will prevent the exploits we describe. We also discuss the process of interacting with DTC genealogy services about these and other security issues, focusing on the case of GEDmatch, a database of more than a million users.

219 A Drosophila telomere protein evolves adaptively to contain telomeric retrotransposons Bastien Saint-Leandre[1], *Mia Levine*[1] 1) Dept of Biology and Epigenetics Institute, University of Pennsylvania.

Intra-genomic conflicts erupt when selfish elements proliferate at the expense of host fitness. The molecular players, mechanisms, and functional consequences of such conflicts are poorly defined. To investigate intra-genomic conflict in molecular detail, we leveraged the Drosophila telomere as a model. While most eukaryotes use telomerase to elongate chromosomes, Drosophila instead relies on specialized retrotransposons that insert specifically at telomeres. We recently reported rapid evolution of both telomeric retrotransposons and telomere-binding proteins, raising the possibility of intra-genomic conflict between the two parties. To empirically test the hypothesis that telomere proteins evolve rapidly to police telomeric retrotransposons, we focused on HOAP, an adaptively evolving telomere protein that protects telomeres from lethal end-to-end chromosome fusions. We cleanly swapped into *D. melanogaster* either a Flag-tagged, *D. yakuba* version of HOAP ("HOAP[yak]") or Flag-tagged *D. melanogaster* version ("HOAP[mel]", our negative control). HOAP[yak] localizes robustly to telomeres, protects chromosomes from end-to-end fusions, and fully rescues viability. However, HOAP[yak] disrupts telomere regulation—we observed depleted H3K9me3 (a silent, heterochromatin histone mark) at chromosome ends and concomitant hypertranscription of the telomeric retrotransposons. We reasoned that more accessible telomeric chromatin may promote telomeric retrotransposon insertions. To address this possibility, we conducted experimental evolution of HOAP[mel] and HOAP[yak] genotypes for 50 generations. We probed retrotransposon proliferation using both whole genome sequencing and cytogenetics. In HOAP[yak] flies only, we observed long telomeres and 'escape events' of typically telomere-restricted elements into non-telomeric locations. Moreover, this long-telomere genotype suffers a female fertility cost. These data are consistent with an ongoing molecular arms race shaping Drosophila telomeres and reveal the molecular players and mechanisms of an intra-genomic conflict. .

220 Identifying sites under positive selection on viral proteins *Jonathan Mah*[1,2], Sarah Hilton[1,2], Jesse Bloom[1,2,3] 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) University of Washington, Seattle, WA; 3) Howard Hughes Medical Institute, Seattle, WA.

RNA viruses, such as influenza, are known for their ability to fix many mutations over a short period of time. Some of these mutations lead to amino acid substitutions in regions of the virus targeted by the immune system. Such changes are often selected for because they confer a fitness advantage by allowing the virus to escape immune response. This rapid evolution is often detrimental to human health as it limits the effective duration of vaccine-conferred immunity. However, rapid evolution in viruses also offers a unique system in which to investigate important basic questions in evolutionary biology, especially given that recent advances in deep sequencing now allow us to observe virus evolution in almost real-time. Here, we use empirical data from a high-throughput functional assay to define null expectations for the evolutionary rate of viruses when subject to only purifying selection. These null models differ from traditional phylogenetic models in that they describe evolutionary constraints on a site-specific basis, offering a major advantage in statistical power. We have implemented a random-effects-likelihood approach to identify sites which deviate from our null models by an unexpectedly high evolutionary rate, i.e., sites under positive selection. We use simulations to rigorously evaluate the statistical power and accuracy of our approach. Preliminary results show that our method outperforms other methods for identifying sites under positive selection. Next, we will apply these methods broadly to several viral proteins, e.g., influenza hemagglutinin, HIV envelope glycoprotein, and Zika virus envelope protein. Identifying sites under positive selection on viral proteins could help predict future circulating strains, inform structure-based vaccine design, and help to understand basic evolutionary questions.

221 Mutualistic interactions shape adaptation in a model yeast-algae community *Sandeep Venkataram*[1], Erik Hom[2], Sergey Kryazhimskiy[1] 1) University of California - San Diego, San Diego, CA; 2) University of Mississippi, Oxford, MS.

Global change and human-assisted migration can generate novel mutualisms between species and even taxa with no prior history of interaction. Studies in model microbial byproduct mutualisms have found strong evidence for rapid evolution, suggesting that novel mutualisms may drastically alter the evolutionary fate of an organism even on short timescales. However, we have little empirical knowledge of how novel mutualisms modify key evolutionary parameters such as the spectrum of adaptive mutations, their distribution of fitness effects and the overall repeatability of evolution. Here we use laboratory evolution experiments of the budding yeast *Saccharomyces cerevisiae* in the presence or absence of a novel mutualistic partner, the alga *Chlamydomonas reinhardtii*, to answer these questions. We further utilize DNA "barcode" technology in the yeast to aid in the isolation and characterization of hundreds of independently evolved adaptive yeast mutants from each treatment. We find that the presence of algae dramatically shifts the spectrum of adaptive mutations in yeast. Surprisingly, this qualitative shift in the targets of adaptation is not accompnied by a substantial shift in their distributions of fitness effects, with most adaptive yeast mutants having similar fitness effects in both monoculture and coculture regardless of the treatment they evolved in. In particular, we find no evidence of fitness trade-offs between the two treatments. Finally, we show that adaptive yeast mutants modify both yeast and algal growth in coculture. The nearly three-fold variation in both yeast and algal density across different yeast mutants suggests that eco-evolutionary feedbacks can arise rapidly and can be highly non-repeatable. Taken together, our results imply that even transient interactions with a strong mutualist could drive historically-contingent adaptation.

222 Most cancers carry a substantial deleterious load due to Hill-Robertson interference *Susanne Tilk*[1], Christina Curtis[1], Dmitri Petrov[1], Chris McFarland[1] 1) Stanford University.

Cancer genomes exhibit surprisingly weak signatures of negative selection. This may be because tumors evolve either under very weak selective pressures ('weak selection') or under conditions that prevent the elimination of many deleterious passenger mutations ('poor efficacy of selection'). The weak selection model argues that the majority of genes are only important for multicellular function. The poor efficacy of selection model argues, in contrast, that genome-wide linkage in cancer prevents many deleterious mutations from being removed via Hill-Robertson interference. Since these linkage effects weaken as mutation rates decrease, we predict that cancers with lower mutational burdens should exhibit stronger signals of negative selection. Furthermore, because linkage affects driver mutations as well, low mutational burden cancers should also show stronger evidence of positive selection in driver genes. Neither pattern — in drivers or passengers — is expected under the weak selection model. We leverage the 10,000-fold variation in mutational burden across cancer subtypes to stratify tumors by their genome-wide mutational burden and used a normalized ratio of nonsynonymous to synonymous substitutions (dN/dS) to quantify the extent that selection varies with mutation rate. We find that appreciable negative selection (dN/dS ~ 0.4) is present in tumors with a low mutational burden, while the remaining cancers (96%) exhibit dN/dS ratios approaching 1, suggesting that the majority of tumors do not remove deleterious passengers. A parallel pattern is seen in drivers, where positive selection attenuates as the mutational burden of cancers increases. Two additional orthogonal lines of evidence support the weak efficacy model: passengers are less damaging in low mutational burden cancers, and patterns of attenuated selection also emerge in Copy

Number Alterations. Finally, we find that an evolutionary model incorporating Hill-Robertson interference can reproduce both patterns of attenuated selection in drivers and passengers if the average fitness cost of passengers is 1.0% and the average fitness benefit of drivers is 19%. Collectively, our findings suggest that the lack of signals of negative selection in most tumors is not due to relaxed selective pressures, but rather the inability of selection to remove individual deleterious mutations in the presence of genome-wide linkage. As a result, despite the weak individual fitness effects of passengers, most cancers harbor a large mutational load (median ~40% total fitness cost) and succeed due to acquisition of additional strong drivers (~5 with an overall benefit of ~130%). Understanding how this deleterious load is overcome may help identify cancer vulnerabilities that may be targeted by new and existing therapies.

Friday, April 24 11:30 AM - 1:00 PM

New Technology and Resources

212 Large-scale phenotypic profiling of yeast subcellular compartments using high-content screening at single-cell resolution *Mojca Mattiazzi Usaj*[1], Nil Sahin[1,2], Myra Masinas[1], Matej Usaj[1], Helena Friesen[1], Charles Boone[1,2], Brenda Andrews[1,2] 1) The Donnelly Centre, University of Toronto; 2) Department of Molecular Genetics, University of Toronto.

The systematic assessment of mutant phenotypes using high-content screening with single cell level readouts is a powerful approach to study the genotype-to-phenotype relationship. We have developed a combined experimental-computational pipeline to explore the effect of thousands of genetic perturbations on the morphology of subcellular compartments in yeast. We used synthetic genetic array analysis (SGA), that automates yeast genetics, to introduce fluorescent markers for 18 subcellular compartments into yeast non-essential and essential gene mutant arrays. High-throughput imaging of the constructed fluorescently tagged arrays was followed by semi-unsupervised phenotype identification, and single-cell phenotype assignment using a compendium of neural network classifiers. The final dataset includes over 1 million 2-channel fluorescence images of >150 million cells classified into one of several dozen mutant phenotypes. This unbiased dataset enables the assembly of multi-parametric phenotypic profiles that allow comprehensive quantification of the penetrance of genetic perturbations, and identification of specific pathways and networks of genes involved in modifying subcellular structures.

213 The design and assembly of synthetic yeast chromosome VIII *Stephanie Lauer*[1], Jingchuan Luo[2], Weimin Zhang[1], Jef Boeke[1] 1) NYU Langone; 2) University of California, San Francisco.

The synthetic yeast genome project (Sc2.0) aims to build the first eukaryotic genome, which is a groundbreaking achievement in the field of synthetic biology. Assembly of 16 designer *Saccharomyces cerevisiae* chromosomes nears completion, and consolidation of these chromosomes into a single synthetic strain is already underway. One priority of Sc2.0 is to maintain wild-type fitness despite the introduction of key design features. Alterations include the recoding of TAG stop codons, the removal of transposable elements, and the use of synonymous substitutions in open reading frames to create PCRTag watermarks. These changes are relatively modest but can result in fitness defects, providing a unique opportunity to probe the flexibility of the yeast genome. Construction of synthetic chromosome VIII (synVIII) was performed using a systematic approach that replaces wild-type sequences with “chunks” of synthetic DNA, alternating auxotrophic markers with each integration. We used this strategy to generate several different strains in parallel, then mated the semi-synthetic strains to produce a single yeast strain with full-length synVIII. In addition to the design elements mentioned above, 11 tRNA genes and 18 introns were removed, resulting in a 10% reduction in chromosome length. A total of 183 *loxPsym* sites were also added, enabling the use of an inducible evolution system called SCRaMbLE (synthetic chromosome rearrangement and modification by *loxPsym*-mediated evolution). Recombination between symmetric *LoxPsym* sites results in gene duplications, deletions, and other complex structural rearrangements. SCRaMbLE has been used for a variety of biotechnology applications, but we are currently testing the effect of iterative genome rearrangement on organism fitness and reproductive potential, which will elucidate the extent to which genome structure shapes phenotypic diversity.

214 Quantifying material tissue properties in cellularizing *Drosophila* embryo using soft bendable cantilevers *Konstantin Doubrovinski*[1], Joel Tchoufag[1,2], Swayamdipta Bhaduri[1] 1) UT Southwestern; 2) University California Berkeley.

How tissue material properties and forces determine the final shape of tissues and organs is a central question in developmental biology. Developing computational models of morphogenesis absolutely requires independent measurements of material tissue properties. If this information is not known from measurements, multiple models may be consistent with the data and therefore an individual model would lack predictive power. To address this problem, our lab has developed a range of techniques that allow direct in vivo measurements of material properties in developing embryos. Here we present a novel technique that is based on the use of micron-sized bendable cantilevers. The cantilever is micro-fabricated from a soft PDMS polymer. It is easily visualized in the interior of the embryo due to the addition of a strongly fluorescent dye. When introduced into a *Drosophila* embryo during cellularization though an incision in the vitelline membrane, the cantilever does not interfere with morphogenesis of the tissue located away from the incision site. Exploiting the fact that embryonic cells are open to the yolk during cellularization, we were able to insert the cantilever into a single cell, and exert relatively large pulling force on a single cellular edge. These manipulations allow us to study local cell rearrangements as well as global tissue deformation arising from very local application of concentrated force. Importantly, this technique directly quantifies the applied force from the instantaneous bending of the cantilever. Based on these measurements, we have constructed a quantitative mechanical model of fruit fly embryonic epithelium which is directly informative about forces that underly morphogenesis in the early embryo.

215 Mapping cell types and gene regulatory networks in the developing Drosophila brain using single-cell transcriptomics and epigenomics *Jasper Janssens*[1,2], Sara Aibar[1,2], Dafni Papasokrati[1,2], Samira Makhzami[1,2], Valerie Christiaens[1,2], Gert Hulselmans[1,2], Maxime De Waegeneer[1,2], Seppe De Winter[1,2], Mariska Reniers[1,2], Ibrahim Ihsan Taskiran[1,2], Carmen Bravo González-Blas[1,2], Stein Aerts[1,2] 1) VIB-KU Leuven Center for Brain & Disease Research; 2) KU Leuven Department of Human Genetics.

Neuronal cell identity is determined by gene regulatory networks (GRNs) in which combinations of transcription factors (TFs) regulate the expression of their target genes. To map gene regulatory networks for the cell types in the Drosophila brain, we previously built a single-cell RNA-seq atlas characterizing the transcriptome of over 80 cell types in the adult brain and inferred the GRNs underlying the major neuronal and glial types using SCENIC. Here we present an extension of this dataset using single-cell chromatin accessibility (scATAC-seq) of the larval, pupal, and adult fly brain.

We have analyzed more than 200,000 cells using topic modelling (cisTopic) and identified over 30 adult cell clusters and their corresponding accessibility profiles. These profiles allow us to link enhancers to Janelia GAL4-lines; to predict novel enhancer targets; to increase the specificity of GAL4 lines; and to improve predictions of master transcription factors in different cell types, including Kenyon Cells and T4/T5 neurons. Linking transcriptome and epigenome data allowed the association of distal enhancers to target genes, and to map developmental trajectories during neuroblast maturation in the larval brain. Finally, analyzing the DNA sequence of co-accessible regions with deep learning methods we identified motifs and candidate transcription factors underlying neuronal and glial differentiation, and further exploited the trained models to predict chromatin accessibility QTLs across a cohort of inbred fly lines.

216 Light-dependent spatiotemporal control of gene expression a la carte: from discrete patterns to emoji-like shapes. *Lorena de Mena*[1], Diego Rincon-Limas[1] 1) University of Florida.

Tools that enable manipulation of gene function are critical to define its contribution to normal development and disease. Unfortunately, current inducible expression systems in flies preclude accurate spatiotemporal control of gene expression and do not allow for sub-territorial manipulations within a given tissue. What if transgene expression could be manipulated a la carte with a switch triggered by light? To address this question, we propose a new and powerful photoactivable gene expression system in Drosophila referred to as PhotoGal4. The light "switch" itself is a sensitive and reversible photosensor called phytochrome B (PhyB), a cytoplasmic chromoprotein that controls growth and development in plants. In response to red light, PhyB is activated, but it returns to the inactive state under far-red light. Thus, we assembled a single protein device consisting of several unrelated modules, based on the heterodimerization of PhyB with its cofactor Pif6. To test the system, we capitalized on the well-characterized GMR enhancer to drive specific expression to the Drosophila eye territory. Thus, we engineered flies containing all the elements required to induce transcription of genes by light and crossed them with a UAS-GFP reporter line to test PhotoGal4 functionality. We found that upon red light stimulation, PhotoGal4 efficiently triggers gene expression in long-term ex vivo cultures of eye discs at different developmental stages. We also found that manipulation of light intensity and duration of the stimuli gives control over reporter dose-response. Then, we used specifically illuminate a defined group of cells within the GMR expression domain, while keeping the rest of the GMR territory in the dark. Strikingly, we found robust GFP expression only within the restricted area of illumination even to specifically engrave a series of "emoji-like" face within the prepupa retina. To our knowledge, this is the first time that a targeted personalized sub-pattern of gene expression is induced in a light-dependent manner within time and space dimensions. Thus, we anticipate that that PhotoGal4 will be a valuable resource for the Drosophila community to investigate complex and multistage biological, developmental and pathological processes with unprecedented resolution. This work was supported by the NIH grant AG059871 to DERL and by an HHMI-LSRF postdoctoral fellowship to LDM.

217 Defining the Geometry of Life Across Model Organisms as a Unifying Framework for Computational Phenomics *Keith Cheng*[1], Santosh Girirajan[2], David Hall[3], Steve Wang[4], Sharon Huang[2], John Liechty[2], Yuxi Zheng[2], Patrick La Riviere[5], Hong Ma[2], Elliot Meyerowitz[6], Vasant Honovar[2], Khai Chung Ang[1], Samarth Gupta[2], Amogh Adishesha[2], Yifu Ding[1], Daniel Vanselow[1], Maksim Yakovlev[1], Spencer Katz[1], Alex Lin[1], Damian van Rossum[1], Carolyn Zaino[1] 1) Penn State College of Medicine, Hershey, PA; 2) Pennsylvania State University, University Park, PA; 3) Albert Einstein College of Medicine, The Bronx, NY; 4) OmniVision Technologies Inc., Santa Clara, CA; 5) University of Chicago, Chicago, IL; 6) California Institute of Technology, Pasadena, CA.

Phenotyping multicellular organisms is central to organismal genetics and lies at the core of medicine, but it is presently impractical to phenotype across all cell types and organ systems. Large scale phenotyping projects would benefit from a Computational Phenomics that enables the comprehensive, computationally accessible, quantitative, objective, and reproducible measures of cellular and tissue features of normal and abnormal microanatomy in whole organisms. Fortunately, cell types in multicellular organisms are finite in number, and cytology and architectural relationships in tissue are conserved across phylogeny. Tissue diagnostics based on histology is based on conservation of tissue-specific, micron-scale cellular and tissue features that characterize physiological and disease states. A "Geometry of Life", in which all cell types, tissues and phenotypes are defined mathematically, may serve this goal.
Based on its phylogenetically-conserved cell types, small body size, and common usage in genetic and chemical screens, we used zebrafish (Danio rerio) to develop a 3D form of histology, X-ray histotomography, based on the principles of micro-CT. The physics of synchrotron X-ray sources, and customizations of sample preparation and imaging parameters allowed the creation of instrumentation to achieve a combination of large field-of-view, high resolution, tissue contrast, and pan-cellular 3D imaging needed for large-scale tissue phenotyping. The generated datasets allow histopathological interpretation from virtual slices of physically intact specimens, as well as novel computational approaches including machine learning, for multi-scale, morphological phenotyping. Creating a synchrotron-based workflow will facilitate genetic and chemical phenome projects, with potential applicability across model and non-model organisms. Data-driven multi-scale characterization of the geometric and morphological features of tissue types across phylogeny will help to reunify biology in our age of specialization. This systems approach to morphological, computational phenotyping will facilitate objectivity, precision, reproducibility, and relevance across model systems. Integration of phenotypes based on morphological analyses with data from additional modalities e.g., gene expression. may facilitate a more comprehensive understanding of the complex interactions of genes, environment, and disease in determining phenotype.

Friday, April 24 11:30 AM - 1:15 PM

Education Session

200 Bear Hair Snares & DNA: Impact of Collaborative Instruction on Molecular Genetics and Mammalogy Students *Julie Hall*[1], Laroy Brandt[1] 1) Lincoln Memorial University.

Often times, students struggle with identifying the connections between the content of their coursework. In these cases, a common student complaint is that they do not understand why they have to take course "A" when it has no bearing on their future careers. For example, as wildlife biology is typically viewed as a field-based discipline, undergraduate wildlife students may often feel that lab-based courses like molecular genetics have no application within their discipline. With this in mind, we developed and implemented a collaborative instruction module between the Molecular Genetics and Mammalogy courses during the fall 2018 semester at Lincoln Memorial University. This instruction module integrated a field-based exercise in our Mammalogy course (non-invasive monitoring of mammal populations) with a laboratory-based exercise in our Molecular

Genetics course (DNA extraction and use in population studies). Specifically, students within the Mammalogy course deployed and monitored bear hair snare stations in southeastern Kentucky, and the students within the Molecular Genetics course processed collected hair samples. Emphasis was placed on the real-world collaborative aspect of wildlife conservation genetics in an attempt to aid students in their understanding of the importance of various courses. The loop of this collaboration was closed with students from the Mammalogy course presenting the molecular results, as it pertained to the field data they collected, to the Molecular Genetics students. To evaluate the impact that this collaborative instruction module had on their understanding of course content, as well as the interrelatedness of these two subjects, students were given a post course survey. Based on our results, we feel that such a collaboration not only enhances student understanding but also provides students a "real world" example of how similar projects are often accomplished.

201 Promoting learn*ing* and learn*er* -centered teaching of genetics and bioinformatics with the Assessment Evaluation Rubric *Rochelle Tractenberg*[1] 1) Georgetown University and the Collaborative for Research on Outcomes and -Metrics.

It is common to create course material for the higher education context that accomplishes content-driven teaching goals, and then develop assessments (quizzes, exams) based on the target content. Content-driven assessment can tend to support teaching- or teacher- centered instruction. Adult learning and educational psychology theories suggest that instead, assessment should be aligned with learning objectives. The Genomics Education Alliance (GEA) was funded 2018-2020 to develop and curate classroom-based undergraduate research experience (CUREs) materials and part of the project is to evaluate the assessments that these materials include. To accomplish this aim, and also to support the alignment of assessments with instruction across the higher education life sciences, the Assessment Evaluation Rubric (AER) was developed. This rubric is intended to support the systematic evaluation of assessments that are included in materials that are curated by the GEA; however the AER can also be utilized to guide the development and evaluation/revision of assessments that are already used, whether or not these relate to genomics or to CUREs. The AER evaluates four features of an assessment: its general alignment with learning goal(s); whether the assessment is intended to/effective as formative or summative; whether some systematic approach to cognitive complexity is reflected; and whether the assessment (instructions as well as results) itself is clearly interpretable. Each dimension (alignment; utility; complexity; clarity) has four questions. Any assessment can be rated "present/absent" or "present/present, needs clarification/absent", or along other dimensions, depending on the user. In the final year of the GEA funding, we are seeking to train assessment evaluators who can use the AER consistently; however, any instructor can use the AER to evaluate their own assessments and ensure that their quizzes and tests promote learn*ing* and learn*er* centered teaching.

202 The Genomics Education Alliance: scalable, sustainable Infrastructure for undergraduate course-based research experiences *Jason Williams*[1], Wilson Leung[2], Anne Rosenwald[4], Vincent Buonaccorsi[3], Rochelle Tractenberg[4], Laura Reed[5], Arthur Hunt[6], Sandesh Subramanya[7], Sarah CR Elgin[2], Emily Wiley[8], Douglas Chalker[2] 1) Cold Spring Harbor Laboratory, DNA Learning Center; 2) Washington University in St. Louis ; 3) Juaniata College; 4) George Washington University; 5) University of Alabama; 6) University of Kentucky; 7) Austin Community College; 8) Claremont McKenna College.

Genomics presents a rich platform for building computational and data literacies for biology students — from high school to undergraduate and beyond. The Genomics Education Alliance (GEA) is a newly NSF-funded research collaboration network (RCN-UBE #1827130). This alliance brings together several established projects in genomics education (e.g. the Genomics Education Partnership, CyVerse DNA Subway, GCAT-SEEK, Genome Solver, Ciliate Genomics Consortium, and others) to combine efforts and seek common solutions. A major goal is to establish a common platform with a core set of computational tools and data resources that can be maintained up-to-date and versioned, facilitating the teaching of genomics in undergraduate biology education. Specifically, we have developed exemplar BLAST, genome browser, and RNA-Seq introductory materials, resolving several technological and pedagogical challenges to provide improved solutions for introducing these common tools.

We highlight the challenges and opportunities already explored by the members as well as technologies and processes under development to create a sustainable network that lowers barriers to teaching genomics to undergraduates at scale. Our focus has been addressing challenges faculty face in combining biology with bioinformatics to develop course-based research experiences (CUREs). Resources being tested by GEA focus on a core set of computational tools, providing instructional materials, assessment instruments, pedagogical methods, and evaluation methodologies. We invite the community of researchers and educators working in genomics and related fields to join us in shaping this alliance with the aim of achieving transformative change, delivering genomics curriculum that can be globally implemented.

Learn more about GEA at https://qubeshub.org/community/groups/gea

203 Crowd-sourcing CRISPR: A course-based research project to investigate the impact of chromatin environment on double-strand break repair while enhancing student learning. *Rebecca Burgess*[1] 1) Stevenson University.

The DNA damage response (DDR) modulates repair outcomes that drive genomic changes in evolution, genome diversification and pathological states. However, the fundamental interplay between the packaging of DNA into chromatin and the choice between use of error-prone vs. error-free pathways is not well understood. We are in a unique position to systematically examine DDR in diverse genomic loci by leveraging the power of multiple parallel undergraduate projects in course-based research. Using a CRISPR-based yeast system, we can introduce double-strand breaks (DSBs) in various pre-existing chromatin environments to be individually analyzed by undergraduates, producing quantitative data on repair outcome across the genome.
Students in a 16-week Course based Undergraduate Research Experience (CURE) select different putative DSB sites, and using publicly available genome-wide histone modification and transcription data, quantitatively define the chromatin "signatures" around their DSB site, learning bioinformatics and genome structure in the process. Students then extract and annotate their genome region, then design and clone unique small guide RNA (gRNA). After students complete molecular cloning and sequence verification of their constructs (8 weeks) they integrate the constructs into yeast. The remaining seven weeks of the semester are dedicated to examining DSB repair pathways. DSB levels are measured using quantitative PCR, and students learn to evaluate their qPCR data for its adherence to QC measures, perform normalization calculations and perform necessary statistical analyses for outliers. In parallel, students perform survivor analysis, a plating assay for cells that have undergone imprecise repair at the DSB site, which is followed by sequencing of DSB sites for mutations. Students perform statistical analysis on multiple survivor analysis trials and learn to interpret data. The laboratory activities designed for this course can be used en bloc or used as single modules for teaching bioinformatics, cloning, sequence analysis, qPCR, and DNA repair concepts.
Through participation in this CURE, we wish to enhance students' science identity by enhancing their technical, analytical, and communication skills.

We used backward-design to define goals for the CURE and determine evidence. The effect on outcomes like science identity and community values is assessed using the Persistence in the Sciences Survey (PITS). Furthermore, this CURE was designed to incorporate the three elements of a successful CURE: Discovery and Relevance, Iteration, and Collaboration. Incorporation of these are assessed using the Laboratory Course Assessment Survey (LCAS). From the results of these two surveys, we will examine if there are correlations between CURE elements and student outcomes

204 The Pipeline CURE: an iterative approach to introduce all students to research throughout a biology curriculum *Teresa Lee*[1], Brandon Carpenter[1], Onur Birol[1], David Katz[1], Karen Schmeichel[2] 1) Emory University, Atlanta GA; 2) Oglethorpe University, Atlanta GA.

Participation in research provides personal and professional benefits for undergraduates. However, some students face institutional barriers that prevent their entry into research, particularly those from underrepresented groups who may stand to gain the most from research experiences. Course-based undergraduate research experiences (CUREs) effectively scale research availability, but many only last for a single semester, which is rarely enough time for a novice to develop proficiency. To address these challenges, we present the Pipeline CURE, a framework that integrates a single research question throughout a biology curriculum. Students are introduced to the research system – in this implementation, *C. elegans* epigenetics research – with their first course in the major. After revisiting the research system in several subsequent courses, students can choose to participate in an upper-level research experience. In the Pipeline, students build resilience via repeated exposure to the same research system. Its iterative, curriculum-embedded approach is flexible enough to be implemented at a range of institutions using a variety of research questions. By uniting evidence-based teaching methods with ongoing scientific research, the Pipeline CURE provides a new model for overcoming barriers to participation in undergraduate research.

205 Performance-Enhanced Biology: an interdisciplinary and inter-institutional experiment in science literacy and communication *Luke Ziegler*[1], Kathy Hendrickson[2], Andrew Arsham[1] 1) Bemidji State; 2) North Hennepin Community College.

Performance-Enhanced Biology: an interdisciplinary and inter-institutional experiment in science literacy and communication
Luke Ziegler, Kathy Hendrickson and Dr. Andrew M Arsham
Communicating with non-specialist audiences is an important aspect of both science and theater. Although superficially different, these two disciplines share core intellectual and expressive tools: curiosity, collaboration, communication, specificity, creativity, and risk. Performance-Enhanced Biology is an interdisciplinary collaboration between Bemidji State University and North Hennepin Community College that creates connections between first-year community college students and advanced university biology majors. The project embraces cognitive disfluency — learning something new in an unfamiliar way. While students are aware of the roles of uncertainty and risk within the disciplines they study, they are surprised to discover their commonalities. The process starts by finding ideas, phrases and skills that are shared across both disciplines. Through warm-up exercises, movement, body langue, and public speaking, students develop vignettes and practice presenting in large groups, ending with a public performance of biological ideas acted out in several scenes on stage with theatrical music and lighting. Students collaborated in mixed groups to clearly and effectively translate scientific theories into performance using rhythm, gesture, voice, poetic movement, and the creation of images. The project helps biology students develop public speaking and science communication skills, while educating and inspiring arts students by increasing their comfort level with science and scientists. Student feedback indicated that groups frequently begin the collaboration with fear and apprehension of the techniques and physical environments of the other and that co-creating the work led to a mutual demystification, accessibility, and respect. Student comments also illustrated the challenges that scientists face when communicating scientific ideas, and that nonscientists face when interpreting technical or scientific data, barriers of keen contemporary importance. The flexibility of the project format allows for variation. In the first year, student presentations focused on research ethics and the responsible conduct of research; second-year projects focused on evolution. Our work suggests that collaborative art-making could be an effective strategy to teach bioethics, evolution, and other topics, and to cultivate science curiosity among non-scientists.

Friday, April 24 1:40 PM - 3:30 PM

Gene Regulation: RNA Features and Functions

235 Unraveling the influence of sequence features and position on uORF activity using massively parallel reporter systems and machine learning. Gemma May[1], Christina Akirtava[1], Matthew Agar-Johnson[1], Jelena Micic[1], John Woolford[1], *Joel McManus*[1] 1) Carnegie Mellon University.

Upstream open reading frames (uORFs) are potent *cis*-acting regulators of translation and mRNA turnover. Ribosome profiling studies suggest that uORFs are quite common, with thousands of uORFs initiating at non-AUG start codons. However, few predicted non-AUG uORF have been tested for regulatory functions. Furthermore, the relative influences of sequence and structural features on uORF functions are currently unknown. To address this, we developed a massively parallel reporter assay (FACS-uORF) to test thousands of AUG- and non-AUG uORFs from three *Saccharomyces* yeast species. While nearly all AUG uORFs were repressors, only 30% of non-AUG uORFs were functional, with roughly equal numbers of enhancers and repressors. These results were validated in a complimentary reporter system that assayed the effects of uORFs on polysome association. Testing our reporter library in a Δ*upf1* yeast strain (lacking NMD) revealed NMD accounts for roughly one third of uORF repressive effects, and showed that stop codon sequence, uORF position, and uORF length influence the strength of NMD induction. Repressive non-AUG uORFs induced NMD, supporting their functional translation. Both enhancer and repressor functions were stronger for uORFs whose start codons were more structurally accessible, supporting their translation. Strong Kozak contexts were associated with the most repressive uORFs but were not required for robust repression. Furthermore, repression was stronger from uORFs located proximal to the 5'-cap, and alternative transcription initiation sites led to corresponding changes in uORF activity. Using machine learning, we developed a model that explains 39% of the variance in uORF function using Kozak sequence, structural accessibility, uORF position, length, codon usage, and peptide charge. Notably, Kozak context and uORF position have similar predictive power. Together, our results define the scope of uORF functions in gene regulation, identify features associated with uORF repression and NMD, and suggest that uORF functions are impacted by the frequency and speed of their translation.

236 Codon usage bias in a complex multicellular organism: one size does not fit all *Scott Allen*[1], Rebeccah Stewart[1], Michael Rogers[2], Oliver Chung[1], Erez Cohen[1], Jessica Sawyer[1], Ivan Jimenez Ruiz[3], Alain Laederach[3], Chris Counter[1], Don Fox[1] 1) Duke University, Durham, NC; 2) Albert Einstein College of Medicine, The Bronx, NY; 3) University of North Carolina at Chapel Hill, Chapel Hill, NC.

For years it was believed that mutations changing one codon to another synonymous codon were “silent” in terms of biological impact. We have since come to appreciate that not all synonymous codons are created equal. Within an organism’s transcriptome, some synonymous codons occur more frequently than others – a phenomenon termed codon usage bias. A prevailing view in the field is that RNAs enriched in rare occurring synonymous codons are less stable and poorly translated compared to their more common counterparts. Most studies supporting this model have been performed in single-celled systems, however, leaving it unclear how codon usage bias impacts distinct tissue biology in complex multicellular organisms. Our lab developed a fluorescent-reporter-based system to identify tissue-level differences in codon usage bias in *Drosophila* and found that the testes, brain, and liver-like oenocytes are resistant to the impacts of rare codon usage. Each tissue exhibits specific developmental windows of time where codon bias resistance is prevalent, and aging appears to impact codon bias resistance as well. Our reporters also enabled us to define important RNA sequence determinants that enable rare codon-enriched RNAs to be translated. We next used bioinformatic approaches to examine endogenous genes in these codon bias-resistant tissues. We find that genes highly expressed and unique to the testis in both *Drosophila* and humans are enriched for rare codons compared to other tissues, suggesting that resistance to codon usage bias is a mechanism to specify male germline identity and/or function, and is likely a conserved feature of protein regulation across species.

237 The splicing factor SFPQ represses the formation of cryptic last exons *Pat Gordon*[1], Fursham Hamid[1], Eugene Makeyev[1], Corinne Houart[1] 1) King's College London, London, United Kingdom.

The splicing factor SFPQ is a ubiquitously expressed member of the major spliceosome that plays a role in transcriptional regulation, alternative splicing, and transport of RNAs into neuronal axons. Loss of *sfpq* is associated with the neurodegenerative diseases ALS and Alzheimer’s. In zebrafish, *sfpq* homozygous null mutants show defects in axon outgrowth and brain boundary formation, and loss of *sfpq* is lethal by four days post fertilization. In order to understand the molecular mechanisms of SFPQ function during embryonic development, we performed RNA-seq analysis on *sfpq* mutant embryos and looked for abnormalities in splicing. We discovered a novel form of alternative splicing and polyadenylation in which a cryptic exon is formed, followed by truncation of the transcript. These cryptic last exons (CLEs) appear preferentially in long introns of genes with neuronal function. Here we show that CLEs form as a direct result of loss of SFPQ binding, that the resulting short peptides cause developmental defects in the *sfpq* mutant, and that the CLEs act as a form of transcriptional regulation. This function of SFPQ is conserved, as we identified CLEs in SFPQ loss models in mouse and human iPS cell models of ALS. These results greatly expand our understanding of SFPQ function in addition to describing an important new regulatory mechanism with relevance to human pathologies.

238 Splicing takes place as RNA polymerase II transcribes past recursive and canonical splice sites in the developing *Drosophila* embryo Pedro Prudencio[1,2], Kenny Rebelo[1], Rui Goncalo Martinho[1,2,3], Maria Carmo-Fonseca[1] 1) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; 2) Center for Biomedical Research, Universidade do Algarve. Faro, Portugal; 3) iBiMED, Departamento de Ciências Médicas, Universidade de Aveiro. Aveiro, Portugal.

Widespread co-transcriptional splicing has been demonstrated from yeast to human. However, measuring the kinetics of splicing relative to transcription has been hampered by technical challenges. Here, we took advantage of native elongating transcript sequencing (NET-seq) to identify the position of RNA polymerase II (Pol II) when exons become ligated in the newly synthesized RNA. We analyzed *Drosophila melanogaster* embryos because the genes transcribed initially during development are short in length with few if any introns (like yeast genes), whereas genes transcribed later contain multiple long introns (more similar to human genes). Moreover, compared to human, the *Drosophila* genome is more compact, making the coverage of NET-seq reads on intragenic regions higher. We detected reads spanning recursive splicing and exon-exon junctions in transcripts connected to the active site of Pol II molecules positioned few nucleotides downstream of recursive and canonical 3' splice sites, indicating that splicing can occur immediately after splice site transcription. Immediate splicing was observed for exons separated by short introns, as well as for short exons separated by very long introns. The latter observation was quite unexpected as it argues that exon definition is not mandatory for metazoan splicing. We further found a significantly higher density of polymerases at the sites where spliced transcripts were detected suggesting a splicing-coupled mechanism that slows down transcription elongation. Taken together our data reveal a tight temporal coordination between splicing and the elongating Pol II complex during embryonic development.

239 mRNAs targeted by silencing small RNAs accumulate in P granules *John Paul Ouyang*[1], Lauren Bernard[1], Geraldine Seydoux[1] 1) Johns Hopkins University School of Medicine.

Regulation of mRNA stability and translation by small RNAs (sRNAs) is a common mechanism to regulate gene expression. In germ cells, many proteins required for sRNA-mediated silencing localize to perinuclear condensates, called P granules in *C. elegans*. Proteins reported to localize to P granules include Argonautes, required for sRNA targeting to mRNAs, and RNA-dependent RNA polymerases (RdRPs), required for sRNA amplification. Whether mRNAs targeted by sRNAs also localize to P granules has not been previously characterized. We have addressed this question using in situ hybridization in *C. elegans*. We find that mRNAs heavily targeted by piRNAs accumulate in P granules. Similarly, mRNAs targeted by siRNAs during RNA-mediated interference (exogenous dsRNA trigger) begin to accumulate in P granules within the first few hours of RNAi treatment. Additionally, we have found that a mutant defective in P granule formation during embryogenesis causes hyper-silencing of mRNAs targeted by piRNAs and transgenerational loss of competence for RNAi. These findings support a link between P granule localization and regulation by sRNAs. We will report on our efforts to uncover the mechanisms that promote mRNA localization to P granules induced by sRNAs.

240 Functional evolution of noncoding RNA for mammalian dosage compensation *Sha Sun*[1], Heather Karner[1], Benjamin Lin[1], Sarah Carmona[1] 1) Univ California, Irvine.

Long noncoding RNAs (lncRNAs) have been identified in all eukaryotes and play important roles in gene regulation and developmental processes. However, functions of the vast majority of lncRNAs are poorly understood. Even less is known about the origin and evolution of lncRNAs for gene regulation. Experimental models for evolutionary analyses of lncRNAs are scarce. Our research focuses on a cluster of lncRNA genes functional for X chromosome inactivation (XCI), the mammalian dosage compensation mechanism to balance sex chromosome content between XX females and XY males. This lncRNA gene cluster has evolved during the divergence of eutherian and marsupial mammals, coincidental with the evolution of XCI. Our previous data have demonstrated that, within this gene cluster in the mouse genome, the lncRNA *Jpx* is important for the activation of lncRNA *Xist* in early embryos and the subsequent control of mouse XCI. It is hypothesized that lncRNAs such as *Jpx* evolve rapidly and that specific molecular features enable the functional roles essential for mechanisms in early developmental processes including XCI.
We present evidence from our transgenic mouse models demonstrating the regulatory mechanisms of lncRNA *Jpx*. We will explain *Jpx*’s allelic origin

of expression in early embryos and show that *Jpx* can influence *Xist* expression using both *trans* and *cis* mechanisms. In addition, we have taken a comparative analysis of the sequence-structure-function relationship for *Jpx* with its human homologous lncRNA *JPX*, whose function in human XCI has long been proposed but not validated. Our detailed understanding of both the function and evolution of the lncRNA *Jpx*, the variation of its roles in mice and humans, makes XCI an excellent experimental model to define the adaptation of lncRNAs for epigenetic regulation and mammalian dosage compensation.

241 RNA abasic sites in yeast and human cells *Vivian Cheung*[1,2], Yaojuan Liu[1], Yesenia Rodriguez[3], Robert Ross[5], Ruoxia Zhao[5], Alan Bruzel[1,2], Dongjun Li[1,2], Jason Watts[1,2], Rajendra Prasad[3], Robert Crouch[4], Patrick Limbach[5], Samuel Wilson[3] 1) Howard Hughes Medical Institute, Chevy Chase, MD; 2) University of Michigan, Ann Arbor, MI; 3) National Institute of Environmental Health Sciences, NIH, Durham, NC; 4) Eunice Kennedy Shriver National Institute of Child Health and Development, NIH, Bethesda, MD; 5) University of Cincinnati, OH.

Abasic sites in RNA are almost unknown except for the abasic site generated by ricin in ribosomal RNA, in contrast to abasic sites in DNA that are well-studied. Here, by genetics, chemistry and physical identification, we show that in yeast and human cells, RNA abasic sites are not as rare as assumed. In studying R-loops, three-stranded nucleic acid structures that include RNA-DNA hybrids and the displaced DNA strands, we found abasic site-processing proteins including apurinic/apyrimidinic endonuclease 1 (APE1) bound to the RNA-DNA hybrids. To examine if there are abasic sites in RNA, we turned to nucleic acid chemistry. When a base is removed from RNA, the remaining ribose is found as a closed-ring or an open-ring sugar with a reactive aldehyde group. We first confirmed that ARP (N'-aminoxymethylcarbonylhydrazino-D-biotin) and methoxyamine bind specifically to the aldehydes on the exposed ribose in control RNAs with abasic sites. Then with ARP and methoxyamine, we showed that there are abasic sites in the nascent RNA, mRNA, and ribosomal RNA of *Saccharomyces cerevisiae* and human cells. Furthermore, aniline that cleaves DNA with abasic sites also cleaves RNA by β-elimination. Next, by liquid chromatography tandem mass spectrometry, we confirmed the presence of RNA abasic sites. To assess these RNA abasic sites, we found that they are coupled to R-loops, as cells with more R-loops have significantly ($P<0.01$) more RNA abasic sites. Then we showed *in vitro* that purified APE1 cleaves RNA-DNA hybrids with RNA abasic sites, and *in vivo* APE1 also processes RNA abasic sites as deletion mutants from yeast had significantly ($P<0.001$) more RNA abasic sites. In this presentation, we will show data from yeast and human cells indicating that R-loops support the formation of RNA abasic sites, which in turn stabilize R-loops. To resolve R-loops, APE1 cleaves RNA with abasic sites. Thus, unlike DNA abasic sites, RNA abasic sites likely play regulatory roles. We will discuss the implications of RNA abasic sites that have eluded attention for over 50 years despite extensive work on DNA abasic sites that spawned the field of DNA damage repair.

Friday, April 24 1:40 PM - 3:40 PM

Genome Integrity

248 Evolutionarily conserved pathways prevent mislocalization of CENP-A and chromosomal instability (CIN) in yeast and human cells *Munira Basrai*[1], Wei Chun Au[1], Jessica Eisenstatt[1], Daniele Fachineti[8], Prashant Mishra[1], Austin Rossi[1], Roshan Shrestha[1], Evelyn Suva[1], Kentaro Ohkuni[1], Tianyi Zhang[1], Michael Costanzo[2], Anastasia Baryshnikova[7], Chad Myers[6], Peter Kaiser[4], Dan Foltz[5], Richard Baker[3], Charles Boone[2] 1) NCI/NIH, Bethesda; 2) University of Toronto, Toronto; 3) University of Massachusetts Med School, Worcester; 4) University of California, Irvine; 5) Northwestern University, Chicago; 6) University of Minnesota, Minneapolis; 7) Cocalico Biologicals, San Francisco; 8) Institute Curie, Paris.

Faithful chromosome segregation prevents chromosomal instability (CIN), a hallmark of aggressive tumors and other diseases. Centromeric localization of evolutionarily conserved centromeric histone H3 variant CENP-A (Cse4 in budding yeast, Cid in flies) is essential for chromosome segregation. Overexpression of Cse4 or Cid causes its ectopic mislocalization to chromosome arms and promotes CIN in yeast and flies respectively. Overexpression and mislocalization of CENP-A have been reported in numerous cancers and is correlated with poor prognosis. Despite these observations, the role of CENP-A overexpression in promoting aneuploidy and tumorigenesis in these cancers is not known. We have used a multi-organismal approach to define the causes and consequences of CENP-A mislocalization in yeast, human cells and a mouse model. A genome-wide synthetic dosage lethality (SDL) screen was used to identify mutants in essential and non-essential genes that exhibit growth sensitivity when Cse4 is overexpressed in budding yeast. Among the top hits are genes that encode the evolutionarily conserved ubiquitin ligase (SCF), replication dependent kinases (DDK) and the replication-independent histone chaperone (HIR) complexes. We determined that SCF-Met30, SCF-Cdc4, DDK and HIR proteins regulate proteolysis of Cse4 to prevent its mislocalization for chromosomal stability. For studies with human cells we used a HeLa cell line stably overexpressing CENP-A and provide the first evidence to show that CIN results from mislocalization of overexpressed CENP-A in human cells (Shrestha *et al.,* 2017). Our ongoing studies have shown that overexpression of CENP-A contributes to aneuploidy, karyotypic heterogeneity and increased invasiness in a stable pseudodiploid DLD1 cells and a mouse model with overexpression of CENP-A. We examined the role of human homologs of yeast genes in preventing mislocalization of CENP-A. Consistent with results from yeast, depletion of HIRA, human homolog of Hir2 or b-TrCP, human homolog of Met30 resulted in mislocalization of CENP-A and CIN phenotypes in human cells. In summary, we provide the first evidence for a role of CENP-A overexpression in promoting aneuploidy and tumorigenesis and define a role of evolutionarily conserved pathways that prevent mislocalization of CENP-A for CIN in yeast and human cells.

249 Rif1 functions in a tissue-specific manner to control replication timing through its PP1-binding motif Robin Armstrong[1], Douradip Das[2], Christina Hill[3], Robert Duronio[1,3,4,5,6], *Jared Nordman*[2] 1) Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC ; 2) Department of Biological Sciences, Vanderbilt University, Nashville TN; 3) Integrative Program for Biological and Genome Sciences, University of North Carolina, Chapel Hill, NC; 4) Department of Genetics, University of North Carolina, Chapel Hill, NC ; 5) Department of Biology, University of North Carolina, Chapel Hill, NC ; 6) Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC .

Replication in eukaryotic cells is regulated in a temporal manner giving rise to a process known as replication timing (RT). RT changes during development to ensure accurate genome duplication and maintain genome stability. To understand the relative contributions that cell lineage, cell cycle, and replication initiation regulators have on RT, we utilized the powerful developmental systems available in *Drosophila melanogaster.* We generated and compared genome-wide RT profiles from mitotic cells of different tissues and from mitotic and endocycling cells of the same tissue. Our results demonstrate that cell lineage has the largest effect on RT, whereas switching from a mitotic cell cycle to the endo cycle has no effect on RT. While RT is highly correlated with transcription, the differences in RT we observed are largely independent of transcriptional changes. Rif1 is a trans acting factor that controls RT from yeast to humans. We employed a genetic approach in these same cell types to understand the effect Rif1 has on

RT during development. Our results demonstrate that Rif1 functions in a tissue-specific manner to control RT. Importantly, the Protein Phosphatase 1 (PP1) binding motif of Rif1 is essential for Rif1 to regulate RT. Together, our data support a model in which the RT program is primarily driven by cell lineage and is further refined by Rif1/PP1 to ultimately generate tissue-specific RT programs.

250 Evidence of pervasive DNA replication mediated class of CNVs *Pieter Spealman*[1], Stephanie Lauer[1], Grace Avecilla[1], Farah Abdul-Rahman[1], Charles Miller[1], Jaden Burrell[1], David Gresham[1] 1) New York University, Center for Genomics and Systems Biology, New York, NY.

Copy **n**umber **v**ariants (**CNV**s) are common, large effect size mutations characterized by the gain or loss of genomic regions. Because they entail amplification or deletion of large regions they are important drivers in evolution, genetic disorders, and cancer.

However, CNVs are not a homogenous class of mutations. Because each class is generated by different mechanisms, such as homologous recombination and DNA replication, the conditions that drive their formation and the rates at which they arise are also different. By better characterizing these CNV classes we hope to be able to distinguish between them by identifying their mutational signatures. In turn, this will help us understand CNV dynamics, their role in evolution, and their effect on health, wellness, and organismal fitness.

One such class of CNV is **O**rigin **d**ependant **i**nverted **r**epeat **a**mplification (**ODIRA**). ODIRA is a replication based, strand-switching, amplification mechanism previously observed at the SUL1 locus in populations of *Saccharomyces cerevisiae* under **l**ong-**t**erm **e**xperimental **e**volution (**LTEE**) to sulfur limited growth conditions.
CNVs were identified using a novel CNV identification method that allows for nucleotide accurate breakpoint resolution from Illumina short-read sequences and validated using Nanopore long-read sequencing.

Here, we describe features of ODIRA generated CNVs at the GAP1, DUR3, and MEP2 loci in LTEE strains adapted to glutamine, urea , or ammonium-sulfate limited growth conditions, respectively. We find ODIRA events at different frequencies at each loci, 6 out of 29 GAP1 CNVs are ODIRA, while 8/9 at DUR3, and 1/3 at MEP2, suggesting local architecture may affect ODIRA formation rates. Furthermore, we find that ODIRA CNVs have short (median 7nt long) neighboring (median 40nt apart) inverted repeats located less than an Okazaki fragment length from each other (~165 bp), consistent with formation by replication stress induced polymerase strand-switching. Using single-cell sorting of fluorescently labelled CNVs we can monitor the rapid gain (~70 generations) and loss (~30 generations) of ODIRA CNVs in response to environmental conditions.

To test whether ODIRA undelies CNV formation in wild strains we analysed the 1011 Yeast Genomes for evidence of GAP1 ODIRA CNVs. Furthermore, we performed long-read sequencing on 6 strains with identified GAP1 amplifications. Interestingly, we did not identify any wild strains with ODIRA GAP1 CNVs, suggesting that ODIRA CNVs may play a role in short term adaptation to stress but are rapidly reverted to wild-type when no longer under selection.

251 Polymerase theta protects against detrimental mitotic recombination *Juan Carvajal-Garcia*[1], Dale Ramsden[1], Jeff Sekelsky[1] 1) University of Nroth Carolina at Chapel Hill.

Chromosome breaks are a lethal form of DNA damage that arise spontaneously during normal cellular processes (e.g. replication), after exposure of cells to exogenous agents (e.g. ionizing radiation), and are induced during meiosis or antibody generation. They are also a key intermediate in Cas9-mediated genome editing. DNA polymerase theta (Pol θ) is central to a chromosome break repair pathway, Theta-Mediated End Joining (TMEJ). When repairing a chromosome break, TMEJ generates short deletions and insertions.

TMEJ is both frequent and essential in the absence of the two main chromosomal break repair pathways, Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR). This is of particular interest in the context of HR deficient (*BRCA1/2* mutant) tumors, as Pol θ is required for their viability. However, the role and relevance of this highly conserved pathway in wild type cells remains unclear.

Using *Drosophila melanogaster*, we have been able to recapitulate the synthetic lethality observed between Pol θ and *BRCA2* observed in mammals. We have also expanded the HR genes that are essential in the absence of Pol θ, adding the Holliday junction resolvases *SLX4* and *GEN1*. TMEJ has been shown that to be genetically downstream of Synthesis Dependent Strand Annealing, the preferred HR pathway. Now, using a newly developed assay to study mitotic crossing-over after Cas9-mediated chromosome breaks, we observe that in the absence of Pol θ, flies have increased mitotic crossovers. Consequently, we have placed TMEJ upstream of Holliday junction resolution. This helps explain the described genetic interaction between Pol θ and the resolvases. Mitotic crossovers result in loss of heterozygosity which can lead to cell death and tumor formation.

Our results show that, in the presence of the canonical chromosome break repair pathways, TMEJ plays a role in protecting cells against deleterious forms of mitotic recombination. We reason that, even if TMEJ is an inherently error-prone pathway, small deletions in DNA may be less detrimental to cells than mitotic crossovers, which can help explain the high conservation of this chromosome break repair pathway.

252 Chromatin modifiers alter repair/rejection outcomes during homologous recombination in *S. cerevisiae* Beata Mackenroth[1], Eric Alani[1] 1) Cornell University.

Repair of DNA double strand breaks (DSBs) by homologous recombination (HR) requires accurate discrimination between allelic and non-allelic loci. Non-allelic HR is highly deleterious because it can result in loss of heterozygosity and gross chromosomal rearrangement events observed in many cancers. During HR, DSB ends are resected to form single strand ends which initially invade a double stranded donor template to form a heteroduplex joint that is called a D-loop. Mismatches in heteroduplex DNA can be indicative of recombination with a non-allelic locus. Such mismatches are recognized by mismatch repair (MMR) proteins, which then recruit factors to disassemble such intermediates in a process called heteroduplex rejection. How the process of heteroduplex rejection occurs in a chromatin landscape has yet to be fully explored. Based on our previous work using an inverted repeat assay developed by the Jinks-Robertson laboratory (Chakraborty et al. Genetics 212:1147), we propose a mechanism where histone deposition at the D-loop structure antagonizes mismatch dependent heteroduplex rejection, stabilizing the D-loop structure and promoting repair. Specifically, we showed that two histone chaperones involved in replication coupled nucleosome deposition, CAF1 and Rtt106, act to suppress heteroduplex rejection. We propose that antagonism with the rejection machinery either directly or through the deposition of nucleosomes

at the D-loop structure suppresses rejection, thus promoting repair. We also observed that two deacetylases, Sir2, and Rpd3, have opposite effects on heteroduplex rejection. Knockout of the main heterochromatin regulator Sir2 results in elevated levels of heteroduplex rejection, indicating a model where Sir2 acts at the D-loop structure during recombination promote efficient repair. Our most recent studies using the inverted repeat recombination assay indicate involvement of the Sir2/3/4 but not the RENT complex. Mutation of RPD3L, but not the RPD3S complex results in a defect in heteroduplex rejection. In contrast to their antagonistic relationship at silent chromatin boundaries, epistasis analysis suggests that the Sir2/3/4 and RPDL complexes act independently of one another. My current work centers around elucidating the mechanisms underlying these observations and modeling how these complexes interact with recombination/MMR machinery to regulate the repair/rejection decision.

253 Regulation of sister chromatid repair maintains genomic integrity during meiosis *Erik Toraason*[1], Marissa Glover[1,2], Cordell Clark[1,3], Anna Horacek[1,4], Diana Libuda[1] 1) University of Oregon, Eugene, OR; 2) University of California Santa Cruz, Santa Cruz, CA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA; 4) National Institute of Child Health and Human Development, NIH, Rockville, MD.

Maintenance of genomic integrity during meiosis, the specialized form of cell division that generates haploid gametes such as sperm and eggs, is critical for fertility. Meiotic cells preferentially utilize recombination to repair DNA double-strand breaks (DSBs) as crossover or noncrossover outcomes. Meiotic recombination with the homologous chromosome has been extensively studied, but the mechanisms of sister chromatid recombination during meiosis are poorly understood. Using a non-allelic sister chromatid repair (SCR) assay that we developed to directly detect sister chromatid repair at a defined DSB site during *Caenorhabditis elegans* meiosis, we reveal mechanisms of sister chromatid recombination during meiotic prophase I progression. We find that the sister chromatid is available as a repair template for both crossover or noncrossover recombination throughout meiotic prophase I, with the sister chromatid as the exclusive recombination partner during late meiotic prophase I. By sequencing the conversion tracts of recombination outcomes from the SCR assay, we have found that: 1) DSBs repaired via sister chromatid recombination are processed similarly throughout prophase I; 2) intersister recombination intermediates do not migrate from site of DSB induction; and, 3) recombination with the sister chromatid appears processive, as we observe no evidence of template switching. Further, we have direct evidence with the SCR assay that the Smc5/6 DNA damage complex is required in late meiotic prophase for efficient recombination with the sister chromatid, and specifically, for formation of intersister crossovers. However, conversion tracts arising from recombination events in *smc-5* mutants are shorter than wild-type tracts in early prophase. These results suggest that SMC-5/6 is differentially engaged during meiotic prophase I to influence both early steps in DSB processing and downstream promotion of recombination with the sister chromatid. Taken together, our research has defined fundamental mechanisms ensuring successful meiosis and preserving genomic fidelity across generations.

254 The meiosis-specific cohesin subunit Rad21l1 is required for oogenesis but is dispensible for spermatogenisis in zebrafish Yana Blokhina[1], An Nguyen[1], Michelle Frees[1], Masuda Sharifi[1], *Sean Burgess*[1] 1) Univ California, Davis.

Meiosis produces haploid gametes through one round of DNA replication and two divisions that will give rise to the next diploid generation. Errors during either division result in aneuploidy, which can lead to miscarriages or birth defects in humans. During meiosis I, a ring-shaped cohesin complex stabilizes sister chromatids and aids in proper segregation of homologous chromosomes. Here we use zebrafish as a model to elucidate the function of a meiosis-specific cohesin submit, Rad21l1, in meiosis and fertility in males and females. Interestingly, knocking out *rad21l1* causes nearly the entire mutant population to develop as fertile males, suggesting the mutation triggers a sex reversal from female to male due to a failure in oocytes production. Both normal and “sex-reverted” *rad21l1* males display normal fertility at sexual maturity. Sex reversal was suppressed in the absence of *tp53* suggesting that defects in meiotic chromosome dynamics induced checkpoint control, specifically in females. Unlike in males, the *rad21l1 tp53* females produced mostly elevated rates of decomposing eggs and deformed offspring, will some notable exceptions. Tp53 encodes a tumor suppressor gene that induces apoptosis in the presence of unrepaired double-strand breaks (DSBs). We tested if *rad21l1* sex reversal was also suppressed in a spo11 mutant that fails to form DSBs, the initiating event of homologous recombination. We were surprised to find that the mutations were not epistatic and all double mutants developed as infertile males. Overall, our data highlight an exceptionally sexually dimorphic phenotype caused by knocking out a meiotic-specific cohesin subunit. We propose that Rad21l1 is required for a specific stage of oogenesis that is under *tp53* checkpoint control.

255 Delineation of the SUMO-Modified Proteome Reveals Regulatory Functions Throughout Meiosis *Neil Hunter*[1,3], Nikhil Bhagwat[1,3], Shannon Owens[1,3], Masaru Ito[1,3], Jeffrey Johnson[2], Nevan Krogan2[2], Owen Davies[4], Sean Collins[1] 1) University California, Davis ; 2) University of California, San Francisco; 3) Howard Hughes Medical Intitute; 4) University of Newcastle, UK.

The Small Ubiquitin-like MOdifier (SUMO) is implicated in orchestrating the complex events of meiotic prophase that ensure accurate chromosome segregation to form haploid gametes. A major impediment to understanding how SUMO regulates meiosis has been the identification of targets, especially the mapping of conjugated lysines. We developed an efficient pipeline for SUMO proteomics in budding yeast by systematically overcoming challenges of low abundance, instability and poor detection in mass spectrometry. In combination with label-free quantification (LFQ) and engineered strains that sharpen cell synchrony, this regimen produced multi-dimensional data that provide unprecedented insights into the landscape and dynamics of SUMO modification during meiosis. 850 targets containing 2747 conjugated lysines were identified, representing the first systematic analysis of SUMO modification across a developmental program. Attesting to the value of this approach, less than 19% of mapped sites could have been identified based on the consensus conjugation sequence (Ψ-K-X-E/D). The cohorts of SUMO conjugates identified by our analysis imply that SUMO regulates all major aspects of meiotic chromosome metabolism, including programmed recombination and chromosome synapsis. This inference was confirmed by acutely inactivating SUMOylation at various stages of meiosis using degron alleles of the SUMO E1 activating enzyme. This approach defined successive molecular execution points for SUMO during meiosis, revealing unanticipated roles in S-phase, the initiation and progression of homologous recombination, and chromosome synapsis. Ongoing studies are focused on understanding SUMOylation of specific targets implicated in various aspects of meiotic recombination.

Friday, April 24 1:40 PM - 3:30 PM

Genetics of Neuronal Development and Behavior

242 The primary cilia gene *Ttc21b* modulates forebrain and orofacial development as a crucial ciliopathy gene *Rolf Stottmann*[1,2], zakia abdelhamed[1], david paulding[1], laura runck[1], sarah salomone[1] 1) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) University of Cincinnati College of Medicine, Cincinnati, OH.

The primary cilium is a critical signaling center and human patients with ciliopathies frequently suffer a number of congenital malformations, including intellectual disability and orofacial phenotypes. In a ciliopathy patient cohort, *TTC21B* was previously found to be the most commonly mutated cilia gene (Davis et al., 2011). We have taken three approaches to identify genes interacting with *Ttc21b*. First, we have attempted to recapitulate the associations from the human cohort with mouse models. We have found that four genes identified in the human genetic studies (*Bbs7*, *Bbs10*, *Cc2d2a*, and *Mks1*) interact with *Ttc21b*. Most intriguingly, we find that heterozygosity for *Ttc21b* extends survival of *Bbs7* and *Bbs10* mutant mice. Doubly homozygous mutants have much more severe phenotypes than either single mutant. Cells from *Ttc21b Bbs7* and *Ttc21b Bbs10* double mutants also show surprisingly drastic effects on ciliogenesis and signal transduction of crucial developmental signaling pathways. These findings have significant implications for models of oligogenic inheritance in ciliopathies.
Secondly, we have previously demonstrated that mice lacking *Ttc21b* have multiple phenotypes, including microcephaly and orofacial clefting. Interestingly, the severity of the microcephaly in *Ttc21b* null mutants is affected by the genetic background. To identify potential genetic modifiers of microcephaly in *Ttc21b* mutants we performed a Quantitative Trait Locus (QTL) analysis and discovered a significant correlation between forebrain size and genetic background on distal chromosome 4. *Gpr63* is a candidate locus for this modifier and has been confirmed with an independent congenic backcross and direct modification of strain-specific *Gpr63* SNPs via CRISPR/CAS9 genome editing. Furthermore, *Ttc21b Gpr63* double mutants have embryonic lethality and spina bifida, further extending the *Ttc21b* phenotypes.
Finally, we have used ENU mutagenesis to try and identify new alleles interacting with *Ttc21b* and found an allele of *Kifap3* which dramatically enhances the *Ttc21b* forebrain neurogenesis phenotype. Together, these studies are beginning to identify crucial components of a *Ttc21b* genetic network leading to ciliopathic disease.

243 Unique homeobox codes delineate all neuron classes of the nematode *Caenorhabditis elegans* *Molly Reilly*[1], Cyril Cros[1], Oliver Hobert[1] 1) Columbia University, New York, NY.

It is presently not known whether neuronal cell type diversity, defined by cell type-specific anatomical, biophysical, functional and molecular signatures, can be reduced to relatively simple molecular descriptors of neuronal identity. We examined the expression of all 88 conserved homeodomain proteins encoded by the *Caenorhabditis elegans* genome, using reporters that contain the full intergenic genomic context of the respective homeobox genes. This analysis revealed that the complete set of 118 *C. elegans* neuron classes can be described individually by unique combinations of this single family of transcription factor proteins, thereby providing the simplest currently known descriptor of neuronal diversity. Furthermore, computational as well as genetic loss of function analysis corroborates that homeodomain proteins not only provide unique descriptors of neuron type, but also play a critical role in specifying neuronal identity. 39 of the 79 neuronally expressed *C. elegans* homeodomain proteins have previously been shown to play a role in neuronal identity specification. We extended this functional analysis by examining 4 homeobox genes that were not previously implicated in neuronal identity specification and found they indeed specify the neurotransmitter identity of neurons where they are expressed. We speculate that the pervasive employment of homeobox genes in defining unique neuronal identities reflects the evolutionary history of neuronal cell-type specification.

244 Axonal initial segment-like regions are localized distal to the intersection of dendrites and axons in active *Drosophila* neurons *Thomas Ravenscroft*[1,2], Jasper Janssens[3,4], Pei-Tseng Lee[1,2], Burak Tepe[1,2], Paul Marcogliese[1,2], Samira Makhzami[3,4], Todd Holmes[5], Stein Aerts[3,4], Hugo Bellen[1,2,6,7,8] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX , USA ; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX , USA ; 3) VIB Center for Brain & Disease Research, KU Leuven, Leuven 3000, Belgium ; 4) Department of Human Genetics, KU Leuven, Leuven 3000, Belgium ; 5) Department of Physiology and Biophysics, School of Medicine, University of California at Irvine, Irvine, CA, USA ; 6) Department of Neuroscience, Baylor College of Medicine, Houston, TX , USA ; 7) Program in Developmental Biology, Baylor College of Medicine, Houston, TX , USA ; 8) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX , USA.

Voltage-gated sodium channels (Nav) in mammalian neurons are located near the cell body, at the site of action potential (AP) initiation in the axonal initial segment (AIS), and distally along the axon at the nodes of Ranvier, at the site of AP propagation. In *Drosophila* the sites of AP initiation and propagation are unclear. *Drosophila* has one Nav gene (*para*) compared to nine genes in humans. The localization and expression pattern of *para* remains elusive due to poor antibodies and low resolution *in situ* data. Using recombination-mediated cassette exchange of a *Minos* mediated integration cassette (MiMIC) in the first coding intron of *para,* we generated lines to characterize *para* gene (*para-T2A-GAL4*) expression and protein (*para-GFSTF)* localization. *para* expression is restricted to ~10% of all neurons in the larval CNS throughout development. In the adult CNS *para* is very broadly expressed. To identify the *para* expressing cells we performed single-cell RNA-seq of the whole third instar larval brain on the 10x Chromium platform and obtained 5056 cells. In addition, we analyzed single-cell RNA-seq from the adult brain. *para* expression positively correlates with markers for fully differentiated, actively firing neurons. This describes most neurons in the adult CNS but only 10% of larval CNS neurons. In both the third instar larval and adult neurons that express *para*, Para is localized to axons distal to the cell body at a clustered region downstream of dendritic innervation, forming a boundary between the somatodendritic and axonal compartments of the axon. This region mimics the hallmarks of the AIS in vertebrates and as such is the likely site of AP initiation in invertebrates. We validated this physiologically using voltage-clamp analysis by isolating tetrodotoxin sensitive Nav voltage evoked currents. We show that the inward activating sodium currents occur distal to the soma. Due to its distal location, we call the site of AP initiation in invertebrates the distal axonal segment (DAS). In addition to Para clustering at the DAS, we observe diffusely expressed Para in the axon between the DAS and the synapses, especially in long axons. At the synapses, Para expression is no longer continual and is distributed before or after each bouton, likely to enable AP propagation to the most distal bouton. The identification of the DAS will improve our interpretation of electrophysiology in invertebrates. In addition, *para-GFSTF* and *para-T2A-GAL4* will allow the mapping of DAS in any neuron.

245 Autophagy-dependent filopodial kinetics restrict synaptic partner choice during *Drosophila* brain wiring *Ferdi Ridvan Kiral*[1], Gerit Arne Linneweber[1,2], Thomas Mathejczyk[1], Svilen Veselinov Georgiev[1], Mathias Wernet[1], Bassem Hassan[1,2], Max von Kleist[3], Peter Robin Hiesinger[1] 1) Free

University Berlin, Berlin Germany; 2) Institut du Cerveau et de la Moelle Epinière (ICM), Paris France; 3) MF1 Bioinformatics, Robert Koch-Institute, Berlin Germany.

Brain wiring is remarkably precise, yet most neurons readily form synapses with incorrect partners when given the opportunity. Dynamic axon-dendritic positioning can restrict synaptogenic encounters, but the spatiotemporal interaction kinetics and their regulation remain essentially unknown inside developing brains. Here we show that the kinetics of axonal filopodia restrict synapse formation and partner choice for neurons that are not otherwise prevented from making incorrect synapses. Specifically, we show that Drosophila R7 photoreceptor neurons form synapses with multiple incorrect partners when filopodial dynamics are artificially stabilized. Using 4D imaging in developing *Drosophila* brains, we show that filopodial kinetics are regulated by autophagy, a prevalent degradation mechanism whose role in brain development remains poorly understood. With surprising specificity, autophagosomes form in synaptogenic filopodia, followed by filopodial collapse. Altered autophagic degradation of synaptic building material quantitatively regulates synapse formation as shown by computational modeling and genetic experiments. Increased filopodial stability enables incorrect synaptic partnerships. Hence, filopodial autophagy restricts inappropriate partner choice through a process of kinetic exclusion that critically contributes to wiring specificity. Our findings suggest that small changes in modulatory mechanisms during brain development - like changing levels of autophagy - can cause selectable and heritable changes in evolutionary programming of neuronal circuits.

246 Neuropeptide VF Neurons Promote Sleep via the Serotonergic Raphe *Daniel Lee*[1], Grigorios Oikonomou[1], Tasha Cammidge[1], Young Hong[1], David Prober[1] 1) California Institute of Technology.

Although several sleep-regulating neuronal circuits have been identified, little is known about how these circuits interact with each other. For example, the serotonergic raphe nuclei (RN) promote sleep in both diurnal zebrafish and nocturnal mice, but it remains unknown how the RN interact with other sleep-promoting nuclei. Here we use zebrafish to describe a neural circuit in which hypothalamic *neuropeptide VF* (*npvf*)-expressing neurons promote sleep via the RN in the hindbrain. Using genetic labeling and *in vivo* calcium imaging, we show that *npvf*-expressing neurons densely innervate, and can robustly activate, serotonergic RN neurons. We also show that optogenetic stimulation of *npvf*-expressing neurons induces sleep in a manner that requires NPVF and is abolished when the RN are ablated or lack serotonin. Finally, genetic epistasis analysis demonstrates that NPVF acts upstream of serotonin in the RN to maintain normal sleep levels. These findings reveal a novel hypothalamus-hindbrain circuit for sleep/wake control.

247 Feedback between sensorimotor and neuromodulatory circuits enables flexible selection of behavioral states in C. elegans *Steven Flavell*[1], Ni Ji[1], Gurrein Madan[1], Casey Baker[1], Alyssa Dayan[1], Guadalupe Fabre[1], Ijeoma Nwabudike[1] 1) Massachusetts Institute of Technology.

To adapt to their environments, animals must generate behaviors that are closely tuned to a dynamically changing sensory world. However, behavioral states such as foraging or mating typically persist over long time scales to ensure proper execution. It remains unclear how neural circuits generate stable activity patterns to drive behavioral states, while maintaining the flexibility to select among alternative states when the sensory context changes. Here, we combine circuit-wide functional imaging in freely-moving animals with genetic and cellular perturbations to elucidate the architecture of a neural circuit controlling the choice between exploration and exploitation states during *C. elegans* foraging. We identify stable activity patterns underlying each behavioral state and show that feedback between a sensorimotor circuit and two antagonistic neuromodulatory inputs underlies the emergence of these network states. We uncover sensory processing neurons that act as key nodes to drive state switching and show that the functional output of these neurons can couple to different neuromodulatory systems to shift the balance between exploration and exploitation upon changes in the sensory context. Our results demonstrate that bi-directional communication between sensorimotor and neuromodulatory circuits allows animals to flexibly select behavioral states appropriate for their sensory context. These neural circuit motifs may be broadly used in a variety of cases where animals need to balance behavioral persistence with sensitivity to environmental change.

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Departures from Additivity: Dominance, Epistasis and GxE (PEQG)

223 Temperature-dependent phenotypic effects of house fly proto-Y chromosomes explain the maintenance of polygenic sex determination in natural populations *Kiran Adhikari*[1], Richard Meisel[1] 1) University of Houston, Houston, TX.

Sex determination is the process by which sexually dimorphic developmental pathways are established. In genetic sex determination systems, a single master regulatory locus determines the sex of an individual. However, some organisms have multiple master sex determining loci in their genome that segregate independently, resulting in polygenic sex determination. Polygenic sex determination is predicted to be an unstable intermediate between monogenic systems, and the factors responsible for maintaining polygenic sex determination are poorly understood. Housefly (*Musca domestica*) has a stable polygenic sex determination system with multiple male and female determiners segregating in natural populations. The male determining factor (*Mdmd*) is commonly found on two different proto-Y chromosomes (Y^M and III^M). Males carrying Y^M are found in colder, northern latitudes whereas III^M males are found in southern, warmer latitudes. This suggests that selection operating on a genotype-by-temperature (GxT) interaction maintains this polymorphism. To test this hypothesis, we raised III^M and Y^M males with otherwise common genetic backgrounds at high and low temperatures, and we studied the resulting GxT effects on two phenotypes: gene expression and extreme temperature tolerance. We found that Y^M males raised at low temperature are the most cold tolerant, suggesting a GxT interaction that includes developmental acclimation. The direction of this GxT interaction is consistent with the Y^M chromosome increasing fitness at colder temperatures. Next, using RNA-seq, we identified 247 genes whose expression in testis and 50 genes whose expression in head depends on GxT interactions. We found that expression of genes involved in the sex determination pathway is independent of GxT interactions, suggesting that GxT effects are the result of genes on the proto-Y chromosomes other than those in the sex determination pathway. Our results therefore support the hypothesis that GxT interactions maintain polygenic sex determination in housefly through temperature-dependent fitness effects of genetic variation on the Y^M and III^M chromosomes. The GxT interactions could be mediated through effects of the proto-Y chromosomes on gene expression.

224 Gene-by-diet interactions modulate the landscape of transcriptional response of individual fruit flies *Luisa Pallares*[1], Julien Ayroles[1] 1) Princeton University, Princeton, NJ.

Exposure to new environmental conditions usually results in shifts in phenotypic mean values. From a quantitative genetics perspective, it has also

been stablished that such mean changes are typically accompanied by an increase in the phenotypic variance of the population. This implies that some individuals are more sensitive than others to environmental changes, however, it is not yet clear to which extent this individual level variation is genetically regulated. To answer this question, we used an outbred population of *Drosophila melanogaster* which genetic composition makes it an ideal mapping population and exposed it to a high sugar diet. *Drosophila* has proven to be a remarkable model to study the phenotypic effects of high sugar diets, and here we set to understand the genetic regulation of the underlying transcriptional response. We collected genome wide SNP data and transcriptional profiles for 2000 individual flies using TM3'seq, a novel low-cost RNAseq library preparation protocol we developed for this project. We performed eQTL mapping using over one million SNPs and identify, not only thousands of eQTLs regulating gene expression levels in control and high sugar diet, but over 10000 GxE eQTLs. These are context-dependent SNPs, SNPs that have no phenotypic effect under control conditions but appear to be regulating gene expression under high sugar diet. Interestingly, the GxE eQTLs not only regulate the context-dependent expression of single genes, but also the correlation between genes. We found that a significant proportion of GxE eQTLs is responsible for strengthening the expression correlation between pairs of genes. This indicates that the function of many of the context-dependent eQTLs might be to buffer the dysregulation of gene expression generated by the exposure to a stressful environment such as high sugar diet. The large amount of context-dependent genetic variation we identify suggests that GxE interactions play a definitive role in modulating not only the overall landscape of transcriptional response in flies, but also the differences in gene expression robustness between individuals.

225 Sign inversion in selection on modifier mutations *Yevgeniy Raynes*[1], Daniel Weinreich[1] 1) Brown University, Providence, RI.

The scaling relationship between population size (*N*) and natural selection has been understood since the early days of population genetics. As population size declines, natural selection is overwhelmed by genetic drift, rendering both beneficial and deleterious mutations selectively neutral. In other words, as *N* declines, the fixation probability of any fitness-affecting mutation (P_{fix}) approaches, but never crosses, that of a neutral mutation (~1/*N*).
In stark contrast to classical theory, we have previously discovered that for mutator mutations – allelic variants of mutation rate modifier loci that elevate the genomic mutation rate – population size determines not just the efficiency of selection but the sign of selection itself. Unlike fitness-affecting mutations, the fixation probability of a mutator mutation is greater than 1/*N* in large populations and less than 1/*N* in small populations. That is, mutators fare better than neutral at large *N* but worse than neutral at small *N* - a behavior we have designated sign inversion. The salient difference between fitness-affecting mutations and mutators responsible for sign inversion is that selection on mutators is mediated not by their inherent fitness effects but by persistent statistical associations with beneficial and deleterious mutations elsewhere in the genome. Using analytical theory, computer simulations, and evolution experiments we have shown that such indirect selection driven by beneficial mutations is more sensitive to drift and is neutralized at a larger *N* than is necessary for drift to overpower selection against the deleterious load.
Intriguingly, we have now found that modifiers of ploidy also exhibit sign inversion. In competition with diploids, haploids fare better than neutral in large populations (i.e., $P_{fix} > 1/N$) but worse than neutral (i.e., $P_{fix} < 1/N$) in small populations. Because higher ploidy masks the fitness effects of new mutations, haploids experience stronger indirect selection than diploids. Similar to mutators, indirect selection on haploids is dominated by associations with beneficial mutations at large *N* and the excess deleterious load at small *N*.
The parallels between modifiers of mutation rate and ploidy suggest that sign inversion may apply broadly to modifiers mutations, revealing a much more general role of population size in evolution. We are now investigating whether the mechanisms of sign inversion in indirect selection on these and other modifiers can be generalized into a single theoretical framework.

226 Goldilocks and the Three Genotypes: Characterizing the Prevalence of Overdominance for Adaptive Mutations that Arise in Diploids *Vivian Chen*[1, 2], Monica Sanchez[1], Gavin Sherlock[2], Dmitri Petrov[1] 1) Stanford University, Stanford, California; 2) Stanford School of Medicine, Stanford, California.

Because new and rare adaptive mutations in diploids tend to be heterozygous, there is a strong bias against the establishment of recessive beneficial mutations. Thus, successful adaptive mutations in diploids must be more fit than the homozygous ancestral (AA) genotype. This pattern is known as Haldane's sieve. Recent theoretical predictions suggest that successful adaptive mutations in diploids, especially mutations of large fitness effect, should often be not only partially or completely dominant (f(Aa) > f(aa)) but also overdominant (f(Aa) > f(AA)). Limited empirical data confirmed these predictions but the data are still currently insufficient to make this claim with confidence. The lack of high throughput experimental methods has until now hampered our ability to measure fitness effects and dominance of a large number of adaptive mutations.
Here we use barcoding and high throughput CRISPR approaches to determine the prevalence of overdominance for a large number of adaptive mutations that arose in diploid evolution experiments. Diploid yeast were barcoded, and evolved under eight different experimental conditions, including non-fermentable carbon sources, antifungal treatment, and change in temperature or pH. We used barcode-based lineage tracking and clone sequencing to track adaptation and to identify over 100 heterozygous, putative adaptive mutations. A subset of these mutations were tested by segregation and found to be recessive lethal - these mutations are by definition overdominant. For the remaining subset, we first used the iSeq 2.0 barcoding system to generate barcoded haploids with the ancestral background and then used CRISPR/Cas9 to engineer the identified mutations into these barcoded haploids. From these haploids, we then generated uniquely barcoded biological replicates of all three genotypes (homozygous, heterozygous, and ancestor) for these adaptive mutations that can be used to measure fitness of all genotypes for all the mutants in a single pool. This high throughput approach allows us to determine the distribution of fitness effects and the dominance (or even overdominance) of a large number of adaptive mutations across a range of conditions. We will discuss our findings and their implications for the maintenance of genetic variation in sexual populations.

227 Evolutionary modification of dominance reversal under seasonal antagonism *Evgeny Brud*[1] 1) University of Pennsylvania.

The maintenance of variation at loci undergoing temporally fluctuating selection is a topic that has gained renewed interest in light of recent observations in Drosophila melanogaster, where temperate populations have been argued to exhibit seasonal oscillations in allele frequencies. Theoretical work on the factors favoring stability of allelic oscillations concludes that a reversal of dominance between seasons is a potent factor in stabilizing polymorphisms. In the evolutionary history of such loci, the existence of dominance reversal may initially owe to fortuitous pleiotropy, in which a gene affects antagonistic components of seasonal fitness and heterozygotes exhibit trait-specific dominance. Subsequently, the magnitude of reversal may be subject to the evolution of modifiers. Here I derive modifier invasion conditions for two-locus models of seasonally antagonistic selection in a bivoltine population, in which a focal fitness polymorphism is subject to the evolution of dominance. In addition, I summarize multilocus simulations that incorporate diminishing returns epistasis with respect to fitness, as well as multivoltinism. These results predict an empirical

pattern of seasonal-reversals of allele-specific expression at oscillating loci.

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Direct Collaborations Between Model Organism Researchers and Clinicians

228 The essential role of model organisms for functional studies of genes and variants linked to human diseases *Ada Hamosh*[1] 1) John Hopkins University.

Model organisms have always played an important role in understanding biological processes on an organismal level or in cells and tissues that are not accessible in the human. The advent of exome sequencing has resulted in an explosion of new potential disease gene discovery. It is no longer acceptable to report presumably pathogenic variation based upon population frequency (or lack thereof) or cell-based assays that are irrelevant to the biological process. Model organisms have become essential to establish the role of observed variation in the disease process. The ability to interrogate biology on an organismal level, during development, and under controlled environments is essential to establish functional relevance without which publication is impossible. Studies using model organisms can further shed light on mechanism of diseases, leading to development of novel therapies. Direct collaborations between clinicians and model organism researchers catalyze new discoveries that can have significant impact on patient care.

229 Solving difficult to diagnose diseases using flies and zebrafish: the Model Organisms Screening Centers of the Undiagnosed Diseases Network *Hugo Bellen*[1,2,3,4], Monte Westerfield[5], John Postlethwait[5], Shinya Yamamoto[1,2,3], Michael Wangler[1,2,6] 1) Neurological Research Institute, BCM; 2) Department of Molecular and Human Genetics, BCM; 3) Department of Neuroscience, BCM; 4) Howard Hughes Medical Instiute, BCM; 5) Institute of Neuroscience, Univeristy of Oregon; 6) Department of Pediatrics, BCM.

Millions of children and adults are affected by diseases that are very difficult to diagnose. The National Institutes of Health (NIH) launched the Undiagnosed Diseases Network (UDN) in 2014. The goal of this project is to diagnose the most difficult medical mysteries using cutting-edge medicine, genomics, and experimental strategies. Physicians typically perform extensive phenotypic characterizations, sequence the genomes or exomes of affected individuals and often the parents, and try to discover a genetic cause. In 2015, we established the UDN Model Organisms Screening Center (MOSC) with two cores: *Drosophila* (Baylor College of Medicine) and zebrafish (University of Oregon). Prior to selecting candidate genes submitted by physicians, we carefully vet them using human genetics and model organism databases through an online tool that we developed, called MARRVEL (http://marrvel.org/). This initial analysis is critical and increases the likelihood that pursuing a candidate gene in a model organism will be productive. We also use GeneMatcher (https://genematcher.org/), a tool developed by the Centers for Mendelian Genomics (CMG), to match researchers and clinicians studying common candidate genes in close collaborations with UDN clinicians. In the fly and zebrafish cores, we developed a battery of strategies and pipelines to study genes and variants that are linked to undiagnosed diseases. Here, we will provide an overview of strategies, activities, and achievements of the cores during Phase I (2015-2018) of the UDN MOSC. During this period we received submissions of 236 variants in 179 genes for 118 cases from UDN clinical sites. From these, we prioritized 72 genes for functional studies in model organisms of which 58 were studied in *Drosophila* and the rest in zebrafish. Efforts have led to disease gene discoveries for *ATP5F1D, IRF2BPL, COG4, TONSL, TBX2, ACOX1* amongst others. The MOSC cores also tackle rare and undiagnosed cases outside the UDN, in particular candidate genes identified in the CMG or by other clinical collaborators around the world. In summary, we have so far successfully used model organisms to help diagnose more than 25 previously unidentified human diseases identified by clinicians and human geneticists in institutes in the US and around the world. The approach has been so successful that in Phase II of the UDN the MOSC was broadened and expanded with a third site that includes a *C. elegans* core and another zebrafish core (Washington University at St Louis). Solving difficult disease cases with model organism approaches is a productive effort.

230 Connecting novel rare disease gene discoveries to functional characterization research in yeast and other model organisms. *Phil Hieter*[1], S. Rogic[1], P. Pavlidis[2], K. Boycott[3] 1) Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada; 2) Department of Pediatrics, Centre de Recherche du CHU Ste-Justine, Montreal, Canada ; 3) CHEO Research Institute, University of Ottawa, Ottawa, ON, Canada Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada.

Implementation of next-generation sequencing technology has greatly accelerated the identification of genetic variants that cause rare, single-gene disorders. However, the discovery of the human variant is inherently a descriptive, hypothesis-generating milestone that requires subsequent studies on the basic function of the gene and the functional consequences of specific gene mutations in a biological context. Model organisms (MOs) such as yeast, worm, fly, zebrafish, and mouse provide powerful experimental tools to investigate the mechanistic basis of rare genetic diseases and to identify and explore potential therapeutic interventions. The key to success is identifying connections and collabirations between clinicians, immediately following the discovery of new disease gene mutations, and scientists able to study equivalent genes and pathways in MOs. For this reason, we established the Canadian Rare Diseases Models and Mechanisms (RDMM) Network.

The RDMM Network built a web-based Canadian directory of MO scientists to identify possible connections and uses a formalized committee process to review clinician-MO scientist collaborations and approve 25K CAD in catalyst funding. This has created a rapid and direct pathway from disease gene discovery to functional characterization studies in MOs. Since October 2014, we have made ~100 clinician-MO scientist connections with catalyst seed funding. These collaborations have resulted in validation of disease gene discovery, identification of possible therapies, and success in obtaining subsequent grant funding. The RDMM concept also serves to educate scientists, clinicians, patient communities and funding agencies about the power of model organisms for developing rational approaches to disease prevention, management, and treatment.

We have recently expanded the impact and reach of the RDMM Network by establishing international linkages with emerging networks modeled on RDMM. To facilitate this, we made the RDMM Registry open-source, portable, and customizable and our committee structures and process freely-available. The emerging network of RDMM networks will enable matches across borders when there is no MO expertise within the country of the gene discovery. With renewed funding through 2023, we will continue to create meaningful collaborations between clinicians and MO scientists, enhance the generation of new knowledge, and advance RD research locally and globally.

231 Leveraging the International Mouse Phenotyping Consortium in collaborative research Lauryl MJ Nutter[1], Anne-Marie Mallon[2], Stephen Mur-

ray[3] 1) The Hospital for Sick Children; 2) MRC Harwell; 3) The Jackson Laboratory.

The International Mouse Phenotyping Consortium (IMPC) aims to produce and phenotype single-gene null alleles for all mammalian protein-coding genes. Each mouse line is subjected to broad-based phenotyping to identify the impacts of gene dysfunction, including viability, developmental, neurological, musculoskeletal, cardiovascular, sensory, immune, and metabolic assays. Members of the IMPC actively collaborate with various human disease consortia, including the Mendelian Genomics Research Centers, the Gabriella Miller's Kids First Program, the Undiagnosed Diseases Network, Care for Rare, and the Province of Ontario Neurodevelopmental Network. Through these collaborations, genes of interest are prioritized for null allele production and phenotyping or custom models are produced to facilitate diagnosis and/or mechanistic research. Mouse lines are made publicly available through the IMPC portal (mousephenotype.org) and the data are also freely available to support novel disease gene discovery. This talk will provide an overview of the IMPC as well as specific examples from various collaborations that have led to the production of new research tools. Examples of data mining that identified new gene candidates for deafness and added new parameters to consider when assessing variants will also be discussed.

232 Modeling rare monogenic human diseases in *C. elegans* *Andy Golden*[1], Peter Kropp[1], Xiaofei Bai[1], Philippa Rogers[1], Ben Nebenfuehr[1] 1) NIDDK/NIH.

There are currently ~7,000 identified rare diseases with more being diagnosed every year. Eighty percent of these diseases are thought to be genetic, and frequently monogenic, in origin. For many of these human diseases, gene orthologs can be readily identified in *C. elegans*. My group models rare human monogenic diseases in *C. elegans* by using CRISPR/Cas9 genome editing to generate and characterize deletion and patient-specific variants in the orthologous gene. The disease-associated alleles we study are selected from published literature as well as variants identified by local clinical collaborators. I will highlight three projects in which we used this approach to understand penetrant, novel, and clinically relevant phenotypes. We are investigating (a) the *lpd-8* gene, an ortholog of human *NFU1*, variants in which cause Multiple Mitochondrial Dysfunction Syndrome 1 (MMDS1), (b) the *seip-1* gene, the ortholog of the human *BSCL2/SEIPIN* gene, variants in which cause lipodystrophy and male infertility, and (c) the *pezo-1* gene, the ortholog of human *PEIZO1/2*, variants in which cause a diverse array of disease, but particularly affects the cardiovascular system. My presentation will show how we are using these models to study metabolism and neuromuscular phenotypes (*lpd-8*), lipid droplet biogenesis in the formation of the *C. elegans* eggshell (*seip-1*), and the forces and signaling involved in ovulation (*pezo-1*). We are characterizing these novel phenotypes for each and hope to uncover the mechanisms by which these phenotypes arise. In the near future, genetic and drug suppressor screens will be carried out to revert these phenotypes.

233 A model organism-based drug discovery pipeline for amyotrophic lateral sclerosis Dina Aggad[1], Kessen Patten[1,2], Gary Armstrong[1, 3], Claudia Maios[1], Lawrence Korngut[4], Richard Robitaille[5], Pierre Drapeau[1], *Alex Parker*[1] 1) Neuroscience, CRCHUM, University of Montreal, Montreal, QC, Canada; 2) INRS-Institut Armand-Frappier, Laval, QC, Canada; 3) Montreal Neurological Institute, McGill University, Montreal, QC, Canada; 4) Neurology, Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada; 5) Neuroscience, University of Montreal, Montreal, QC, Canada.

Amyotrophic lateral sclerosis (ALS) is a fatal, incurable neurodegenerative disease characterized by a progressive loss of motor neurons in the brain and spinal cord. A better understanding of disease pathogenesis is crucial for disease diagnosis and intervention strategies. To this end, there has been a revolution in identifying the genetic causes of both sporadic and familial ALS. ALS is caused by mutations in a growing number of genes, one of which being *TARDBP*, which codes for TDP-43 and is a major component of neuronal inclusions. Since TDP-43 is evolutionarily conserved, we turned to the model organisms *C. elegans* and zebrafish to learn more about their biological functions and screen for potential therapeutic modifiers. We generated *C. elegans* and zebrafish models expressing wild-type or mutant human TDP-43 that reflect aspects of ALS. To explore the potential of our models in identifying chemical suppressors of mutant TDP-43 neuronal toxicity, we screened a set of chemical libraries for FDA-approved compounds with potential neuroprotective properties. Chemical libraries were screened using motility and stress response assays with mutant *TARDBP* worms and hits were validated in zebrafish using motility assays. The expression of mutant TDP-43 in worm motor neurons produced robust, adult onset motility defects and in both models this was caused by motor neuron deficits. We isolated a number of chemical suppressors of mutant *TARDBP* toxicity.
The most potent drug was pimozide, which blocked T-type Ca2+ channels in these simple models and stabilized neuromuscular transmission in zebrafish and enhanced it in mice. Pimozide was then tested in a short randomized controlled trial of sporadic ALS subjects who demonstrated stabilization of motility and evidence of target engagement. Pimozide is now being tested in a longer, Canada-wide Phase 2 clinical trial for ALS. Thus, model organisms are useful in identifying promising compounds for the treatment of ALS, and this approach holds promise for other neurodegenerative diseases.

234 Strategies and resources to facilitate direct collaborations between clinicians and model organism researchers on a global scale *Shinya Yamamoto*[1,2,3], Sanja Rogic[4,5], Zhandong Liu[3,6], Michael Wangler[1,3], Kym Boycott[7], Paul Pavlidis[4,5], Phil Hieter[4] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 3) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX; 4) Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada; 5) Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada; 6) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 7) CHEO Research Institute, University of Ottawa, Ottawa, ON, Canada.

Clinical researchers and basic scientists have complementary knowledge, skill sets, resources and networks. Direct collaborations between clinicians and model organism scientists can greatly facilitate basic and translational biomedical research as evident by the success of large reserach consorita such as the Undiagnosed Diseases Network (UDN) in the USA and Rare Diseases Models and Mechanisms Network (RDMM) in Canada. Although the need for functional studies of genes and variants associated with human diseases are greatly increasing in the human genetics field in many parts of the world, most model organism biologists are not involved in this type of research because they are not well connected with clinical researchers. In addition, cultural and linguistic barriers that exist between basic scientific and medical fields make such collaboration difficult to initiate, maintain or expand. In this presentation, I will discuss different ways in which model organism researchers can identify potential clinical collaborators by utilizing tools and resources that faciliates communication between clinicians and scientists. In addition to discussing the utility of existing tools and platforms such as the GeneMatcher website and RDMM software, I will introduce ModelMatcher (https://www.modelmatcher.net/), a new online matchmaking platform that UDN anad RDMM researchers are co-developing to facilitate collaborations at a global scale. In addition, I will touch upon some of the challenges that one may face when initiating and maintaining a cross-decipline collaboration. By under-

standing the values and responsibilities that are associated with collabortive research with clinicians and taking advantages of informatic tools and publically available genetic resources, model organism researchers can facilitate disease diagnosis and therapeutic research to make a direct impact on patient care and advacement of biomedical sciences.

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Genomics and Systems Biology

270 A distinct class of condensin II sites are required to establish long-range interactions between distal heterochromatic sites following mitotic exit *Randi Isenhart*[1], Leah Rosin[1], Olivia Crocker[1], Son Nguyen[1], Yemin Lan[1], Margaret Shaw[1], Eric Joyce[1] 1) University of Pennsylvania, Philadelphia, PA.

Metazoan genomes are arranged into a nested hierarchy of unique structural features, driven by a division of labor amongst the few architectural proteins that we know of (e.g. CTCF, cohesin, condensin). During interphase, cohesin and CTCF are essential for chromatin looping while recent work from our lab and others suggests that the condensin II complex is important instead for large-scale chromosome folding. However, the mechanism by which condensin II mediates chromosome folding during interphase remains unknown. In Drosophila, mouse, and human interphase cells, a well-described subset of condensin II is localized to active promoters. We have identified a novel subpopulation of condensin II sites that are not enriched at promoters or active chromatin. We found that these sites are associated with the gypsy insulator complex and longer-range intra-chromosomal interactions by Hi-C. Specific loss of condensin II at these sites via depletion of condensin II loader PP2A phenocopies the disrupted chromosome folding observed following condensin II depletion. Similar results are observed following depletion of the gypsy insulator component Su(Hw). These condensin II and gypsy sites are enriched in silent chromatin including regions marked by H3K9me2. Hi-C and FISH data confirm that long-range interactions between these chromatin types are particularly disrupted after depletion of condensin II. This indicates that condensin II and gypsy collaborate early in the cell cycle to mediate long-range interactions between heterochromatic regions. Preliminary data suggest that these interactions are important for proper silencing of distal, but not proximal, heterochromatic sequences. Together, these studies are revealing a mechanism underlying condensin II-dependent folding of interphase chromosomes that have implications for gene regulation and genome integrity.

271 Functional Analysis of the Mysterious Germline-Restricted Chromosome in Zebra Finch (*Taeniopygia guttata*) *Kathryn Asalone*[1], Michelle Biederman[1], Megan Nelson[1], Colin Saldanha[1], John Bracht[1] 1) American University, Washington D.C..

Developmentally programmed genome rearrangements are rare in vertebrates but have been reported in scattered lineages including the zebra finch (*Taeniopygia guttata*). In the finch, a well-studied animal model for neuroendocrinology and vocal learning, genome rearrangement involves a Germline-Restricted Chromosome, or GRC, which is found in germlines of both sexes but eliminated from mature sperm. Transmitted only through the oocyte, it displays uniparental female-driven inheritance, and early in embryonic development it is apparently eliminated from all somatic tissue in both sexes. The GRC comprises the longest finch chromosome at over 120 million basepairs. In 2018, we report the first protein-coding gene from the GRC using computational methods such as subtractive transcriptomics. Recent publications have indicated that there are over 115 coding genes. Here we introduce an *in vitro* model for examining the mechanisms of GRC elimination. We are able to differentiate the zebra finch primordial germ cells into mature sperm to examine how the GRC is expelled. This also gives us a way to begin to examine the function of the germline restricted chromosome, which is hypothesized to be involved in reproduction, development, and sex determination. Finally, this model can also be used to study the mechanism of genome rearrangements which can help us better understand this process which occurs in some cancers.

272 DeepArk: sequence-based models of cis-regulatory logic for model organisms *Evan Cofer*[1,2], João Raimundo[1], Michael Levine[1,3], Olga Troyanskaya[1,4,5] 1) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ ; 2) Graduate Program in Quantitative and Computational Biology, Princeton University, Princeton, NJ ; 3) Department of Molecular Biology, Princeton University, Princeton , NJ; 4) Flatiron Institute, Simons Foundation, New York City, NY; 5) Department of Computer Science, Princeton University, Princeton , NJ.

Model organisms are a cornerstone of both basic and translational research, and allow unparalleled resolution with which to investigate regulatory genomics. To enable large-scale functional analyses of regulatory genomics of model species, we have developed DeepArk, a set of deep learning models of the cis-regulatory codes of four widely-studied model species: *Caenorhabditis elegans*, *Danio rerio*, *Drosophila melanogaster*, and *Mus musculus*. Unlike existing approaches, which often scan genomic regions for conserved transcription factor binding sites, DeepArk integrates information from a wide sequence context. For a given 4 kb genomic sequence, DeepArk predicts tissue- and stage-specific activity of a collective 6558 transcription factors, histone marks, and chromatin states - a number of which are broad regulatory features unamenable to conventional approaches. Importantly, DeepArk enables species- and context-specific prediction of regulatory activity, cross-species comparisons of regulatory logic, and functional interpretation of genomic variants. As a case study of DeepArk's predictive quality and direct experimental utility, we performed in silico mutagenesis of the mesodermal enhancer for *T48* in *D. melanogaster*, and used *in vivo* live imaging to identify the allele-specific effects on expression during nuclear cycle 14. DeepArk accurately predicts binding of Zelda, with the observed transcriptional output of the allelic series tested *in vivo*. We also demonstrate a number of other applications that DeepArk uniquely enables. For instance, we used DeepArk to annotate regulatory regions in non-traditional model species, and accurately predict the effects of thousands of regulatory variants tested in a high-throughput reporter assay. Finally, we have made DeepArk accessible to all biomedical researchers via a free and user-friendly GPU-accelerated web application.

273 Biological Robustness: genetic compensation and transcriptional adaptation Mohamed El-Brolosy[1], Zacharias Kontarakis[1], Andrea Rossi[1], Vahan Serobyan[1], Gabrielius Jakutis[1], Jordan Welker[1], *Didier Stainier*[1] 1) Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany.

Genetic robustness, or the ability of an organism to maintain fitness in the presence of harmful mutations, can be achieved via protein feedback loops. Previous work has suggested that organisms may also respond to mutations by transcriptional adaptation, a process by which related gene(s) are upregulated independently of protein feedback loops. However, the prevalence of transcriptional adaptation and its underlying molecular mechanisms are unknown. Here, by analyzing several models of transcriptional adaptation in *C. elegans*, zebrafish and mouse, we uncover a requirement for mutant mRNA degradation. Alleles that fail to transcribe the mutated gene do not exhibit transcriptional adaptation, and these alleles give rise to more severe phenotypes than alleles displaying mutant mRNA decay. Transcriptome analysis in alleles displaying mutant mRNA decay reveals the upregulation of a substantial proportion of the genes that exhibit sequence similarity with the mutated gene's mRNA, suggesting

a sequence-dependent mechanism. Furthermore, using the *C. elegans* model, we uncover a requirement for factors known to be involved in small RNA maturation and transport into the nucleus including Argonaute proteins and DICER, and confirm these findings in mouse models. These results have implications for our understanding of disease-causing mutations, and will help in the design of mutant alleles with minimal transcriptional adaptation-derived compensation.

274 The dynamics of global acetylation remodeling during the yeast heat shock response Rebecca Hardman[1], Aaron Storey[2], Alan Tackett[2], Jeffrey Lewis[1] 1) University of Arkansas; 2) University of Arkansas for Medical Sciences.

All organisms experience stress and must rapidly adapt to changing conditions. Thus, cells have evolved sophisticated rapid-response mechanisms such as post-translational protein modification to rapidly and reversibly modulate protein activity. Our focus has been on post-translational lysine acetylation, where recent proteomic studies have identified thousands of acetylated proteins across diverse organisms. While the sheer size of the "acetylome" is striking, we have little idea regarding the function of acetylation for the vast majority of proteins. Here, we show that acetylation plays a previously unappreciated role in the heat shock response of *Saccharomyces cerevisiae*. We find that dysregulated acetylation renders cells heat sensitive, and moreover, that the acetylome is globally remodeled during heat shock over time. Using quantitative acetyl-proteomics, we identified ~400 high confidence acetyl marks across ~200 proteins that significantly change in acetylation when cells are shifted to elevated temperature. Proteins with significant changes in lysine acetylation during heat shock strongly overlap with genes induced or repressed by stress. Thus, we hypothesize that protein acetylation augments the heat shock response by activating induced proteins and inactivating repressed proteins. Intriguingly, we find nearly 40 proteins with at least two acetyl marks that significantly change in the opposite directions. These proteins are strongly enriched for chaperones and ribosomal proteins, suggesting that these two key processes are coordinately regulated by protein acetylation during heat shock. Moreover, we hypothesize that the same type of activating and inactivating marks that exist on histones may be a general feature of proteins regulated by acetylation. Overall, this work has identified a new layer of post-translational regulation that likely augments the classic heat shock response.

275 Disome profiling reveals genome-wide targets of ribosome quality control Sezen Meydan[1], *Nicholas Guydosh*[1] 1) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

Ribosomes encounter obstacles during protein synthesis, such as mRNA structures, combinations of poor codons, and damaged bases, which slow the ribosome or cause it to stall, blocking further translation of the message. Failures in resolving unwanted ribosome slowdowns result in mitochondrial dysfunction, aggregation of abnormal proteins and neurodegeneration. To address this problem, cells have evolved a ribosome-associated quality control (RQC) system that can recognize stalled ribosomes and degrade aberrant proteins and mRNAs. The conundrum for the RQC system is to discriminate between transient or programmed ribosome pauses, such as those required for protein localization or protein folding, from cases of detrimental ribosome stalling. Recent structural and biochemical data proposed that the complex formed by two collided ribosomes ("disome") is the substrate detected by the RQC pathway and the RQC protein Hel2/ZNF598 plays a major role in recognizing disomes. However, the extent of disome formation in the cell and the repertoire of endogenous mRNAs where disomes are formed has not been characterized. To study this question in yeast, we used ribosome profiling, a method where deep sequencing of ribosome-protected footprints is used to reveal a global snapshot of translation in the cell. By specifically isolating mRNAs that were footprinted by disomes, rather than single ribosomes, we were able to adapt this approach to reveal the genome-wide positions of disomes in the cell. Disome profiling showed that collided ribosomes are present on many endogenous mRNAs at sites encoding poly-Lys and poly-Arg peptides, which are known RQC targets. In other cases, we found disomes enriched on Pro-, Gly- and Asp- rich sequences that are known to slow the ribosome but not thought to be triggers for RQC. Strikingly, deletion of Hel2 decreased global disome occupancy at all these sites, suggesting that, after recognition, collided ribosomes on endogenous mRNAs are stabilized regardless of the sequence context. Recognition by Hel2, however, did not cause degradation of the disome-associated mRNAs. Interestingly, loss of Hel2 activated Gcn4, which is the master regulator of stress response in yeast. These results suggest Hel2-mediated stabilization of disomes on endogenous mRNAs may not always trigger the downstream RQC pathway, but it could rather facilitate co-translational events that are important for cellular homeostasis.

276 A large accessory protein interactome is rewired across environments Zhimin Liu[2], Darach Miller[1], Fangfei Li[1], Xianan Liu[1], *Sasha Levy*[1,2] 1) Joint Initiative for Metrology in Biology, SLAC National Accelerator Laboratory, Stanford University, Stanford, CA; 2) Stony Brook University, Stony Brook, NY.

A network of protein-protein interactions (PPIs) executes most cellular processes and its rewiring is likely to underlie the structural, biochemical, and behavioral differences between cells in different environments or tissues. To characterize PPI network rewiring in the yeast *Saccharomyces cerevisiae*, we combined a protein fragment complementation assay, pooled competitive growth, and barcode sequencing to quantify the *in vivo* PPI abundance of 1.6 million protein pairs across 9 growth conditions with replication for a total of 44 million measurements. Our multi-condition screen identified 13,764 specific PPIs, a 3-fold increase over PPIs identified under standard growth conditions. Only a small fraction of "core" PPIs were stable across conditions, with most network remodeling being due to gains or losses of previously under sampled "accessory" PPIs that are rarely present across conditions. However, coordinated losses of core PPIs involved in presumably unneeded cellular processes were also observed. Proteins that participate in PPIs that are unstable across conditions were more likely to be disordered, quickly evolving, essential, and have a large number of genetic interactions, suggesting that their environment-dependent folding and binding is critical to cell function. Changes in PPI abundance were well explained by changes in protein abundance for only a minority of PPIs, suggesting that localization, post-translational modifications, and competitive binding have a major role in driving protein interactome dynamics.

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Developmental Genetics: Organ Systems

262 Single cell sequencing the lateral plate mesoderm origins of mesothelial membranes Karin D. Prummel[1,2], Helena Crowell[2,3], Susan Nieuwenhuize[1,2], Eline Brombacher[2], Stephan Daetwyler[4], Charlotte Soneson[2,3], Jan Huisken[4,5], Mark D. Robinson[2,3], *Christian Mosimann*[1,2] 1) Department of Pediatrics, Section of Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, USA; 2) Institute of Molecular Life Sciences, University of Zürich, Zürich, Switzerland; 3) Swiss Institute of Bioinformatics, University of Zürich, Zürich, Switzerland; 4) Max Planck Institute of

Molecular Cell Biology and Genetics, Dresden, Germany; 5) Morgridge Institute for Research, Madison, Wisconsin, USA.

The mesothelium in vertebrates forms epithelia that line the peritoneal cavities, close the body wall, and surround the intra-abdominal organs. Widely distributed within the body as peritoneal membranes, mesothelium and mesothelial stem cells contribute to organ homeostasis and regeneration, and aberrant regulation of mesothelial cells can result in tumors and congenital herniation syndromes. Nonetheless, the embryonic ontogeny of mesothelia and their developmental regulation remain unresolved.
Here, we combine genetic lineage tracing, *in toto* live imaging, and single-cell transcriptomics in zebrafish to track mesothelial progenitor origins from lateral plate mesoderm (LPM). While mapping LPM-descendant lineages, we consistently found LPM-derived genetic lineage labeling in the mesothelial layers covering the body cavities and surrounding the major internal organs. Single-cell transcriptome analysis revealed a post-gastrulation gene expression signature centered around *hand2* that delineates a LPM lineage distinct from the other LPM-derived progenitors forming kidney, vasculature, and blood fates. We further uncovered gene expression patterns that outline distinct segments within the emerging mesothelium forming the prospective pericardium, parietal, and visceral peritoneum. Combining gene expression analysis and imaging of transgenic reporter embryos, we charted the origin of mesothelial progenitors to the *hand2*-expressing lateral-most stripe within the emerging LPM, consolidating previous observations in zebrafish, chick, and mice. In lightsheet-based time-lapse imaging of transgenic *hand2* reporter embryos, we captured zebrafish mesothelium formation *in toto*, documenting the coordinated cell movements that close the body wall and the pericardium. Functionally, and adding to the well-documented cardiac and forelimb defects, we found that loss of *hand2* in zebrafish causes mesothelial defects characterized by ventral herniation as a result of perturbed migration of mesothelium progenitors.
Taken together, our findings define the developmental context of LPM formation towards mesothelial progenitors and indicate that Hand2 function provides critical cellular properties to a highly specialized mesodermal progenitor pool that forms mesothelia. Our work defines a genetic and developmental signature of mesothelial orgins with implications for our understanding of malignancies affecting the mesothelium.

263 Regulation of blood cell transdifferentiation by oxygen sensing neurons through atypical guanylyl cyclases Sean Corcoran[1], Anjeli Mase[1], Yousuf Hashmi[1], Jordan Augsburger[1], Katelyn Kukar[1], *Katja Brückner*[1] 1) University of California San Francisco.

Transdifferentiation gives rise to differentiated cell types independent of progenitors or stem cells. Transdifferentiation of functionally specialized blood cell types has been reported in vertebrates and other species across the animal kingdom including the invertebrate *Drosophila melanogaster*. However, in most systems it remains unclear how transdifferentiation is regulated in vivo. Here we study a model of transdifferentiation between two *Drosophila* blood cell types, from macrophage-like plasmatocytes to crystal cells that execute melanization. Using functional lineage tracing, we provide new in vivo evidence for direct plasmatocyte-to-crystal cell transdifferentiation in the *Drosophila* larva. Interestingly, we find that this transdifferentiation process is promoted by neuronal activity of a specific subset of sensory neurons, in the sensory cones at the caudal end of the larva; this is confirmed by specific neuron ablation, and the silencing or activation of these neurons through transient expression of *Kir2.1* or *TrpA1*, respectively. Consistent with this, crystal cells develop in clusters surrounding the sensory cones. Strikingly, we reveal functional links of environmental sensing with blood cell transdifferentiation: oxygen sensing through atypical guanylyl cyclases (Gyc88E, Gyc89Da, Gyc89Db) that are specifically expressed in sensory cone neurons drives plasmatocyte-to-crystal cell transdifferentiation. We demonstrate this by reduced crystal cell numbers and loss of transdifferentiation under hypoxic conditions (5% O2), and the specific requirement of *gyc* function by in vivo RNAi. Our findings reveal an unexpected functional link how oxygen sensing, through sensory neurons and their activity, regulates blood cell transdifferentiation in vivo, suggesting similar principles in other species.

264 Molecular regulation of vascular smooth muscle cell recruitment to arteries during development *Amber Stratman*[1,2], Brant Weinstein[2] 1) Washington University School of Medicine, St. Louis, MO; 2) NICHD/NIH, Bethesda, MD.

The preferential recruitment of vascular smooth muscle cells (vSMCs) to arteries versus veins during early development is a well-described phenomenon that has traditionally been attributed to higher levels of shear stress and blood flow rates through the arterial vasculature. Although the preferential recruitment of smooth muscle to arteries has been appreciated for very centuries, little is known about the molecular pathways responsible for this preference. We show that the *cxcl12* ligand and its receptor *cxcr4* are both expressed on embryonic arteries during stages of vSMC acquisition. Using zebrafish and mouse genetic mutants, RNA/DNA over expression studies in the fish, and *in vitro* mechanistic analysis, we find that *cxcl12/cxcr4* signaling within arterial endothelial cells leads to increased *pdgf-bb* ligand production, resulting in increased vSMC recruitment to arteries. Shortly after the onset of blood flow, expression of *klf2a,* a well-characterized flow–regulated gene that negatively regulates *cxcr4* expression, transiently becomes heavily polarized to veins. This inhibits vSMC recruitment to veins and limits expression of *cxcr4* and *pdgf-bb* to the arterial vasculature. Together, our findings illuminate an early developmental molecular signaling axis driving preferential recruitment of smooth muscle to the arterial vasculature.

265 *The sexy heart*: sex-specific differences during mouse cardiac development Daniel Deegan[1], Rob Kulathinal[2], *Nora Engel*[1] 1) Fels Institute for Cancer Research, Temple University School of Medicine; 2) Department of Biology, Temple University.

Expression patterns between males and females vary in every adult tissue, even in organs with no conspicuous dimorphisms such as the heart. In fact, clinical presentation of cardiovascular disease differs substantially between men and women. Although sexual dimorphisms are usually attributed to hormonal influences, expression and epigenetic sex biases observed at early embryonic stages cannot be accounted for in this way. Many congenital heart defects exhibit sex biases in presentation, mortality and morbidity and are primarily due to disruptions occurring *before* gonad formation. We hypothesize that these imbalances are established primarily by the sex chromosome constitution and that, independently of hormonal influence, sex chromosome-linked genes and their downstream targets contribute to the male- and female-specific transcriptomes and epigenomes across the lifespan. We previously reported expression and epigenetic differences between male and female mouse embryonic stem cells, indicating that the male and female genomes are epigenetically poised for divergent pathways at the molecular level. Here we investigated how sex-specific gene expression networks wax and wane throughout mouse cardiac development, generated sex-specific co-expression networks and examined the presence of sex-biased chromatin domains, beginning at early embryonic stages. Our studies reveal sex-biased gene expression at every stage in heart development, with a subset of genes exhibiting the same bias across multiple stages. We identify key transcriptional factors that set up epigenetic differences in early cardiogenesis that persist after lineage determination and organogenesis. We also explore whether these sexual dimorphisms predict distinctions in response to environmental signals and whether they foreshadow sex-specific health-related outcomes after birth. Our results have implications for understanding the developmental origins of disease and sex-appropriate disease treatment.

266 Endocardial cell dynamics, modulated by cardiac function and Acvrl1a signaling, shape the cardiac outflow tract *Pragya Sidhwani*[1], Giulia Boezio[2], Hongbo Yang[1], Neil Chi[1], Beth Roman[3], Didier Stainier[2], Deborah Yelon[1] 1) University of California, San Diego, La Jolla, CA; 2) Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany; 3) University of Pittsburgh, Pittsburgh, PA.

Physical forces are important participants in the cellular dynamics that shape developing organs. During heart formation, for example, contractility and blood flow generate biomechanical cues that influence patterns of cell behavior. Here, we address the interplay between function and form during the assembly of the cardiac outflow tract (OFT), a crucial connection between the heart and vasculature that develops while circulation is underway. In zebrafish, we find that the OFT expands via accrual of both endocardial and myocardial cells. However, when cardiac function is disrupted, OFT endocardial growth ceases, accompanied by reduced proliferation and reduced addition of cells from adjacent vessels. The flow-responsive TGFβ receptor Acvrl1 is required for addition of endocardial cells, but not for their proliferation, indicating distinct modes of function-dependent regulation for each of these essential cell behaviors. Together, our results indicate that cardiac function modulates OFT morphogenesis by triggering endocardial cell accumulation that induces OFT lumen expansion and shapes OFT dimensions; moreover, these morphogenetic mechanisms provide new perspectives regarding the potential causes of cardiac birth defects.

267 Cholinergic nerve dependent regeneration in the gut *Afroditi Petsakou*[1], Norbert Perrimon[1] 1) Harvard Medical School.

The regenerative capacity of organs and tissues is highly dependent on neuronal signals, however the neuronal mechanisms involved are largely unexplored. We examined the role of the enteric nervous system in the *Drosophila* gut and demonstrate that cholinergic enteric neurons (ChEN) are involved in the recovery process during regeneration. Specifically, upon injury, cytokines are released from the proliferating gut and sensitize ChEN to render them more excitable. In turn, ChEN innervations that form direct junctions with gut epithelial cells, release the neurotransmitter acetylcholine through varicosity-like structures. Cholinergic signaling triggers differentiation and reduce cell proliferation by modifying the cation influx properties of epithelial cells. This cholinergic neuronal pathway acts as the first response in protecting the gut from over-proliferating and is required for the initiation of the recovery state of regeneration. This mechanism is reminiscent of the role of cholinergic neurons in regulating immune cell signaling during the inflammatory reflex in mammals. Since the gut is the organ that constantly regenerates due to its frequent contact with viruses and toxins via food intake, our findings which provide a direct link between ChEN and gut regeneration could potentially explain why neurodegenerative diseases like Parkinson, ALS and Alzheimer have early manifestation in the gut and why gut dysbiosis occurs in those diseases.

268 A role for PAR polarity proteins in microtubule reorganization as intestinal epithelial cells divide *Maria Sallee*[1], Jessica Feldman[1] 1) Stanford University.

Dividing and differentiating cells require different arrangements of microtubules to function. Mitotic cells establish centrosomes as microtubule organizing centers (MTOCs), producing radial microtubule arrays that are critical for chromosome segregation. In contrast, polarized epithelial cells form parallel arrays of microtubules emanating from a non-centrosomal MTOC, the apical membrane, that promote cell polarity and intracellular transport. During development and tissue homeostasis, some polarized epithelial cells divide, presenting an important but poorly understood obstacle: microtubules must temporarily cycle between the apical surface and the centrosomes. The developing *C. elegans* intestine provides an excellent *in vivo* epithelial model to study how this microtubule reorganization is achieved. After the 16-cell embryonic intestine polarizes and establishes an apical MTOC, exactly four "E16*" cells divide again. The E16* divisions involve a rapid change in microtubule organization from apical to centrosomal as cells enter mitosis, and back to apical upon mitotic exit (Yang and Feldman 2015). Using fluorescent markers, genetic screens, and tissue-specific protein depletion with live imaging, we are testing the hypothesis that apical polarity proteins control microtubule reorganization during the E16* divisions. During mitosis, we observe that, like microtubules, MTOC-associated proteins also leave the apical membrane as the centrosome becomes the MTOC. However, the apical PAR polarity proteins remain at the apical membrane during the E16* divisions, suggesting that they may act as a memory mark and help direct the return of microtubules and MTOC proteins after division. Consistent with this model, we have found that intestine-specific depletion of the apical polarity proteins PAR-6 and PKC-3/aPKC disrupts MTOC reformation following the E16* division. A pilot forward genetic suppressor screen has isolated a suppressor of the MTOC defects caused by PAR-6 depletion. These experiments reveal a role for PAR proteins in returning MTOC function to the apical membrane following mitosis, a critical step in epithelial cell divisions across organisms.

269 An abundant quiescent stem cell population protects principal cells from kidney stones in adult *Drosophila* Malpighian tubules *Chenhui Wang*[1,2], Allan Spradling[1,2] 1) Howard Hughes Medical Institute Research Laboratories; 2) Department of Embryology, Carnegie Institution for Science, Baltimore, MD.

Adult Drosophila Malpighian tubules have low rates of cell turnover but are vulnerable to damage caused by stones, like their mammalian counterparts, kidneys. Using a new surgical assay and a *Drosophila* stone disease model, we show that Drosophila renal stem cells (RSCs) comprise a unique, unipotent regenerative compartment. RSCs respond only to loss of nearby principal cells (PCs), cells critical for maintaining ionic balance. RSC daughter cells do not migrate to the upper tubules to replenish stellate cells or principal cells. Perhaps due to the large size of PCs they are outnumbered by RSCs, which replace each lost cell with multiple PCs of lower ploidy. RSCs share a developmental origin with highly active intestinal stem cells (ISCs), and like ISCs generate daughters by asymmetric Notch signaling, yet RSCs remain quiescent in the absence of damage. Nevertheless, the capacity for RSC-mediated repair extends the lifespan of flies carrying kidney stones, highlighting the importance of RSC-mediated regeneration. We propose that abundant, RSC-like stem cells exist in other tissues with low rates of turnover where they may have been mistaken for differentiated tissue cells.

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Mechanistic Intracellular Dynamics

277 Patched regulates lipid homeostasis by controlling cellular cholesterol levels Carla Cadena del Castillo[1], J. Thomas Hannich[2], Andres Kaech[3], Hirohisa Chiyoda[4], Masamitsu Fukuyama[4], Nils Faergeman[5], Howard Riezman[2], *Anne Spang*[1] 1) University of Basel, Basel, Switzerland; 2) University of Geneva, Geneva, Switzerland; 3) University of Zürich, Zürich, Switzerland; 4) University of Tokyo, Tokyo, Japan; 5) University of Southern Denmark, Odense, Denmark.

Hedgehog (Hh) signaling is essential during development and in organ physiology. In the canonical pathway, Hh binding to Patched (PTCH) relieves

the inhibition of Smoothened (SMO). Yet, PTCH may also perform SMO-independent functions. While the PTCH homolog PTC-3 is essential in *C. elegans*, worms lack SMO, providing an excellent model to probe non-canonical PTCH function. Here, we show that PTC-3 is a cholesterol transporter. *ptc-3(RNAi)* leads to accumulation of intracellular cholesterol and defects in ER structure and lipid droplet formation. These phenotypes were accompanied by a reduction in acyl chain (FA) length and desaturation. *ptc-3(RNAi)*-induced lethality, fat storage and ER morphology defects were rescued by reducing dietary cholesterol. We provide evidence that cholesterol accumulation modulates the function of nuclear hormone receptors such as of the PPARα homolog NHR-49 and NHR-181, and affects FA composition. Our data uncover a novel role for PTCH in organelle structure maintenance and fat metabolism.

278 P*la2g12b* affects serum cholesterol levels via the lipoprotein biogenesis pathway *James Thierer*[1], Meredith Wilson[1], Tabea Moll[1,2], Maggie Shen[1], Elisabeth Busch-Nentwich[3], Steven Farber[1,2] 1) Carnegie Institution for Science, Baltimore MD; 2) Johns Hopkins University, Baltimore MD; 3) Department of Medicine, University of Cambridge, Cambridge UK.

Cardiovascular diseases such as heart attack and stroke are driven by the accumulation of lipid-rich particles called lipoproteins in the arterial wall. There is thus significant impetus to identify genetic and pharmacological factors that can be used to lower the level of lipoproteins in the bloodstream, which could result in significant reduction in cardiovascular disease risk. We have developed transgenic zebrafish that produce glowing atherogenic lipoproteins (LipoGlo) to facilitate genetic and small-molecule screens to identify new modulators of lipoprotein size and abundance. Genetic screening revealed that *pla2g12b*, a catalytically inactive phospholipase gene with unknown function, has a dramatic influence on the size, abundance, and localization of atherogenic lipoproteins. We show that larval zebrafish lacking functional Pla2g12b produce fewer lipoproteins overall, and the lipoproteins produced are smaller and lipid-poor. Further, electron microscopy revealed that excess lipid accumulates in lipid droplets within the ER-lumen, suggesting that a large amount of intracellular lipid cannot be efficiently transferred to lipoproteins. A series of mutant alleles of *pla2g12b* were used in rescue experiments, which highlighted at least three functional protein domains. Further, protein interaction studies have shown that these functional domains interact directly with known components of the lipoprotein biogenesis pathway. We therefore propose a model whereby Pla2g12b serves as a molecular scaffold that is essential for efficient lipidation of atherogenic lipoproteins. Mice with mutations in *Pla2g12b* display healthy body weights and morphology, yet showed approximately 90% lower levels of serum cholesterol and 50% lower levels of triglycerides. Ongoing studies are evaluating whether mutations in *Pla2g12b* are able to prevent atherosclerosis in a mouse model of cardiovascular disease. We therefore conclude that Pla2g12b is an essential and highly conserved component of the lipoprotein biogenesis pathway, and may also represent a valuable new therapeutic target for prevention of cardiovascular disease.

279 ERM-1 phosphorylation and NRFL-1 redundantly control lumen formation in the *C. elegans* intestine in concert with the Ste20-like kinase GCK-4 João Ramalho[1], Jorian Sepers[1], Ophélie Nicolle[2], Grégoire Michaux[2], *Mike Boxem*[1] 1) Utrecht University, Utrecht, The Netherlands; 2) Univ Rennes, CNRS, Rennes, France.

Reorganization of the plasma membrane and underlying actin cytoskeleton into specialized cortical domains is essential for the function of most polarized cells in animals. ERM proteins are conserved regulators of cortical specialization, that function both as membrane-actin linkers and organizers of molecular hubs. Regulation of ERM protein activity involves a conformational switch from an inactive cytoplasmic form into an active membrane- and actin-bound form, which is thought to be mediated by sequential PIP_2-binding and phosphorylation of a conserved C-terminal threonine residue. However, *in vivo* data supporting this model is scarce, and results on the essentiality of phosphorylation are conflicting. Here, we use the single *C. elegans* ERM ortholog, ERM-1, to study the contribution of these regulatory events to ERM activity and tissue formation *in vivo*. Using CRISPR/Cas9-generated *erm-1* mutant alleles we demonstrate that PIP_2-binding is critically required for ERM-1 function. In contrast, dynamic regulation of C-terminal T544 phosphorylation is not essential, but instead modulates ERM-1 apical localization and dynamics in a tissue-specific manner, to control cortical actin organization and drive lumen formation in epithelial tubes. We also investigated the contribution of ERM-1 associated proteins to lumen formation in the *C. elegans* intestine. We show that the interaction between ERM-1 and the NHERF1/2 ortholog NRFL-1 is conserved in *C. elegans*, and that ERM-1 T544 phosphorylation and NRFL-1 act in parallel to drive lumen formation. Finally, we identify GCK-4, the *C. elegans* homolog of LOK/SLK kinases, as an ERM-1 kinase, and show that ERM-1 promotes intestinal lumenogenesis both via ERM-1 T544 phosphorylation dependent and independent mechanisms.

280 Connecting the lamin dots: Lmnl3 orchestrates chromosome segregation and replication timing during zebrafish cleavage stages *Dana Klatt Shaw*[1,2], David Grunwald[2] 1) Washington University, St. Louis, MO; 2) University of Utah, Salt Lake City, UT.

Many animals, including fishes, amphibians, and diptera, undergo an early cleavage stage, which includes rapid mitoses accompanied by cell divisions wholly dependent on maternally supplied factors. Maternal-effect screens in zebrafish have confirmed that specialized maternally supplied components are devoted to facilitating the unique challenges presented by cleavage stage development. We have investigated cleavage stage-specific roles of the nuclear lamina, a fibrillar network of intermediate filaments that gives structural support to the nuclear envelope. *Lamin* mutations in humans lead to a broad spectrum of poorly understood laminopathies and have been tenuously linked to cancer. Therefore, lamina is also implicated in more broad functional roles in gene regulation, DNA replication, cell division, and cellular signaling. We generated and analyzed the effects of two different loss-of-function alleles in *lamin-like 3* (*lmnl3)*, which ecodes a predominant lamin of the cleavage stage embryo. Both mutations eliminate the production of functional Lmnl3 product and produce maternal-effect lethal phenotypes. *MZlmnl3* mutants display defects in chromosome segregation during cleavage stage mitoses as evidenced by lagging chromosomes and anaphase bridges between daughter nuclei. In the absence of maternal wildtype Lmnl3, cell divisions are also disrupted and embryos containing multi-nucleate and anucleate cells are produced. Double-stranded DNA breaks are present at micronuclei and chromosome bridges in *MZlmnl3* embryos, suggesting a role for Lmnl3 in genome stability. DNA replication initiation is delayed in *MZlmnl3* mutants and replication continues delayed into prophase, while chromosomes are beginning to condense for the next mitotic division. This work implicates nuclear lamins as a primary player in maintaining the integrity of the genome through assisting chromosome segregation and maintaining the timing of DNA replication. These more broad functional roles of lamins may help explain the poorly understood spectrum of laminopathies caused by defects in the lamin genes, which range from cardiomyopathies to premature aging to cancer.

281 Ire1 Phosphorylates Pumilio to protect XBP1 mRNA from RIDD *Fatima Cairrao*[1], Adrien LeThomas[2], Cristiana Santos[1], Avi Ashkenazi[2], Pedro Domingos[1] 1) ITQB-Antonio Xavier, UNL, Oeiras, Portugal; 2) Cancer Immunology, Genentech Inc, South San Francisco, CA, USA.

Homeostasis in cells during the Unfolded Protein Response (UPR) requires that the mRNA coding the transcription factor Xbp1 be properly localized

to the endoplasmatic reticulum (ER) and modulated to respond adequately to changes in demand for protein folding and ER stress. We noted that the Drosophila Xbp1 3'UTR contains putative binding sites for the RNA binding protein Pumilio, matching the 8nt core consensus sequence previously described. We therefore hypothesized that Xbp1 mRNA may be a target for post-transcriptional regulation by Pumilio. Our studies demonstrate that Pumilio stabilizes $Xbp1^{spliced}$ mRNA and its expression. TAP-RNA affinity purification of Xbp1 mRNAs from adult drosophila eyes showed association with the RNA binding domain of Pumilio. Furthermore, Pumilio's phosphorylation state affected Xbp1 regulation during the UPR. Indeed, Drosophila Pumilio showed an increase in phosphorylation during ER stress in S2 cells. Kinase assays using a recombinant human IRE1α and purified recombinant forms of Pumilio revealed that Ire1α, which harbors a kinase moiety, mediates phosphorylation of both drosophila and human Pumilio proteins. These results suggest an evolutionarily conserved mechanism involving Ire1-mediated phosphorylation of Pumillio to regulate Xbp1 mRNA stability.

282 Phenomic screen implicates the yeast lysine acetyltransferase NuA4 in regulation of glycogen synthesis and mitochondrial morphology through the PKA inhibitor Bcy1 *Elizabeth Walden*[1,2], Roger Fong[1,2], Trang Pham[1,2], Hana Knill[1,2], Mary Ellen Harper[1,2], Kristin Baetz[1,2] 1) University of Ottawa Faculty of Medicine, Ottawa Canada; 2) Ottawa Institute of Systems Biology, Ottawa Canada.

Cellular metabolism is tightly regulated by many signaling pathways, including by the post translational modification lysine acetylation. While in many cases lysine acetyltransferase (KAT)-dependent acetylation of enzymes impacts their activity, there is also growing evidence that lysine acetylation can impact protein localization. As the *Saccharomyces cerevisiae* KAT complex NuA4 has been implicated in a variety of metabolic processes, here we explore whether NuA4 is regulating the localization and/or abundance of metabolic proteins. We performed a high-throughput GFP-based microscopy screen of over 400 metabolic proteins and identified 23 proteins whose localization and/or abundance changed upon deletion of the NuA4 scaffolding subunit, *EAF1*. This included 3 proteins required for glycogen synthesis and 14 proteins associated with the mitochondria. We determined that in *eaf1Δ* cells the transcription of glycogen biosynthesis genes is upregulated resulting in increased proteins and glycogen production. Further, our study shows that in the absence of *EAF1* mitochondria are hyperelongated, and chaotically distributed with a three-fold increase in mitochondrial volume relative to wildtype cells. Despite aberrant structure, *eaf1Δ* mitochondria still respond to environmental stress and extracellular flux analysis indicates that mitochondrial function is intact. We determined that the increased glycogen synthesis and mitochondrial structure changes in *eaf1Δ* cells are dependent on Bcy1, the yeast negative regulator of PKA. Surprisingly we find that in the absence of *EAF1*, Bcy1 localization changes from being largely nuclear to cytoplasmic. This localization of Bcy1 is dependent on NuA4-dependent lysine acetylation at residue K313 of Bcy1. Together our work shows that NuA4 acetylation of Bcy1 at the K313 site impacts Bcy1 localization and its downstream regulation of mitochondrial morphology and glycogen synthesis. As NuA4 is highly conserved with the human Tip60 complex, our work may prove to be directly transferable to human disease biology, revealing new avenues to investigate the role of Tip60 in metabolic regulation and by extension the deregulation that may occur in disease.

283 Translational induction of ATF4 mRNA during Integrated Stress Response requires noncanonical initiation factors eIF2D and DENR Deepika Vasudevan[1], Amy Yang[1], Sarah Neuman[2], Lea Lough[1], Timothy Cardozo[1], Arash Bashirullah[2], *Hyung Don Ryoo*[1] 1) New York University SoM; 2) University of Wisconsin, Madison.

The Integrated Stress Response (ISR) is a signaling pathway that is activated by conditions that include amino acid deprivation and excessive protein misfolding in the endoplasmic reticulum (ER). The kinases that mediate ISR help metazoan cells adapt to these stresses by phosphorylating a canonical translation initiation factor, eIF2, thereby limiting the availability of initiator methionyl-tRNA to ribosomes. While such conditions reduce overall mRNA translation in cells, they paradoxically stimulate the translation of ATF4 mRNA through a regulatory 5' leader sequence with multiple upstream Open Reading Frames (uORFs), thereby activating stress-responsive gene expression. Here, we report the identification of critical regulators of such ATF4 induction during ISR signaling. Specifically, we identified two related noncanonical initiation factors, *eIF2D* and *DENR*, as regulators of ATF4 in *Drosophila* and human cells. Loss of *eIF2D* and *DENR* in *Drosophila* results in increased vulnerability to amino acid deprivation, susceptibility to retinal degeneration caused by endoplasmic reticulum (ER) stress, and developmental defects similar to *ATF4* mutants. eIF2D requires its tRNA-binding motif for regulating the 5' leader-mediated ATF4 translation. Consistently, eIF2D and DENR deficient human cells show impaired ATF4 protein induction and reduced ribosome occupancy at the ATF4 main ORF in response to ER stress. Altogether, our findings indicate that *eIF2D* and *DENR* are critical mediators of ATF4 translational induction and stress responses in vivo.

284 α-Arrestins Regulate Autophagy *Allyson O'Donnell*[1], Karandeep Chera[1], Ray Bowman[1], Donna Stolz[2] 1) Dept. of Biologicals Sciences, University of Pittsburgh; 2) Dept. of Cell Biology, University of Pittsburgh .

Cells adapt to changes in their environment through selective reshuffling of their proteome. This reorganization is regulated in part, by a highly conserved family of protein trafficking adaptors called the α-arrestins. To identify specific regulators of the α-arrestins and to aid in defining new α-arresitn functions, we generated and utilized the *Saccharomyces cerevisiae* Ubiquitin Interactome (ScUbI) library. The ScUbI library is a unique subset of the deletion collection that contains knockout strains for all non-essential genes annotated as being important for ubiquitination and ubiquitin interaction. Ubiquitination is a post-translational modification known to impact the function of α-arrestins, but for most α-arrestins the mechanism of this regulation is unclear. We used this library as part of an undergraduate Cell & Molecular Biology lab to screen for factors that altered α-arrestin-mediated resistance to the TORC1-inhibiting drug rapamycin. Changes to the α-arrestin-induced resistance to rapamycin were most enriched in cells lacking autophagy components, or *ATG* genes. The *ATG* genes comprise the machinery for the self-degradative process of autophagy, a functional pathway whereby cells recycle aging or damaged components or reclaim key nutrients during starvation and implicated in human disease. We have defined the genetic network that links α-arrestins and the *ATG* family. To elucidate the mechanism connecting the α-arrestins with autophagy, we employed live cell imaging and biochemical analyses, which demonstrated that autophagic flux is impaired in the absence of select α-arrestins. Potentially as a mechanism to improve autophagy in the absence of α-arrestins, the *ATG8* gene is transcriptionally upregulated in strains lacking α-arrestins. Electron microscopy revealed a reduction in autophagosomes in the vacuole, indicative of a defect prior to autophagosome fusion. We also observed in EM images, fewer autophagosomes accumulating in the cytosol in the absence of α-arrestins, suggesting a defect in autophagosome biogenesis. We further find a reduced lipid droplet reserve in the absence of α-arrestins, which could impede autophagosome formation by limiting the availability of lipids needed to generate the autophagosome. Together, these data support an exciting novel role for the α-arrestins as regulators of autophagy, expanding their known suite of functions in sensing and responding to nutrient stress.

Friday, April 24 3:45 PM - 5:45 PM

Complex Trait Adaptation

256 Characterizing strong adaptation in an admixed population over 20 generations Iman Hamid[1], Katharine Korunes[1], Sandra Beleza[2], *Amy Goldberg*[1] 1) Duke University; 2) University of Leicester.

How fast can selection shape genetic variation? We use one of the strongest proposed selection pressures in human history, the malaria pathogen *Plasmodium vivax*, as a case study to test this question. We consider the admixed human population of Cape Verde, founded in the 1400s by Portuguese colonizers and African slaves. Selection post-admixture constrains the potential timing of selection, strengthening the test of our question. Additionally, admixture provides one of the fastest scenarios for adaptation by introducing a potentially beneficial allele at a high frequency. Current approaches to identify regions under selection in admixed populations are largely based on ancestry-outlier detection, which discard a lot of information and produce false positives. Instead, we use the length and distribution of ancestry within an individual, between individuals in a population, and between populations to infer the timing and strength of selection. Combining genome-wide simulations with data from 564 admixed Cape Verdeans across five regions, we find evidence of strong selection at the *DARC* gene containing the classic malaria-protective Duffy-negative allele. Notably, we only find large allele frequency deviations and long African ancestry tracts on one island consider, Santiago. Indeed, Santiago is the only island with a substantial history of malaria incidence. Under a variety of realistic simulation scenarios, we demonstrate how selection has dramatically altered genetic variation in this population over just 20 generations. More generally, we highlight how, on short timescales, strong selection on a single locus can bias demographic history inferred from whole genomes.

257 Decoding wheat adaptation by genus-level population sequencing *Fei Lu*[1,2,3], Xuebo Zhao[1,2], Yao Zhou[1], Aoyue Bi[1,2], Changbin Yin[1], Jing Wang[1] 1) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences; 2) University of Chinese Academy of Sciences; 3) CAS-JIC Centre of Excellence for Plant and Microbial Science (CEPAMS).

Bread wheat (*Triticum aestivum*. ssp. *aestivum*) expanded its habitats from a small core area of the Fertile Crescent to global environments within ~10,000 years. Genetic mechanisms of this remarkable evolutionary success are not well understood. By whole-genome sequencing of populations from 25 subspecies, a total of 414 accessions within genera Triticum and Aegilops, we constructed a comprehensive whole-genome genetic variation map of wheat (VMap I), consisting of ~104M SNPs. By comparing bread wheat and its wild relatives, including wild emmer, domesticated emmer, free-threshing tetraploids, and *Aegilops tauschii*, we found that 13%~36% of the bread wheat genome was contributed by alien introgression, which tremendously increased the genetic diversity of bread wheat and allowed its divergent adaptation worldwide. Meanwhile, signatures of convergent adaption to human selection were identified in Triticum species despite drastic differences in ploidy levels and growing zones, indicating the vital importance of adaptive constraints in the success of bread wheat. These results showed the genetic necessities of wheat as a global crop and provided new perspectives about leveraging adaptation success across species for crop improvement.

258 The genomic basis of adaptation in a ninety year long barley experiment *Daniel Koenig*[1], Jacob Landis[1], Keely Brown[1], Jill Marzolino[1], Angelica Guercio[1], Chris Fiscus[1] 1) University of California Riverside.

The rapid diversification of domesticated species is an important model of adaptive evolution. However, our understanding of adaptation is limited by the obscure evolutionary history of individual varieties. Here, we directly record genetic change during adaptation to a stochastic field environment over the course of a 90 years in the barley Composite Cross (CC) experiments. We find massive, envrionment dependent restructuring of genetic diversity over time in the CCs. Evolutionary change was initially driven by strong selection on developmental genes, but then it transitioned to a gradual fixation of locally adapted alleles throughout the genome. At least one of the targeted developmental factors is the domestication locus Vrs1, suggesting that mutations implicated in domestication can rapidly fix in agricultural environments absent human mediated selection. The later stages of the experiment are characterized by the rapid increase of a few multilocus haplotypes ultimately accounting for 30-40% of the population. We link these genetic changes to strong phenotypic shifts in the population and identify the footprint of directional selection targeting loci that control these traits. The unique oppurtunity to observe evolution in action the CCs opens exciting new avenues for research to understand adaptation and diversification in domesticated plants.

259 The strength and pattern of natural selection on rice gene expression *Simon Groen*[1], Irina Calic[2], Zoe Joly-Lopez[1], Adrian Platts[1], Jae Choi[1], Rahul Satija[1,3], Georgina Vergara[4], Amelia Henry[4], Steven Franks[2], Michael Purugganan[1] 1) New York University, New York, NY, USA; 2) Fordham University, Bronx, NY, USA; 3) New York Genome Center, New York, NY, USA; 4) International Rice Research Institute, Los Banos, Laguna, Philippines.

Levels of gene expression underpin organismal phenotypes, yet the nature of selection on gene expression, and its role in adaptive evolution, remain elusive. We assayed gene expression in rice *Oryza sativa*, and used phenotypic selection analysis to estimate the type and strength of selection on levels of >15,000 transcripts. Variation in most transcripts appears neutral/near-neutral or under very weak stabilizing selection in wet paddy conditions, with median standardized selection differentials near zero, but greater under drought. More transcripts look to be conditionally neutral (2.83%) than antagonistically pleiotropic (0.04%). Also, transcripts displaying lower levels of expression and stochastic noise and higher levels of plasticity were under stronger selection. Selection strength was further weakly negatively associated with levels of *cis*-regulation, and network connectivity. Multivariate analysis suggests that selection acts on the expression of photosynthesis genes, but that its efficacy is genetically constrained under drought. Drought selected for earlier flowering and higher expression of *OsMADS18*, a known regulator of early flowering, marking this MADS-box transcription factor as a drought escape gene. The ability to estimate selection strengths provides insights into how selection can shape molecular traits at the core of gene action.

260 Fitness and environmental patterns in maize landraces identify beneficial alleles at single gene resolution *Daniel Gates*[1], Dan Runcie[1], Garret Janzen[2], Alberto Romero Navarro[3], Martha Willcox[4], Kai Sonder[4], Samantha Snodgrass[2], Fausto Rodríguez-Zapata[5], Ruairidh Sawers[6], Rubén Rellán-Álvarez[5], Edward Buckler[3], Sarah Hearne[4], Matthew Hufford[2], Jeffrey Ross-Ibarra[1] 1) UC Davis; 2) Iowa State University; 3) Cornell University; 4) International Maize and Wheat Improvement Center (CIMMYT); 5) North Carolina State University; 6) Penn State University.

A fundamental goal of evolutionary biology is to understand the genetic basis of adaptation. The loci that confer adaptation to various environments also hold great potential to improve and adapt crop breeding populations to changing climates. In our research we identify loci with patterns of local adaptation using multiple methods and demonstrate how this information complements experimental results designed to identify maize

lines with superior adaptation to different stresses. We use data from over 2500 traditional maize landraces broadly representing genetic diversity of maize in Mexico to evaluate plant fitness in 13 common gardens across a range of environments to show patterns of local adaptation. Using genome-wide genotyping-by-sequencing data, we then identify locally adapted loci based upon genotype by environment interactions across experiments conducted in different environments. We further identify genetic associations with environment across landrace origins in Mexico, Central, and South America. These environmentally associated loci are highly predictive of adaptive variation in yield and flowering time in our field trials, and we show that alleles associated with low precipitation environments also predict performance in drought trials. Our results indicate that the genetic variation necessary to adapt crops to changing climate exists in open pollinated landraces that have been subject to ongoing environmental adaptation and can be identified by both environmental associations and environmental stress trials. Furthermore, the high diversity and rate of linkage decay of landraces combined with the high marker density of modern genotyping means that GWAS approaches can deliver exceptional precision allowing identification of individual adaptive genes in landrace germplasm.

261 A full-likelihood method to disentangle selection on genetically-correlated traits using whole-genome genealogies *Aaron Stern*[1], Leo Speidel[2], Noah Zaitlen[3], Rasmus Nielsen[1] 1) UC Berkeley, Berkeley, CA, USA; 2) University of Oxford, UK; 3) UC Los Angeles, Los Angeles, CA, USA.

A key aim of genetic research is to identify selection on complex traits and its impact on human genome evolution. The lack of strong signals of selection at individual loci, combined with the insight from genome wide association studies (GWAS) that most human traits of interest are highly polygenic, suggests that polygenic adaptation is likely the dominant mode of human adaptation, as it would allow traits to differentiate rapidly while leaving only subtle signals of selection at each causal locus.

To this end, we present a full-likelihood method to estimate polygenic adaptation from contemporary DNA sequence data. Our method works by combining population genetic data and GWAS summary statistics from up to thousands of nucleotide sites throughout the genome. Building on Stern, *et al.* (2019), we estimate the likelihood by conducting importance sampling on the ancestral recombination graph, which we sample using the program Relate. We then aggregate these likelihoods across loci, along with their GWAS SNP effects, in order to estimate the selection gradient on traits.

Furthermore, our method controls for pleiotropy, which typically adds a significant complication; traits which have no effect on fitness may still show signs of selection if there is genetic correlation (r_g) with another trait that is under selection. Current methods only test for selection on traits marginally, which we show gives biased estimates of selection for traits with appreciable rg. To validate our method, we conduct population genetic simulations which show that our method accurately disentangles selection even among traits with very high genetic correlation ($|r_g| = 0.8$; c.f. Schizophrenia & Bipolar Disorder). Our method is robust to uncertainty and ascertainment bias in SNP effects estimated through GWAS ($N=10^5$), uncertainty in the causal SNP, and multiple linked causal SNPs. Our method is well-powered at modest sample sizes ($n=200$) and can control for non-equilibrium demography. We apply our method to scan >100 polygenic traits for signs of adaptation using summary statistics from UK Biobank, Psychiatric Genomics Consortium, and other groups. We also re-examine evidence for polygenic adaptation of height and/or its genetic correlates.

Friday, April 24 6:00 PM - 7:05 PM

Keynote Session 2

285 Stem Cells In Silence, Action and Cancer *Elaine Fuchs*[1] 1) Rockefeller University.

abstract is not available at the time of print

286 2020 Morgan Medal *David Botstein*[1] 1) Calico Labs.

abstract is not available at the time of print

287 A liquid-like organelle at the root of motile ciliopathy *John Wallingford*[1] 1) University of Texas, Austin.

Motile ciliopathies are characterized by defects in cilia beating that result in chronic airway disease, subfertility, ectopic pregnancy, and hydrocephalus. Many patients harbor mutations in the dynein motors that drive cilia beating, but the disease also results from mutations in so-called Dynein Axonemal Assembly Factors (DNAAFs) that act in the cytoplasm. Here, we will describe a novel, cell-type specific liquid-like organelle, which we term DynAPs, for Dynein Axonemal Particles. These organelles provides a privileged space for enrichment of DNAAFs, general chaperones, and dyeing subunits. By exploring DynAP assembly and function using in vivo imaging and proteomics, we provide a unifying cell biological framework for a poorly understood class of human disease genes and add motile ciliopathy to the growing roster of human diseases associated with disrupted biological phase separation.

Saturday, April 25 11:00 AM - 1:00 PM

System Biology of Yeast (Yeast)

311 Towards a systematic map of the functional role of protein phosphorylation Bede P. Busby[1,2], Cristina Viéitez[1,2], David Ochoa[2], André Mateus[1], Marco Galardini[2], Areeb Jawed[1], Danish Memon[2], Clement M. Potel[1], Sibylle Vonesch[1], Chelsea Szu Tu[1], Mohammed Shahraz[1], Frank Stein[1], Lars M. Steinmetz[1], Mikhail M. Savitski[1], Athanasios Typas[1], Pedro Beltrao[1,2] 1) European Molecular Biology Laboratory, Genome Biology Unit; 2) European Molecular Biology Laboratory, European Bioinformatics Institute.

Phosphorylation is an essential post-translational modification involved in the regulation of almost all cellular processes. To date, over 20,000 phosphorylation sites have been identified in *S. cerevisiae*, however, less than 5% have a known molecular function. We implemented a chemical genetic approach to study the functional relevance of phosphorylation in *S. cerevisiae*. We generated a library 474 phospho-deficient mutants that, along with the yeast knockout (KO) library, were screened for fitness in 102 diverse stress conditions. This analysis showed that 42% of phospho-deficient mutants exhibited conditional growth phenotypes, suggesting they are likely to have a molecular function. We were able to infer their function

based on the similarity of their growth profiles with that of the gene KO's. Some phospho-deficient mutants showed loss of function phenotypes, similar to that of their corresponding gene KO. In contrast, a higher number exhibited phenotypes not observed in the corresponding gene deletion, which is suggestive of a gain of function. For nine phospho-deficient mutants, we carried out additional molecular characterization using thermal proteome profiling and shotgun lipidomics, which further elucidated the functional relevance of these sites. Furthermore, in addition for phospho-sites which have an orthologous site in humans, the phenotypes observed in *S. cerevisiae* can be indicative of their functional relevance in humans.

312 Species-wide survey of background-dependent phenotype across yeast natural populations *Jing Hou*[1], Guihong Tan[1], Brenda Andrews[1], Charlie Boone[1] 1) University of Toronto.

The same mutation does not always cause the same phenotype in different individuals due to differences in their genetic backgrounds. Such background effect may constitute an inherent feature of biological traits, complicating our ability to predict phenotypes from genomic information. In model systems, a classic example of background effect is conditional gene essentiality, which occurs when the loss-of-function of a gene causes lethality in one background but not another. Between two yeast strains, S288c and ∑1278b, ~1% of all genes were conditional essential. Understanding the genetic basis of conditional essentiality offers a great opportunity to dissect the origin of background effects - a potential source of "missing heritability" in complex traits.
Over the past few years, an expanding number of natural yeast isolates in the *Saccharomyces cerevisiae* species has been completely sequenced. These isolates are originated from various ecological and geographical sources and are genetically diverse, with maximum nucleotide divergence ~1.5%. Taking advantage of these resources in the yeast system, our goal is to survey the entire species for background-dependent phenotypes related to gene deletion mutations. We generated a collection of ~450 strains that are diploid, euploid and homozygous, which were originated from monosporic segregants of over 1,000 wild yeast isolates. To efficiently create gene deletion mutants in these wild strain backgrounds, we developed a CRISPR-Cas9 based plasmid library that allows for one-stop PCR-free gene deletion across the whole genome, for any background of interest across the *S. cerevisiae* species. Each deletion mutant generated using this method will carry barcodes that allow for precise identification in pool-based strategies. This powerful resource will greatly benefit the yeast community in studying background-specific mutation effects, higher-order genetic interactions and gene-environment interactions.

313 Gene regulatory network reconstruction using single-cell RNA sequencing of barcoded genotypes in diverse environments *David Gresham*[1] 1) New York University.

Understanding how gene expression programs are controlled requires identifying regulatory relationships between transcription factors and target genes. Gene regulatory networks are typically constructed from gene expression data acquired following genetic perturbation or environmental stimulus. Single-cell RNA sequencing (scRNAseq) captures the gene expression state of thousands of individual cells in a single experiment, offering advantages in combinatorial experimental design, large numbers of independent measurements, and accessing the interaction between the cell cycle and environmental responses that is hidden by population-level analysis of gene expression. To leverage these advantages, we developed a method for scRNAseq in budding yeast (Saccharomyces cerevisiae). We pooled diverse transcriptionally barcoded gene deletion mutants in 11 different environmental conditions and determined their expression state by sequencing 38,285 individual cells. We benchmarked a framework for learning gene regulatory networks from scRNAseq data that incorporates multitask learning and constructed a global gene regulatory network comprising 12,228 interactions.

314 Connecting novel rare disease gene discoveries to functional characterization research in yeast and other model organisms. *Phil Hieter*[1], S. Rogic[1], P. Pavlidis[2], K. Boycott[3] 1) Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada; 2) Department of Pediatrics, Centre de Recherche du CHU Ste-Justine, Montreal, Canada ; 3) CHEO Research Institute, University of Ottawa, Ottawa, ON, Canada Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada.

Implementation of next-generation sequencing technology has greatly accelerated the identification of genetic variants that cause rare, single-gene disorders. However, the discovery of the human variant is inherently a descriptive, hypothesis-generating milestone that requires subsequent studies on the basic function of the gene and the functional consequences of specific gene mutations in a biological context. Model organisms (MOs) such as yeast, worm, fly, zebrafish, and mouse provide powerful experimental tools to investigate the mechanistic basis of rare genetic diseases and to identify and explore potential therapeutic interventions. The key to success is identifying connections and collabirations between clinicians, immediately following the discovery of new disease gene mutations, and scientists able to study equivalent genes and pathways in MOs. For this reason, we established the Canadian Rare Diseases Models and Mechanisms (RDMM) Network.

The RDMM Network built a web-based Canadian directory of MO scientists to identify possible connections and uses a formalized committee process to review clinician-MO scientist collaborations and approve 25K CAD in catalyst funding. This has created a rapid and direct pathway from disease gene discovery to functional characterization studies in MOs. Since October 2014, we have made ~100 clinician-MO scientist connections with catalyst seed funding. These collaborations have resulted in validation of disease gene discovery, identification of possible therapies, and success in obtaining subsequent grant funding. The RDMM concept also serves to educate scientists, clinicians, patient communities and funding agencies about the power of model organisms for developing rational approaches to disease prevention, management, and treatment.

We have recently expanded the impact and reach of the RDMM Network by establishing international linkages with emerging networks modeled on RDMM. To facilitate this, we made the RDMM Registry open-source, portable, and customizable and our committee structures and process freely-available. The emerging network of RDMM networks will enable matches across borders when there is no MO expertise within the country of the gene discovery. With renewed funding through 2023, we will continue to create meaningful collaborations between clinicians and MO scientists, enhance the generation of new knowledge, and advance RD research locally and globally.

315 Introduction of Jonathan Weissman for the Ira Herskowitz Presentation *Orna Cohen-Fix*[1] 1) NIH/NIDDK.

abstract is not available at the time of print

316 Ira Herskowitz Award Presentation *Jonathan Weissman*[1] 1) University of California, San Francisco.

abstract is not available at the time of print

Saturday, April 25 11:00 AM - 1:00 PM

Insights into Cellular Dynamics and Functions (Zebrafish)

317 The recycling endosome protein Rab25 coordinates actomyosin network maintenance, mitosis and cytokinesis to regulate epithelial tissue spreading in the zebrafish gastrula *Morley Willoughby*[1], Jessica Yu[1], Molly Allen[1], Tianhui Chen[1], Rodrigo Fernandez-Gonzalez[1], Ashley Bruce[1] 1) University of Toronto.

How cell shape changes and tissue integrity are coordinated during epithelial morphogenesis is not fully understood. In the zebrafish gastrula, a single-cell thick epithelial sheet spreads by thinning and expanding in a process termed epiboly. We identified a potential molecular regulator of this conserved process, the epithelial specific recycling endosome protein Rab25. Rab25 expression is restricted to the epithelium and its subcellular distribution is dynamic, with N-terminally fluorescently tagged protein localizing to the plasma membrane and in cytosolic puncta. Tagged Rab25 co-localizes with the apical junctional complex and additionally becomes enriched around the centrosomes upon entry into mitosis. Maternal-zygotic (MZ) *rab25* mutants show delayed epithelial tissue spreading and exhibit multinucleated cells with increased apical surface area and junctional curvature, resulting in an abnormal number of cell-cell contacts. Live imaging shows mitotic defects, cytokinesis failures, cell fusions and basal cell extrusions in MZ*rab25* embryos. The large, multinucleate cells contain numerous centrosomes resulting in multipolar, misoriented spindles and multiple cleavage furrows. Cytokinesis fails due to incomplete cleavage furrow ingression. Cytokinesis also fails when apical intercellular bridges persist after mitosis, apparently due to failed abscission. In mutant embryos, epithelial cells in close proximity can be connected by intercellular bridges and, presumably due to morphogenetic stress, the bridges regress, leading to large scale cell fusions. Furthermore, in MZ*rab25* embryos adherens (AJ) and tight junction (TJ) proteins are abnormally distributed and junctional actomyosin is reduced. These defects correlate with reduced junctional tension, which was measured using laser ablation, and suggest that force transmission across the epithelium is likely abnormal in mutant embryos. Perturbing actin specifically in the epithelium is sufficient to disrupt tissue spreading, suggesting that the epiboly delay in mutants could be due to cell autonomous defects in the actin network. Interestingly, AJ, TJ and actomyosin are also disrupted in cells which undergo normal proliferation and cell shape changes in MZ*rab25* embryos, suggesting that Rab25 remodels cell-cell junctions independently of its role in mitosis and cytokinesis. Thus, we propose that Rab25 controls epithelial tissue spreading through cell-cycle dependent and independent mechanisms.

318 Dynamic actomyosin pulses induce visco-elastic heterogeneity to drive epithelial cell extrusion *Youmna Atieh*[1], Thomas Wyatt[2], Ana-Maria Zask[3], Prerna Malaney[4], George Eisenhoffer[1] 1) Department of Genetics, The University of Texas MD Anderson Cancer Center, Houston, TX; 2) Laboratoire Matière et Systèmes Complexes, UMR 7057 CNRS & Université Paris Diderot, 10 rue Alice Domon et Léonie Duquet, 75013, Paris, France; 3) Atomic Force Microscopy Service Center, The University of Texas Health Science Center, Houston, TX; 4) Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX.

Extrusion is a mechanism used to eliminate unfit cells from epithelial tissues. Cell culture studies where damaged or oncogenic single cells are surrounded by WT neighbors have shown that extrusion depends on the contractility of the neighbors that push out the damaged cell through an organized assembly of an actomyosin ring. However, how this mechanical signal is integrated at the scale of complex tissues and embryos where cells are stochastically damaged is still unknown. While we start to have a better understanding on the influence of mechanics on cell differentiation and proliferation, how mechanical stresses can also lead to cell death has been much less explored so far.
Using the zebrafish fin epidermis as an *in vivo* model, we show that cell extrusion is preceded by a mechano-sensing event in the form of pulsatile actomyosin contractions that drive cell shape changes. Atomic force microscopy measurements on whole embryos reveal that tissue remodeling is facilitated by an overall decrease in stiffness. Analyzing the dynamics of cell area fluctuation during contractions predicts that long pulses yield less reversible deformations and therefore lead to extrusion. We show that pulsing correlates with an enrichment of junctional actomyosin in epithelial cells, inducing tension redistribution across the tissue whereas pulsing areas display high tension and non-pulsing areas low tension. Finally, we demonstrate that fluctuations in physical forces are controlled by sphingosine 1 phosphate (S1P) enrichment, thus defining a temporal causality between actomyosin pulses and cell extrusion.
Altogether, we describe a novel mechano-sensing mechanism that orchestrates the coordination between physical forces in damaged epithelia and predicts local extrusion spots to facilitate elimination of unfit cells in a living vertebrate epithelial tissue.

319 Genetic analysis of ileal identity in the zebrafish intestine *Jia Wen*[1], Alyssa Volland[2], Gilberto Padilla Mercado[1], Colin Lickwar[1], Jason Ridlon[2], John Rawls[1] 1) Duke University; 2) University of Illinois Urbana-Champaign.

Digestive physiology in the intestine in achieved through the coordinated function of distinct regions along its cephalocaudal axis. The ileum is a specialized region of the intestine that actively absorbs bile salts, amphipathic molecules that act as lipid emulsifiers and signaling molecules. However, the mechanisms determining ileal identity remain largely unknown. We recently found that the intestinal regionality, including the ileum, is conserved between zebrafish and mammals. Many conserved markers of ileal epithelial cells are targets of the bile salt activated transcription factor Farnesoid X receptor (Fxr/Nr1h4). We generated *fxr* mutant zebrafish which exhibited diminished expression of conserved ileal markers including *fabp6*, suggesting that Fxr may be required to establish ileal identity. Single-cell RNA-seq analysis of intestinal epithelial cells from wild-type and *fxr* mutant zebrafish revealed that *fxr* is not required for development of ileal epithelial cells, but does promotes distinct gene expression programs in ileal and other cell types. We next evaluated zebrafish *cyp7a1* and *slc10a2* mutants that have defective synthesis or uptake of bile salts, respectively. The activity of a *fabp6:GFP* reporter in the ileal region was reduced in both mutants, suggesting that bile salts are required for Fxr-mediated expression of ileal markers. In mammals, primary bile acids are modified by gut microbiota into secondary bile acids with varying activities as Fxr ligands. We found that the primary bile salts in zebrafish consist largely of bile alcohols with few bile acid species, and that those primary bile salts undergo multiple modifications by zebrafish microbiota. We also identified a zebrafish microbiota member *Acinetobacter* sp. capable of modifying a major bile salt species thus altering its potency for Fxr activation. Together, these results establish that the ileum and bile salt modifications by gut microbiota are conserved features in the intestine of fishes and mammals, and that Fxr directs distinct gene expression programs in the ileum and other intestinal epithelial cell types.

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Regulation of protrusive behavior during collective cell migration *Hannah Olson*[1], Hillary McGraw[2], Alex Nechiporuk[1] 1) Oregon Health and Science University, Portland, OR; 2) University of Missouri, Kansas City, Kansas City, MO.

Cells migrate individually or in groups in a process known as collective cell migration. These cohorts of cells maintain cell-cell contact, group polarization and exhibit coordinated behavior. Collective cell migration is important for numerous processes during development including blood vessel branching and neural crest cell migration as well as in adulthood in wound healing and cancer invasion. During individual and collective cellular migration, cells must extend protrusions to interact with the extracellular environment, sense chemotactic cues, and act as points of attachment. The mechanisms and regulators of protrusive behavior have been widely studied in individually migrating cells; however, how this behavior is regulated throughout collectives is not well understood. To study protrusive behavior during collective cell migration, we use the zebrafish posterior lateral line primordium (pLLP) as a model. The pLLP is a cluster of ~100 cells that migrates along the zebrafish trunk, depositing groups of cells that will become sensory organs. To define protrusive behavior during pLLP migration, we performed mosaic analysis to sparsely label cells within the pLLP with a transgene marking filamentous actin. This approach revealed an abundance of brush-like actin-based protrusions throughout the pLLP. We found that these brush-like protrusions orient in the direction of migration and are larger in area in cells in the front of the pLLP in comparison to cells in the trailing region of the pLLP. Further, inhibition of branched actin networks prevents the formation of brush-like protrusions and inhibits pLLP migration, suggesting that brush-like protrusions are made up of branched actin networks and are necessary for migration. Mosaic labeling of both cellular actin-based protrusions and cell membranes within the pLLP indicate that these protrusive structures interact with cellular membranes of cells within the pLLP. These results suggest that protrusions in the pLLP could be playing a role in inter-cellular communication among the cells of the pLLP. Finally, inhibition of Fgf signaling, a signaling pathway necessary for proper morphogenesis of the pLLP, results in a loss of brush-like protrusions indicating that Fgf signaling regulates these protrusive structures. Our results suggest that these brush-like protrusions are made up of branched actin networks, are regulated by Fgf signaling and are necessary for proper migration of the pLLP.

321 Studying meningeal development and function using the zebrafish *Marina Venero Galanternik*[1], Ryan Gober[1], Andrew Davis[1], Daniel Castranova[1], Steven Coon[1], Ryan Dale[1], Sydney Hertafeld[1], Joe Zoeller[1], Luois Dye 3rd[1], Brant Weinstein[1] 1) NICHD, National Institutes of Health.

The meninges are a complex vascularized connective tissue that surrounds the Central Nervous System, protecting it from mechanical shock, supporting brain buoyancy, and maintaining brain homeostasis. Despite the critical role of this tissue, the molecular identity, developmental origins, and functional properties of different meningeal cell types remain poorly characterized. We are using the zebrafish to carry out a comprehensive anatomical, molecular, and genetic characterization of the meninges, their cellular constituents, and their roles in brain homeostasis. To examine the anatomical structure of the meninges and the morphology of its resident cell types, we are using histology, electron microscopy, and super-resolution confocal imaging. Our studies have shown that the adult zebrafish meninges are a complex highly vascularized three-layered tissue containing several unusual cell types. Using single-cell RNA-seq from dissected meninges, we are profiling these cells and correlating our expression and anatomical data to define the morphological and molecular identities and interrelationships between the different meningeal cell populations. Our studies thus far have confirmed that “Fluorescent Granular Perithelial” cells (FGPs), a novel macrophage-like scavenger perivascular cell population, reside within the inner meningeal layer, where they clear waste from the cerebrospinal fluid. Besides FGPs, we have also identified a meningeal cell population expressing high levels of *ependymin (epd)*, a meningeal cerebrospinal fluid glycoprotein with a poorly understood function. Our preliminary super-resolution imaging data suggests that Ependymin-high expressing cells (EPDs) are large flat cells that ensheath meningeal blood vessels and FGPs. Together, our ongoing studies using the powerful tools and methods available in the fish are facilitating comprehensive understanding of meningeal development and function.

Saturday, April 25 11:00 AM - 1:00 PM

Developmental Genetics (*Drosophila*)

294 Intercellular feedback in the growing *Drosophila* germline cluster *Caroline Doherty*[1,2], Manisha Kapasiawala[2,3,5], Rocky Diegmiller[2,3], Elizabeth Gavis[1], Stanislav Shvartsman[1,2,3,4] 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Lewis-Sigler Institute of Integrative Genomics, Princeton University, Princeton, NJ; 3) Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ; 4) Center for Computational Biology, Flatiron Institute, New York, NY; 5) Department of Bioengineering, California Institute of Technology, Pasadena, CA.

In many species, formation of a healthy oocyte requires the donation of organelles and macromolecules from connected germline cells. Regulation of the growth and collective biosynthetic capacity of these supporting cells, called nurse cells (NCs), is not well understood. In contrast to the prevailing view that the oocyte is merely a recipient of NC products, we show that the *Drosophila* oocyte plays an active role in regulating NC growth. Previous work uncovered a patterned hierarchy of NC volumes based on proximity to the oocyte, such that NCs closer to the oocyte are larger than NCs farther away from the oocyte. Within the 16-cell germline cyst this generates four cell-network-based groups. We demonstrate that the same grouping is true for DNA content, where NC ploidy depends on distance to the oocyte. From live and fixed imaging of egg chambers with endogenously tagged Cyclin E, we observe that the timing of NC cell cycles also occurs in these groups. Because DNA content is correlated with cell volume, this points to an oocyte-centric model for control of collective growth via cell cycle regulation. This model is supported by analyses of the CDK inhibitor Dacapo, which regulates Cyclin E during the NC endocycles. Using photoconversion of Dacapo endogenously tagged with Dendra2, we show that, while *dacapo* transcripts are localized to the oocyte, Dacapo protein moves back to the NCs. Moreover, targeted degradation of Dacapo results in the disruption of the NC volume hierarchy, establishing a direct link between endocycle regulation and collective growth. We summarize our results with a simple computational model that captures the essential features of the system and propose that bidirectional communication between the oocyte and NCs regulates the amount of material provided by the NCs to the oocyte.

295 A new conserved modulator of immune cell tissue invasion induces a metabolic program through concerted shifts in transcription and translation Shamsi Emtenani[1], Elliott Martin[2], Attila Gyoergy[1], Prashanth Rangan[2], *Daria Siekhaus*[1] 1) Institute of Science and Technology Austria; 2) SUNY Albany.

The developmental establishment of tissue resident macrophages, the protective responses of immune cells, and the metastatic spread of cancer all depend on these cells’ ability to move through tissue barriers. Our lab uses the developmental migration of *Drosophila* macrophages as they penetrate the extended germband as a system to investigate tissue invasion, working to identify new mechanisms required in invading and invaded cells.

We have found that a previously unexamined nuclear protein, CG9005, which we call Atossa, is required in macrophages for their tissue invasion. Live imaging reveals that the invasion defect in the *atossa* mutant is due to a delay in tissue entry and a decrease in speed of the first two macrophages, which take turns acting as invasion pioneers. Atossa has conserved domains, one with an unknown function and one previously linked to chromosome segregation which we show are required for its ability to promote invasion. RNA seq analysis shows that Atossa induces changes in the transcription of several metabolic enzymes and a helicase that we call Porthos, each of which are also required for normal amounts of invasion. Polysome profiling reveals that the helicase enhances the occupancy on translating ribosomes of a set of RNAs with a 5' TOP sequence, many of which are linked to metabolism and ATP production. The importance of the helicase in this process is underscored by its ability to substantially rescue the invasion defect when expressed in macrophages mutant for *atossa*. Additionally, live imaging of macrophages with reduced levels of the helicase show a similar defect in pioneer cell invasion as that seen in the absence of Atossa. This unusual control mechanism for a metabolic program appears to be evolutionarily conserved, as the two vertebrate orthologs of Atossa can rescue the invasion phenotype, and one of them is enriched in immune cells. We thus identify a novel conserved nuclear factor that activates a program for initial tissue entry through concerted changes in the transcription and translation of metabolic enzymes which may be generally important for immune cell responses.

296 Positioning a stem cell niche during organogenesis *Lauren Anllo*[1], Lindsey Plasschaert[1], Stephen DiNardo[1] 1) University of Pennsylvania.

Stem cells are required for tissue homeostasis, as well as for regeneration after damage or due to aging. Accomplishing such tasks often requires intimate association between stem cells and their niche. Unfortunately, we know little about how niches are positioned within tissues during development. We study the *Drosophila* testis, which is a long-standing paradigm in niche-stem cell biology, and have succeeded in live-imaging niche development as the gonad first forms (Anllo et al., 2019). We found that niche progenitor (pro-niche) cells extend protrusions to pull themselves out to the gonad periphery, where they migrate anteriorly along extracellular matrix. Imaging also revealed that the niche assembles with a stereotypic inward tilt with respect to the body axis, suggesting that some tissue external to the gonad guides niche formation. Learning these dynamics has motivated us to seek the extrinsic cues and intrinsic factors necessary for niche morphogenesis. To identify a source for the extrinsic cue, we genetically ablated various tissues adjacent to the gonad. We found that niche assembly was disrupted when the visceral mesoderm (Vm) was removed, and in particular that those pro-niche cells specified furthest from the gonad anterior were unable to reach the proper location. Interestingly, well before niche assembly we found that pro-niche cells are in direct contact with a subset of Vm cells, suggesting that this extrinsic cue could be contact-dependent. While exploring extrinsic cues, in parallel we have been identifying factors required intrinsically for niche formation. We found that the transcription factor *islet* is expressed in niche cells, its expression is dependent on the Vm, and the niche does not assemble properly in *islet* mutants. From previous cis-regulatory analysis of *islet* (Boukhatmi et al, 2014), we identified an enhancer sufficient for *islet* expression in pro niche cells. We are currently testing whether conserved binding sites within this element might help identify candidates for a signal delivered by the Vm. Since *islet* can regulate the expression of guidance receptors and a cellular response to guidance in motor neurons (Santiago et al., 2017), we are testing whether *islet* plays a similar role in pro-niche cells. This research is among the first describing how the niche is positioned correctly during its development, and bridges an important gap in our knowledge of stem cell-niche biology.

297 Ecdysone dependent maturation of the epithelial barrier limits Dilp8 signaling in *Drosophila* wing imaginal discs. *Danielle DaCrema*[1], Rajan Bhandari[1], Ryunosuke Yano[1], Faith Karanja[1], Adrian Halme[1] 1) University of Virginia.

Many organisms lose the ability to regenerate following damage during development. In *Drosophila melanogaster*, the loss of regenerative capacity occurs at the end of the third instar of larval development prior to pupariation due to increasing levels of the steroid hormone ecdysone, which also triggers pupariation. It is not clear how ecdysone regulates this process. Prior to regeneration restriction, damage to the imaginal discs (larval precursors to adult organs) induces expression of *Drosophila insulin-like peptide 8* (*dilp8*). Dilp8 functions in the brain and prothoracic gland to inhibit ecdysone production, thereby extending development to allow time for regeneration. Although Dilp8 functions in the brain, it is secreted into and accumulates in the imaginal disc lumen. We found that Dilp8 is sequestered in the imaginal disc lumen by the imaginal disc epithelial barrier, which limits *dilp8* signaling. To determine if the sequestering of Dilp8 is correlated with regeneration restriction, we observed the localization of components that form the epithelial barrier (septate junctions in *Drosophila*). The components Neurexin, Kune-Kune, and Coracle increasingly localize to the septate junctions during the third instar, but only Coracle is diffusely localized along the basolateral membrane before regeneration restriction and becomes completely localized to the septate junctions after regeneration restriction. To determine how the function of the barrier changes, we developed an *ex-vivo* method to quantify barrier function using 10 kD fluorescent conjugated dextran. We found that before regeneration restriction the epithelial barrier is functional, but that the function changes during the third instar. Since ecdysone regulates the change in regenerative ability, we asked if the function of the epithelial barrier is also ecdysone dependent. We fed the larvae ecdysone to prematurely increase circulating ecdysone, which was sufficient to induce barrier maturation. To determine if ecdysone is necessary for barrier maturation, we locally expressed a dominant negative allele of *ecdysone receptor A*. We found that ecdysone is not necessary for the earlier barrier but is necessary for the late barrier. Together these data indicate that ecdysone regulates the maturation of the epithelial barrier in the late third instar, possibly by localizing Coracle to the septate junctions, and that the late epithelial barrier sequesters Dilp8 in the imaginal disc lumen to limit *dilp8* signaling. These data may indicate that the maturation of the epithelial barrier is one way that ecdysone ultimately induces the loss of regenerative capacity. These data also bring into question the possibility that the maturing epithelial barrier may concentrate other signaling peptides within the lumen of the imaginal disc or prevent signals from acting in other parts of the body.

298 The Integrity of the mitotic nuclear lamina is required for stem cell maintenance *Tingting Duan*[1], Rebecca Cupp[1], Pamela Geyer[1] 1) University of Iowa.

Homeostasis of Drosophila germline stem cells (GSCs) depends upon the integrity of the nuclear lamina (NL). GSCs possess a NL checkpoint, whose activation is associated with a thickened and lobulated NL structure. Activation of the NL checkpoint involves two kinases, the ATM- and Rad3-related (ATR) kinase and the Checkpoint kinase 2 (Chk2). The NL checkpoint is activated upon loss of either the NL LEM-domain (LEM-D) protein D-emerin/Otefin or its partner Barrier-to-autointegration factor (BAF), leading to a block of germ cell differentiation and GSC death. As both D-emerin/Otefin and BAF are involved in nuclear reformation during mitosis, we investigated whether NL checkpoint activation is associated with failed NL reformation. To begin these analyses, we examined sequential mitotic events of GSCs in wild type ovaries. Surprisingly, we found that the NL does not disseminate during mitosis. Instead, the NL forms a structure that surrounds centrosomes and the mitotic spindle. These observations indicate that GSCs undergo an atypical "semi-closed" mitosis that is similar to mitosis in yeast. Notably, loss of D-emerin/Otefin causes irregularities in the lamin network and thickens lamin deposition around the centrosomes. These structural defects are linked to a failure to recruit Chk2 to centro-

somes during mitosis, correlating mitotic events with the NL checkpoint activation. Our data represent the first report of a semi-closed mitosis in a stem population and suggest that the integrity of this process is required for stem cell maintenance.

299 Cells with loss-of-heterozygosity after exposure to ionizing radiation in *Drosophila* are culled by p53-dependent and p53-independent mechanisms Jeremy Brown[1], Justine Bozon[2], Inle Bush[3], Tin Tin Su[1] 1) University of Colorado, Boulder, CO; 2) The University of Rouen, Rouen, France; 3) College du Leman, Versoix, Switzerland.

Loss of Heterozygosity (LOH) typically refers to a phenomenon in which diploid cells that are heterozygous for a mutant allele lose their wild type allele through mutations. LOH is implicated in oncogenesis when it affects the remaining wild type copy of a tumor suppressor. *Drosophila* has been a useful model to identify genes that regulate the incidence of LOH, but most of these studies use adult phenotypic markers such as *multiple wing hair* (*mwh*). Here, we describe a cell-autonomous fluorescence-based system to monitor LOH that may be used in larval, pupal and adult stages. This system is based on the QF/QS transcriptional module and, therefore, may be used in conjunction with the GAL4/GAL80 system to modulate gene expression. Using the QF/QS system, we were able to detect the induction of cells with LOH by X-rays in a dose-dependent manner in the larval wing discs, and to monitor their presence through subsequent development in pupa and adult stages. We tested the genetic requirement for changes in LOH cell number, using both classical mutants and GAL4/UAS-mediated RNAi. Our results identify distinct culling phases during development that eliminate cells with LOH, as well as p53-dependent and p53-independent mechanisms for culling.

300 Rescue of a missing heart: The role of ribosomal proteins in congenital heart disease *Tanja Nielsen*[1], Anaïs Kervadec[1], Zeng X-X I[1], Schroeder Analyne[1], Jeanne Theis[2], Timothy M. Olson[2], Karen Ocorr[1], Paul Grossfeld[3], Alexandre R. Colas[1], Georg Vogler[1], Rolf Bodmer[1] 1) Sanford Burnham Prebys Medical Research Institute, La Jolla, CA, USA; 2) Mayo Clinic, Rochester, MN, USA; 3) UCSD School of Medicine, La Jolla, CA, USA.

Hypoplastic Left Heart Syndrome (HLHS) represents the most lethal Congenital Heart Disease (CHD) and is characterized by a severely underdeveloped left ventricle. HLHS is likely of oligogenic origin; however, the causal genes and disease mechanisms involved are poorly understood. We recently identified ribosomal protein (RP) genes *RpL13* and *RpS15A* as novel candidate genes involved in CHD pathogenesis. Heart-specific knockdown (KD) of the fly orthologs of either gene results in a complete or partial loss of the adult fly heart; and KD in human iPSC-derived cardiomyocytes (hiPSC-CMs) leads to reduced CM proliferation (Schroeder et al., 2019, *HMG*). Furthermore, we found enrichment of other RPs in 25 sporadic, poor-outcome HLHS proband-parent trios. A growing number of RP mutations have been linked to tissue-specific human clinical phenotypes including anemia or congenital limb defects; however, the specific role of RPs in heart development and their contribution to HLHS/CHD has not been investigated.
We focused on *RpS15Aa* as a candidate gene, which harbored a promoter variant and exhibited reduced expression in iPSC-CMs derived from a HLHS patient. In both fly and hiPSC-CMs, the dosage of *RpS15Aa* was a critical factor in the phenotype's severity. Mechanistically, neither 3rd instar larvae or adult flies displayed induction of apoptosis in CMs and blocking apoptosis by *diap-1* overexpression (OE) in a *RpS15A*a KD background was not sufficient to protect flies from partial heart loss. In iPSC-CMs, however, we found evidence that simultaneous KD of *p53* and *RpS15A* rescued the proliferation phenotype. Conversely, heart loss in the fly can be partly restored not by *p53* KD, but by OE of *yorkie*, the fly *YAP* ortholog of the Hippo pathway, or by KD of the proto-oncogene *myc* in the heart. Both *p53* and *yorkie/myc*-modulated phenotypes point towards a role for cardiac growth as a critical component.
Furthermore, we knocked down *RpS15A* ortholog in zebrafish by morpholino antisense oligonucleotides and found a reduced number of CMs at 72 hours-post fertilization. Morphant hearts lack cardiac looping and exhibited significant fractional area shortening, without affecting the overall embryonic development. The cardiac phenotypes in the zebrafish system are consistent with the hiPSC-CM proliferation deficit and fly heart loss upon *RpS15A* KD.
In this study, we aim to characterize the roles of RPs in cardiogenesis and to investigate RPs as a novel class of potential genetic effectors in HLHS.

Saturday, April 25 11:00 AM - 1:00 PM
Neurogenetics/New Technology (*Drosophila*)

301 Non-autonomous regulation of *Drosophila* neuroblast proliferation via glia lipid mediated hedgehog signalling *Qian Dong*[1,2], Francesca Froldi[1,2], Michael Zavortink[1], Tammy Lam[1], Louise Cheng[1,2,3] 1) Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia; 2) Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria, Australia; 3) The Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, Australia.

The finite size of the adult central nervous system (CNS) is determined by the size of neuronal lineages generated by neural stem cells (NSCs) in the developing brain. NSCs reside within a specialised microenvironment called the glia niche. During early neurogenesis, glia cells relay the nutritional status of the animal to NSCs to trigger their entry into the cell cycle from quiescence (Sousa-nunes et al., 2011, Chell et al., 2010); past critical weight, glia cells sustain NSC proliferation under nutrient, hypoxic and oxidative stress (Cheng et al., 2011, Bailey et al., 2015). However, under physiological conditions, the role of the glial niche on NSC proliferation remains unclear. We show that lipid droplets, which reside within the glia niche are closely associated with the signalling molecule Hedgehog (Hh). Under physiological conditions, Hh is maintained at low levels in the niche to allow NSCs to progress through the cell cycle. Upon increased glia size via FGF activation, we observed an overall increase in lipid droplet number as well as Hh levels; this in turn caused activation of hedgehog signalling in NSCs, slowing down the rate at which NSCs cycle. It is known signalling molecules such as Hh and Wingless (Wg) undergo lipid modification. Here we show lipid metabolism are involved in hh palmitoylation and localization in the glia niche, which inturn affects NSC hedgehog signalling. Together, our data suggest that the glia niche non-autonomously regulates NSC proliferation and lineage size via modulation of lipid metabolism and hedgehog signalling.

302 Walking and Singing: Closed-loop modulation of *Drosophila* song *Osama Ahmed*[1], Xiao-Juan Guan[1], Rucha Alur[1], Lucas Encarnacion-Rivera[1], Mala Murthy[1] 1) Princeton University.

Locomotion, such as walking or flying, is vitally important for the survival and fitness of practically all animals, many of which have evolved to execute diverse behaviors as they move. While locomotion is known to modulate neural systems and behavior, the neural mechanisms underlying the integration and coordination of locomotion with other ongoing behaviors remain unknown. Addressing this question will inform our understand-

ing of how neural pathways interact to mediate coherent behaviors. Here we test how walking behavior modulates acoustic signal production in *Drosophila melanogaster*. Males of this species use wing vibration to produce a complex song to court females, and many of the neurons controlling this behavior have been identified. Male flies sing while they move and their walking velocity accounts for much of the variability in song structure – however, the neural mechanisms underlying this modulation remain a mystery. We optogenetically activated descending neurons (DNs) contingent on specific features of courtship song, and analyzed concomitant changes in song dynamics using computational methods. We specifically tested DNs hypothesized to alter walking behavior. Our results reveal that DN-activation influences song production and patterning, in a DN-specific way. Activation of some DNs alters song production while activation of other DNs modulates specific features of song. These results provide an important avenue for studying activity within the song motor pathway while manipulating locomotor behavior. To that end, we developed a 2-photon imaging setup to record neural activity in walking, singing flies. These results will uncover how two distinct motor pathways, for locomotion and song production, interact in real time to coordinate behaviors**.**

303 Proteomics of protein trafficking by *in vivo* tissue-specific labeling *Ilia Droujinine*[1], Dan Wang[1,2], Yanhui Hu[1], Namrata Udeshi[3], Luye Mu[4], David Rocco[1], Namrata Bali[5], Rebecca Zeng[1], Tess Branon[6], Areya Tabatabai[1], Justin Bosch[1], John Asara[7,8], Alice Ting[6,9], Steven Carr[3], Kai Zinn[5], Norbert Perrimon[1,10] 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Department of Entomology, China Agricultural University, Beijing, China; 3) Broad Institute of Harvard and MIT, Cambridge, MA; 4) Department of Electrical Engineering, Yale University, New Haven, CT; 5) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA; 6) Department of Chemistry, Massachusetts Institute of Technology, Cambridge MA; 7) Department of Medicine, Harvard Medical School, Boston, MA; 8) Division of Signal Transduction, Beth Israel Deaconess Medical Center, Boston, MA; 9) Departments of Genetics, Biology, and Chemistry, Stanford University, Stanford, CA; 10) Howard Hughes Medical Institute, Boston, MA.

Secreted interorgan communication factors encode key regulators of homeostasis. However, there are long-standing questions surrounding their origins/destinations, mechanisms of interactions, and the number of proteins involved. Progress has been hindered by the lack of methodologies for these factors' large-scale identification and characterization, as conventional approaches cannot identify low-abundance factors or the origins and destinations of secreted proteins. We established an *in vivo* global proteomic platform to investigate secreted protein trafficking between organs, whereby engineered promiscuous biotin ligase BirA*G3 biotinylates all proteins in a subcellular compartment of one tissue (e.g. endoplasmic reticulum (ER)), and biotinylated proteins are affinity-enriched and identified from distal organs using quantitative mass spectrometry. Hence, we simplify the proteome, enabling detection of low-abundance factors, their origins and destinations. Using this platform in *Drosophila*, we identified 51 muscle-secreted proteins from heads and 269 fat body (FB)-secreted proteins from the muscle organ (here defined as muscle tissue and innervations/ neuromuscular junctions (NMJs)), of which 60-70% have human orthologs. Among these were FB proteins that have known receptors and/or were previously demonstrated to bind to muscles. Here I describe a previously-uncharacterized FB-derived secreted factor, cDIP (common Dpr-interacting protein), a leucine-rich repeat protein that binds to most members of the Dpr-DIP network of interacting cell surface proteins expressed at the NMJ. We demonstrate that cDIP is induced in FB by starvation, glucagon, and foxo signaling, and that cDIP binds directly and specifically to the muscles near the NMJ. Furthermore, cDIP signals the starved state of the FB to the Dpr10 receptor on muscles to regulate their activity and increased neurite coverage. This establishes a direct link between these two organs to signal low energy storage, which is significant because such factors have not been previously identified. In addition to these data, and of relevance to the broader TAGC community, I will describe our *in vivo* secretome trafficking studies using the novel BirA*G3-ER mice. Altogether, these results suggest that our approach can identify specific ligand-receptor interactions and remote action by secreted proteins. This method should be widely applicable to studies of interorgan, local and intracellular protein trafficking networks including unconventional secretion, in both arthropod and mammalian systems, in healthy or diseased states.

304 Microbiome High-throughput Screening System in Drosophila: an Opportunity to Understand Colonization *Maria Jaime*[1], Ren Dodge[1], Brian Oliver[2], William Ludignton[1] 1) Carnegie Science, Embryology Department, Baltimore, MD; 2) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

Drosophila melanogaster has become a powerful model system for the gut microbiome, due to its simple and tractable microbiome, with on the order of 1 to 10 species. To understand the mechanisms of colonization, such as host individual response to different microbes, we have developed a high-throughput screening (HTS) system using the Whole Animal Feeding FLat (WAFFL), which permits the individual feeding of Drosophila in a 96 well microplate format. This system facilitates the transfer of the flies from one condition to another in seconds, making the screening process at least 10 times faster than traditional methods. Our system provides the opportunity to evaluate different fly and bacterial genotypes in large populations of individually colonized flies, accounting for the variation between individual organisms within a population. We are using the WAFFL to study the genetics of colonization and the ecological mechanisms of community assembly after antibiotic perturbation.

Saturday, April 25 11:00 AM - 1:00 PM

Germ Line (*C. elegans*)

288 Lessons learned from a genetic law-breaker *Diane Shakes*

abstract is not available at the time of print

289 mRNA localization is linked to translation regulation in the *Caenorhabditis elegans* germ lineage *Dylan Parker*[1], Lindsay Winkenbach[1], Sam Boyson[1], Matthew Saxton[1], Camryn Daidone[1], Zainab Al-Mazaydeh[1, 2], Marc Nishimura[1], Florian Mueller[3], Erin Osborne Nishimura[1] 1) Colorado State University, Fort Collins, CO; 2) Hashemite University, Zarqa, Jordan; 3) Institut Pasteur, Paris, France.

In *C. elegans*, the first embryonic cell division is asymmetric. Hundreds of mRNAs are asymmetrically abundant in the resulting daughter cells despite lacking zygotic transcription. This diversification of transcriptomes without active transcription provides an opportunity to explore long-standing questions regarding the interplay of translation regulation, post-transcriptional regulation, and downstream cell functions.

In exploring mechanisms driving cell-specific transcriptome diversification, we observed subcellular localizations for many maternally-loaded transcripts suggesting that subcellular localization might direct cell-specificity at these pre-transcriptional stages. For example, the AB-enriched mRNA

erm-1 concentrates at the cell cortex whereas *imb-2* mRNAs localize to the nuclear periphery when imaged by single-molecule Fluorescence In Situ Hybridization. Intriguingly, these are the destinations where the encoded proteins will ultimately function. In contrast, the P_1-cell enriched mRNAs *chs-1*, *nos-2*, *clu-1*, and *cpg-2* localized to P granules (ribonucleoprotein particles, RNPs, associated with germline function), and P-bodies (RNPs associated with RNA processing).

We aimed to identify sequences and machinery required for subcellular mRNA localization. We found 3'UTR sequences were sufficient to direct subcellular localization of P granule localized transcripts, but not membrane localized transcripts. Further, P granule localized transcripts showed varying dependencies on RNA binding proteins for localization. In testing the role of RNA binding proteins in P granule localization we found a strong, but imperfect correlation between low translational status and recruitment of mRNA to P granules. This finding unravels a long-standing question: Are mRNAs driven to P granules for the purpose of repressing translation or does translational repression drive mRNA into P granules? We found that repression of translation precedes localization to P granules and can occur independently of P granule localization. Moreover, translational repression of symmetrically distributed transcripts is sufficient to direct recruitment to P granules and down the P lineage. Altogether, we demonstrate that transcripts important for development are sent to P granules through translational repression, in turn directing their enrichment in the progenitor germ lineage where they can ultimately function upon relief of repression.

290 DNA damage repair is altered in aging C. elegans oocytes. *Victoria Adler*[1], Erik Toraason[1], Diana Libuda[1] 1) University of Oregon.

Reproduction in women declines with age as is observed by decreased oocyte quality and reduced fertility. Oocytes are generated during fetal development and are held at the end of meiotic prophase I until fertilization. Multiple factors have been suggested to contribute to reduced oocyte quality with aging, including mitochondrial dysfunction, cohesion loss, and misregulated gene expression. Here we exploit young and old oocytes from *Caenorhabditis elegans fog-2* mutant females, which hold and age their oocytes in meiotic prophase I, to assess the dynamics of DNA damage and repair in aging oocytes. Using a computational pipeline for analyzing immunofluorescence images of individual nuclei within whole aging *C. elegans* germlines, we found that aged *fog-2* oocytes exhibit increased double strand DNA breaks (DSBs), as indicated by foci of the recombinase RAD-51. Moreover, we find that increased levels of DSBs are specifically observed in oocyte nuclei in the proximal germline of older worms. To assess if aging oocytes exhibit a reduced capability to repair exogenous DNA damage, we exposed young and old *fog-2* females to ionizing irradiation and assessed their germlines for persistent DSBs 48 hours later. Intriguingly, we observed more nuclei with high levels of RAD-51 foci in the distal germline of younger worms, while older animals exhibit these highly damaged nuclei in the proximal germline. Further, we found a bimodal distribution of nuclei within irradiated germlines: 1) nuclei with little to no DSBs; and, 2) nuclei with extremely high levels of DSBs. These observations indicate age-dependent variance in the response to exogenous DNA damage between young and old worms. Our future work will identify the sources of elevated DNA damage in aged germlines and define the DNA repair pathways that are differentially regulated during oocyte aging. Taken together, our work is uncovering reproductive DNA repair phenotypes associated with reproductive aging.

291 SPE-36 is an EGF-motif containing secreted sperm protein required for fertilization in *C. elegans* *Amber Krauchunas*[1], Matthew Marcello[2], Andrew Singson[1] 1) Rutgers University, Piscataway, NJ; 2) Pace University, New York, NY .

Irrespective of the species, the ultimate function of a sperm once it contacts an egg is to recognize it as an egg, bind to the egg, and fuse with the egg. Through the power of forward genetics we know of ten genes necessary for sperm function in *C. elegans*. Most recently, our lab has identified the first secreted proteins required for fertilization. One of these proteins, SPE-36, is encoded by the gene *F40F11.4* (hereafter referred to as *spe-36). spe-36* mutants display a classic Spe phenotype: hermaphrodites are self-sterile and lay unfertilized eggs, but produce progeny when mated to wild type males. We also find that *spe-36* males are sterile. Sperm from *spe-36* mutants are present in the spermatheca, are able to undergo sperm activation, and have normal morphology, indicating that sterility is due to a defect in fertilization. The predicted SPE-36 protein contains a signal peptide and an epidermal growth factor (EGF) domain, suggesting that SPE-36 is secreted. Despite this, SPE-36 appears to act cell autonomously. Due to its cell autonomous behavior and EGF domain, it is tempting to speculate that SPE-36 binds to proteins on the surface of the sperm helping to confer organization or functionality at the interface between the sperm and the egg. Our discovery of secreted sperm function proteins expands our model of the fertilization synapse and challenges our expectations of the types of proteins that mediate the interactions of sperm and egg.

292 DAF-18/PTEN inhibits germline zygotic gene activation during primordial germ cell quiescence Amanda Fry[1,2], E. Jane Hubbard[1,2,3] 1) New York Univ Sch Medicine; 2) Skirball Institute; 3) Department of Cell Biology.

Quiescence, an actively-maintained reversible state of cell cycle arrest, is not completely understood. PTEN is one of the most frequently lost tumor suppressors in human cancers and regulates quiescence of stem cells and cancer cells. In a striking parallel, the *C. elegans* PTEN homolog, DAF-18*,* suppresses proliferation of primordial germ cells (PGCs). *C. elegans* PGCs are born during embryogenesis and normally exit quiescence mid-way through the first larval stage, provided that food is present. PGCs in *daf-18* mutant worms exit quiescence and divide even when the worms are starved. Genetic studies in *C. elegans* place TOR downstream of *daf-18* in PGC quiescence regulation. The *C. elegans* PGCs and DAF-18/PTEN provide a powerful model system to explore the regulation of cell cycle quiescence in response to food, and since PGCs arrest in G2 of the cell cycle, they may offer a model to better understand exit from G2 arrest.

We further investigated the role of *daf-18* in maintaining PGC quiescence. We found that quiescence is maintained by either maternal or zygotic *daf-18* function, that *daf-18* can regulate PGC quiescence either germline- or soma-autonomously, and that *daf-18* also affects the PGC cell cycle in fed animals. We found a genetic interaction between *daf-18* and *top-2*, a topoisomerase that affects PGC quiescence*.* Additional results implicate *daf-18* in gene activation. In wild-type worms, PGCs begin transcription upon feeding, as indicated by histone modifications associated with active transcription and phosphorylation of serine 2 on the RNA Pol II C-terminal tail. We found that PGCs in starved *daf-18* mutant worms display these marks of active transcription while PGCs in starved wild-type worms do not. Consistent with these findings, inhibiting RNA Pol II with α-amanitin partially suppresses inappropriate PGC divisions in *daf-18* mutant worms. Although PGCs in starved *daf-18* mutants divide in the absence of food, they are likely specified properly since somatic gene activity is not observed. However, we observe inappropriate zygotic germline gene activation in PGCs of starved *daf-18* mutants, similar to that observed upon feeding. Together, our results suggest that *daf-18* in prevents inappropriate germline zygotic gene activation in the absence of food.

293 Effects of Polyploidy in *C. elegans* *Mara Schvarzstein*[1,2], Gunar Fabig[3], Katharina Kupsch[3], Tara Vanvarong[1], Brandon Ely[2], Thomas Müeller

Reichert[3] 1) City University of New York, Brooklyn College, Brooklyn, NY, USA ; 2) The Graduate Center CUNY, NY, NY, USA; 3) Experimental Center, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Germany.

Polyploidy is a common condition in plants and some animals, of possessing more than two complete sets of chromosomes. Despite the importance of polyploidization in development, evolution, and disease, there is no multicellular laboratory animal model to study whole organism polyploidization. Studies of this process have been mostly limited to plants, related animal species with different ploidy, or the ancestral remnants of this process in genomes. We developed a methodology to easily generate viable and fertile tetraploid animals devoid of unwanted genetic markers from any genetic background that allows for the generation of multiple of tetraploid strains per diploid. Using multiple tetraploids, we have surveyed effects of polyploidization on; meiosis and mitosis, and biological scaling and scaling relationships. The effects of increased genome on biological scaling and cell divisions are context dependent. Comparison of tetraploid and diploid strains meiotic and mitotic divisions, show that the effects of polyploidy on centrosome size, final spindle length, and the speed of chromosome segregation, were different either between the two meiotic divisions or in the first mitosis of the embryo. All tetraploid animals are larger than the diploids they were derived from. Interestingly, the extent of scaling relative to the animal size depends on the cell type and is also organ dependent in tetraploids. This differential scaling results in body shape differences between diploids and tetraploids that are not due to a proportional size change. Surprisingly, two diploid strains with significantly different body length gave rise to tetraploids of similar size, suggesting that there may be a limit to the increase in body length tetraploidy can cause. Also surprising is that tetraploid hermaphrodites with 4 X chromosomes (24 chromosomes in total) instead of 3 X chromosomes (23 chromosomes in total) are bigger than expected if all chromosomes contributed according to their size. Organ size is not significantly different between 4X and 3X tetraploids. This may suggest that in tetraploids gene expression from the X chromosome may contribute to animal size, but not organ size, to a greater extent than gene expression from autosomes. This and other data suggest that specific-gene expression rather than global increase in gene expression might be at lease in part responsible for changes in biological size and scaling in *C. elegans* tetraploids. At this meeting we will present our initial comparison between diploid and tetraploid strains transcription profiles. In addition to furthering our understanding of the role of polyploidization on biological scaling, this method provides an unprecedented opportunity to query polyploidy establishment and short- and long-term consequences in multicellular animals.

Saturday, April 25 11:00 AM - 1:00 PM

Divergence, Hybridization and Reproducible Isolation (PEQG)

305 Assembly of a young vertebrate Y chromosome reveals convergent signatures of sex chromosome evolution *Catherine Peichel*[1,2], Shaugnessy McCann[2], Joseph Ross[2,3], Alice Shanfelter[4], James Urton[2,3], Jennifer Cech[2,3], Jane Grimwood[5], Jeremy Schmutz[5], Richard Myers[5], David Kingsley[6], Michael White[2,4] 1) Institute of Ecology and Evolution, University of Bern, Bern, Switerland; 2) Divisions of Human Biology and Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Graduate Program in Molecular and Cellular Biology, University of Washington, Seattle, WA; 4) Department of Genetics, University of Georgia, Athens, GA; 5) HudsonAlpha Institute for Biotechnology, Huntsville, AL; 6) Department of Developmental Biology and Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA .

Heteromorphic sex chromosomes have evolved repeatedly across diverse species. Suppression of recombination between young X and Y chromosomes leads to rapid degeneration of the Y chromosome. However, these early stages of degeneration are not well understood, as complete Y chromosome sequence assemblies have only been generated across a handful of taxa with relatively ancient sex chromosomes. Here we describe the assembly of the threespine stickleback (*Gasterosteus aculeatus*) Y chromosome, which is less than 26 million years old. Our previous work identified that the non-recombining region between the X and the Y spans ~17.5 Mb on the X chromosome. Here, we combined long-read PacBio sequencing with a Hi-C-based proximity guided assembly to generate a 15.87 Mb assembly of the male-specific region of the Y chromosome. Our assembly is concordant with cytogenetic maps and Sanger sequences of over 90 Y-chromosome clones from a bacterial artificial chromosome (BAC) library. We find three evolutionary strata on the Y chromosome, consistent with the three inversions identified by our previous cytogenetic analyses. The young threespine stickleback Y shows convergence with older sex chromosomes in the retention of haploinsufficient genes and the accumulation of genes with testis-biased expression, many of which are recent duplicates. However, we found no evidence for the large amplicons found in other sex chromosome systems, like the older mammalian Y. We also report an excellent candidate for the master sex-determination gene in the threespine stickleback: a translocated copy of *Amh* (*Amhy*). Together, our work shows that the same evolutionary forces shaping older sex chromosomes can cause remarkably rapid changes in the overall genetic architecture on young Y chromosomes.

306 A supernumerary chromosome produces a 0W/00 sex determination system in a cichlid fish *Erin Peterson*[1], Abby Cozart[1], Melissa Lamm[1], Natalie Roberts[1], Aldo Carmona Baez[1], Reade Roberts[1] 1) North Carolina State University, Raleigh, NC.

Genetic sex determination is a relatively conserved process among many vertebrates, but appears to evolve rapidly in some taxa. East African cichlids are one such group, where sex can be determined by a variety of genetic loci within and among species. B chromosomes, often referred to as supernumerary chromosomes, are characterized by highly repetitive DNA and in East African cichlid fish show homology with autosomal sequences. In this study, we examine a species of cichlid, *Labidochromis caeruleus*, whose sex determination relies entirely on the presence or absence of a B chromosome, resulting in a female-determining 0W/00 system. While other species of cichlids have been shown to have interactions of autosomal sex determiners with these feminizing B chromosomes, this is the first instance of a B chromosome serving as the sole sex determiner. Using whole genome sequencing of a female *L. caeruleus*, we identified B chromosome sequences and B-specific SNPs. Using B-specific primers, we genotyped for the presence or absence of this supernumerary chromosome in both pure species and in hybrid crosses. In pure *L. caeruleus* families, we observe sex associating perfectly with the presence of a B chromosome (n = 44). In two interspecific hybrid mapping crosses, we find lowered transmission of the B chromosome to the F2 offspring, while maintaining the B chromosome as the primary sex determiner. In an intergeneric hybrid cross we observe only 7% female F2 (n = 474), and perfect association of the B chromosome with sex. However, in an intrageneric hybrid cross between two Labidochromis species, we observe 34% female F2 (n = 162), and a strong but imperfect association of sex with B chromosome status. Our results provide the strongest evidence of a 0W/00 sex determination to date, and suggest an effect of genetic background leading to differential transmission of the B chromosome.

307 Odorant receptor tuning contributes to the evolution of sexual signaling in perfume-collecting orchid bees. *Philipp Brand*[1], Thomas Eltz[2], Santiago Ramirez[1] 1) University of California, Davis; 2) Ruhr-University, Bochum.

Variation in sexual signaling has been linked to the origin and maintenance of reproductive isolation. However, the molecular mechanisms underlying the evolution of sexual communication systems and their impact on the speciation process remain poorly understood. Male orchid bees collect fragrances from various environmental sources to concoct species-specific perfume mixtures that are subsequently exposed to females during courtship. As a result, orchid bees rely exclusively on the sense of smell to accumulate and detect perfumes. Here, we tested the hypothesis that chemosensory gene divergence underlies the evolution of perfume communication and reproductive isolation in orchid bees. We conducted a population-level analysis of two recently diverged sibling species, *Euglossa dilemma* and *E. viridissima*, including 366 bees sampled throughout the distribution ranges. Using gas-chromatography mass spectrometry and population genomics analyses we found that the two lineages exhibit distinct chemical perfume compositions and are reproductively isolated despite low levels of genetic differentiation. Whole-genome re-sequencing revealed two highly divergent regions in the genome exhibiting selective sweep signatures, both harboring tandem arrays of odorant receptor (*Or*) genes. While most of the 43 olfactory genes exhibited low divergence, we identified one receptor (*Or41*) with significantly elevated dN/dS values along the *E. dilemma* branch, revealing signatures of strong positive selection in this lineage. Using functional assays, we demonstrate that the derived variant of *Or41* in *E. dilemma* is specific towards its major species-specific perfume compound, whereas the ancestral variant in *E. viridissima* is broadly tuned to multiple odorant compounds. Our study provides chemical, genetic, and functional evidence in support of the hypothesis that divergence in chemical tuning in a key odorant receptor gene contributed to the evolution of pre-mating reproductive barriers in these bee lineages. The linkage between male traits (perfume composition) and female preference (perfume preference) in orchid bees may have facilitated the rapid evolution of assortative mating and the formation of new species via changes in few chemosensory receptor genes.

308 The selective forces and genetic basis of mating interactions that contribute to the rapid evolution of reproductive isolation *Dean Castillo*[1,2], Bozhou Jin[2], Daniel Barbash[2] 1) School of Biological Sciences, University of Utah; 2) Department of Molecular Biology and Genetics, Cornell University.

To understand how reproductive barriers evolve during speciation we need to understand which traits confer reproductive isolation at the earliest stages of divergence. We are leveraging geographically differentiated lineages of *Drosophila melanogaster* (Zimbabwe [Z] and cosmopolitan [M]) that show partial reproductive isolation to determine the genetic basis of divergent mating interactions. In this system Z type females strongly reject M type males, whereas M type females mate randomly. We have investigated three aspects of this isolation. First, female mate preference is a critical behavior for reproductive interactions, but we know little about the evolution and genetic basis of this trait. The desaturase gene *desat2* has been previously implicated in controlling Z type behavior but caveats of previous students left its role in female mate preference unresolved. We have unambiguously demonstrated that *desat2* contributes to Z female preference and are disentangling the roles that this gene has on pheromone production and female mate preference. Second, to identify additional candidate genes we used population genetic patterns to identify loci that are resistant to gene flow in an admixed population using a formal cline analysis. Two candidate genes, *Neuroglian (Nrg)* and *alan shepard (shep)* have known roles in female mating behavior. Virgin females that are homozygous null for either gene refuse to mate in general. We have demonstrated that these genes contribute to Z female mate preference. Third, for females to discriminate between potential mates, male signals and mating behaviors must be distinguishable between lineages. In the Z and M system divergence in male traits was previously undocumented. We quantified the traits that differentiate Z and M males for courtship behavior and pheromone (CHC) composition. We found that Z males perform additional behaviors and have increased diversity in their CHCs compared to M males. These traits correlate with differences in mating success, suggesting differences in selective pressures between these populations. We have also demonstrated that males of both genotypes can shift their courtship behavior depending on the female genotype they are courting, which can explain asymmetries in reproductive isolation. By simultaneously examining these interactions from both the female and male perspectives we have identified selective forces and genetic mechanisms leading to the rapid evolution of mating interactions.

309 Repeated evolution of circadian clock dysregulation in cavefish populations *Katya Mack*[1], James Jaggard[2], Jenna Persons[3], Courtney Passow[4], Bethany Stahl[2], Estephany Ferrufino[2], Dai Tsuchiya[3], Sarah Smith[3], Brian Slaughter[3], Johanna Kowalko[5], Nicolas Rohner[3,6], Alex Keene[2], Suzanne McGaugh[4] 1) Biology, Stanford University, Stanford, CA, USA ; 2) Department of Biological Sciences, Florida Atlantic University, Jupiter, FL, USA ; 3) Stowers Institute for Medical Research, Kansas City, MO, USA ; 4) Ecology, Evolution, and Behavior, University of Minnesota, Saint Paul, MN, USA; 5) Wilkes Honors College, Florida Atlantic University, Jupiter FL, USA ; 6) Department of Molecular and Integrative Physiology, The University of Kansas Medical Center, Kansas City, KS, USA.

The circadian clock is a highly conserved internal timing mechanism that permits organisms to synchronize behavior and physiological processes to predictable daily environmental changes. These clocks are nearly ubiquitous throughout the animal kingdom, suggesting they are critical for survival in diverse environments. Investigating clocks in animals that have adapted to environments that lack daily environmental rhythms has potential to uncover fundamental principles underlying the function and evolution of circadian clocks. The tetra, *Astyanax mexicanus*, exists as surface populations that live in rivers with robust light and temperature rhythms, and at least 30 cave populations that live in perpetual darkness with limited fluctuations in temperature or other environmental cues. Here we characterize and compare the circadian transcriptome of *A. mexicanus* surface fish with that of three cave populations under constant darkness. We find widespread changes in the circadian transcriptome, with cave populations showing convergent reductions and losses of transcriptional oscillations and alterations in the phase of key circadian genes. Population genetic analyses indicated that a number of genes in the circadian transcriptome are also genetically differentiated between the surface and one or more cave populations. Finally, we investigated circadian genes with convergent reductions in rhythmic expression through CRISPR/Cas9 mutant surface fish, and find that these genes are involved in the regulation of sleep, phenocopying reductions in sleep seen in multiple cave populations. Our results suggest that the circadian clock, a highly conserved timing mechanism across most metazoans, has been repeatedly disrupted at the molecular level across cavefish populations, with potential consequences for organismal physiology and behavior.

310 The genetics of reproductive isolation through host switching in experimentally evolved pigeon lice (Columbicola columbae) *James Baldwin-Brown*[1], Emiko Waight[1], Scott Villa[2], Sarah Bush[1], Dale Clayton[1], Michael Shapiro[1] 1) University of Utah, Salt Lake City, UT; 2) Emory University, Atlanta, GA.

A classic contradiction in evolutionary biology is the fact that parasites are adapted very narrowly to match their host environments, but host switching is frequently observed in nature. Adding to this, host switching is closely associated with speciation, as in the famous case of the hawthorn fly, which diverged into two species after a host switch to apples.

We have generated a set of experimentally evolved parasitic pigeon lice (*Columbicola columbae*) specifically for the investigation of the interac-

tions of host switching, population and quantitative genetics, and speciation. Here, we use whole genome sequencing of populations of lice in 6 month time intervals to directly measure genotypic evolutionary change immediately after a host switching event.

Pigeon lice live on their hosts, the domestic rock pigeon *Columba livia*. Prior studies show that lice match their color to that of their host and match their thorax width to their hosts' feather inter-barb space. We transplanted a large, outbred population of lice from feral pigeons to breeds of domestic pigeon with dramatically different feather sizes and colors, producing four replicate populations of controls, dark birds, light birds, large birds, and small birds. We collected lice every 6 months from these populations and sequenced all time points from the 4-year experiment either in pools or individually, depending on the time point. In addition, we generated a high contiguity (N50 22Mb) reference genome assembly and Oxford Nanopore RNAseq-derived full-length-transcript annotation to further analysis of this data.

We now present the results of the population genetic analysis of this data. We used multiple methods to identify sites with replicated rapid allele frequency change and used an analysis of temporal covariance to characterize the total number of selected sites and the total amount of selection acting across the genome. We find a set of genes that appear to be under strong selection for either body size or color, and also characterize the genetic architecture of the size and color traits based upon the overall level of linked selection detectable in our data. This result brings us closer to understanding the genetic basis of speciation during host switching.

Saturday, April 25 1:30 PM - 3:30 PM

Dynamics and Regulation of Cellular Organization (Yeast)

352 A non-canonical Hippo pathway regulates spindle disassembly and cytokinesis during meiosis II in *Saccaromyces cerevisiae* Scott Paulissen[1], Cindy Hunt[1], Christian Slubowski[1], Yao Yu[2], Dang Truong[1], Xheni Mucelli[1], Hung Nguyen[1], Shayla Newman-Toledo[1], Aaron Neiman[2], *Linda Huang*[1] 1) University of Massachusetts Boston; 2) Stony Brook University.

Sexual reproduction requires meiosis to produce haploid gametes from a diploid precursor cell. Meiosis in budding yeast is used to create haploid yeast spores within a diploid mother cell. During meiosis II in yeast cells, the prospore membrane is synthesized *de novo* and will mature to become the plasma membrane of the haploid spore. Prospore membranes initiate growth at the modified spindle pole bodies, grow around the haploid nuclei, and fuse to close at the side of the nuclei away from the spindle pole bodies; this process results in the capture of a single nucleus (along with some cytoplasmic material) within its own membrane and the formation of four spores within an ascus. Exit from meiosis II includes spindle disassembly and cytokinesis, the latter which occurs by closure of the prospore membrane. We have previously demonstrated that *SPS1*, which encodes a STE20-family GCKIII kinase, is involved in timely prospore membrane closure. To identify genes that may activate *SPS1*, we screened for genes that exhibited a histone phosphorylation defect similar to that of *sps1* mutants, and identified *CDC15*. *CDC15* encodes a Hippo-like kinase that acts in the Mitotic Exit Network used to regulate exit from mitosis. We find that Sps1 complexes with Cdc15, and that Sps1 phosphorylation requires Cdc15. Furthermore, *CDC15*, like *SPS1*, is required for timely prospore membrane closure. We also find that *SPS1*, like *CDC15*, is required for meiosis II spindle disassembly, and that both *CDC15* and *SPS1* are required for the sustained anaphase II release of the Cdc14 phosphatase. For mitotic exit, the NDR-kinase complex acts downstream of *CDC15*. However, we see that in meiosis II, the *DBF2 DBF20 MOB1* encoded NDR-kinase complex members are not required for spindle disassembly, timely prospore membrane closure, or sustained anaphase II Cdc14 release. Instead, in meiosis, the NDR-kinase complex regulates spore number control independent of *CDC15* and *SPS1*. Taken together, our results suggest that exit from meiosis II involves a rewired Mitotic Exit Network in which *SPS1* replaces the NDR-kinase complex downstream of *CDC15*.

353 Measuring load-bearing interactions between the Dam1 complex and its multiple binding sites in the Ndc80 complex *Rachel Flores*[1], Zachary Peterson[1], Charles Asbury[1], Trisha Davis[1] 1) University of Washington.

Accurate chromosome segregation requires the kinetochore to stably attach to the dynamic tip of a spindle microtubule. The Ndc80 and Dam1 complexes are the two main microtubule binding components in the kinetochore. Interaction between the Ndc80 and Dam1 complexes enhance the load-bearing ability of the kinetochore. We previously found that three different components in the Dam1 complex each binds to a different site in the Ndc80 protein. Electron micrographs show that the Ndc80 complex bridges two Dam1 complex rings *in vitro*. Mutations in any of these three sites disrupt the ability of the Ndc80 complex to bridge two rings *in vitro* and disrupt proper Dam1 complex localization to the rest of the kinetochore *in vivo*. However, which interaction sites between the Ndc80 and Dam1 complexes play a role in load-bearing is still unknown.
As part of error correction, Aurora B kinase disrupts the interaction between the Dam1 and Ndc80 complexes by phosphorylation at any of the Dam1 complex sites that interact with the Ndc80 complex (named A^{Dam1p}, B^{Ask1p}, and C^{Spc34p}). I utilized the optical trap to measure the strength of the Ndc80 complex attachment to an assembling microtubule in the presence of Dam1 complex phosphorylated at each site. Phosphorylation at either region A^{Dam1p} or B^{Ask1p}, inhibits the load-bearing interaction between the Ndc80 and Dam1 complexes on an assembling microtubule. Phosphorylation at region C^{Spc34p} did not cause this defect.
To test whether the same regions in the Ndc80 complex play a role in establishing load-bearing interactions with the Dam1 complex, I have generated two lethal insertion mutations along the Ndc80 protein (B^{Ndc80p} and C^{Ndc80p}). The addition of an insertion mutation at region B^{Ndc80p} showed a partial defect in the ability of the Ndc80 complex to form load-bearing interactions with the Dam1 complex, while an insertion mutation in region C^{Ndc80p} did not show any defect. To test whether region C^{Ndc80p} plays a role in establishing load-bearing interactions with the Dam1 complex on disassembling microtubules. The detachment rate on disassembling microtubule tips for wild-type Ndc80 complex with Dam1 complex was 15-fold lower than for C^{Ndc80p} with Dam1 complex.

In conclusion, my results show that regions A and B form load-bearing interactions between Dam1 and Ndc80 complexes on assembling microtubule tips; whereas region C^{Ndc80p} forms load-bearing interactions with the Dam1 complex on disassembling microtubule tips.

354 Spatial segregation of repair pathways within the pericentromere *Kerry Bloom*[1], Diana Cook[1], Colleen Lawrimore[1], Sarah Long[1], Patrick Cusick[1], Katie Salisbury[1], Sarah Grant[1] 1) University of North Carolina at Chapel Hill.

DNA double-strand breaks arise when a chromosome with two centromeres segregates in mitosis. The double-strand breaks generate phenotypic diversity due to the range of monocentric derivative chromosomes that become fixed in the population. To explore the nature of the repair pathways we have analyzed a suite of dicentric chromosomes in which the distance between the two centromeres ranges from 6.8 kb to 40 kb. Single-strand annealing is the predominant repair pathway in when centromeres are 6.8 kb apart. Reciprocal cross-overs via homologous recombina-

tion are the predominant pathway in chromosomes whose centromeres are 40 kb apart. The length of the homology (338 bp CEN3) is considerably shorter than typically observed for homologous recombination. For chromosomes whose centromeres lie between ~10-20 kb from one another, non-homologous end-joining is the preferred pathway.

There are several unique aspects of the repair of DNA damage after dicentric chromosome breakage. Firstly, the centromere (125 bp) and flanking 250 bp are sufficient to provide a template for homologous recombination, despite their extremely short length. Secondly, when both centromeres are within the pericentromere (as defined by the 3X enrichment of cohesin and condensin in the ~30 kb flanking the centromere) homologous recombination is suppressed and NHEJ is the dominant repair pathway. The pericentromere exhibits biochemical properties much like the nucleolus with respect to the preference for NHEJ, and unlike HR repair pathways most often encountered by genes outside the pericentromere.

355 Ubiquitin hydrolase regulation of membrane scission by ESCRT-III Dalton Buysse[1], Anna-Katharina Pfitzner[2], Matt West[1], Aurélien Roux[2], *Greg Odorizzi*[1] 1) University of Colorado; 2) University of Geneva.

The ESCRT-III protein complex executes reverse-topology membrane scission. The scission mechanism is unclear but is linked to remodeling of ESCRT-III complexes at the membrane surface. At endosomes, ESCRT-III mediates the budding of intralumenal vesicles (ILVs). In *Saccharomyces cerevisiae*, ESCRT-III activity at endosomes is regulated through an unknown mechanism by Doa4, a ubiquitin hydrolase that deubiquitinates transmembrane proteins sorted into ILVs. We report that the non-catalytic N terminus of Doa4 binds Snf7, the predominant ESCRT-III subunit. Through this interaction, Doa4 acts non-catalytically to alter Snf7 assembly status and inhibit ILV membrane scission. In vitro, the Doa4 N terminus inhibits Snf7 association with Vps2, which functions with Vps24 to arrest Snf7 polymerization and remodel Snf7 polymer structure. In vivo, Doa4 overexpression inhibits Snf7 interaction with Vps2 and with Vps4, an ATPase recruited by Vps2/Vps24 to remodel ESCRT-III complexes by catalyzing subunit turnover. These data suggest a mechanism by which the deubiquitination machinery regulates ILV membrane scission by interfering with ESCRT-III remodeling factors.

356 Comprehensive protein architecture of the yeast epigenome at high resolution *Frank Pugh*[1], Matthew Rossi[1], William Lai[1], Prashant Kuntala[1], Gretta Kellogg[1] 1) Penn State University, University Park, PA.

The complete arrangement of proteins along a eukaryotic genome that informs us of gene and genome regulation is not known. Here we mapped, using ChIP-exo, the precise positional and structural organization of ~400 distinct proteins across the Saccharomyces genome at near single-bp resolution. What resulted was a clear picture of the positional placement of proteins around centromeres, replication origins, X-elements, LTRs, and RNA polymerase I, II, and III transcription units, that suggest functional mechanisms. In particular, we will discuss how constitutive, induced, and insulated transcription arise from chromatin organization, transcription factors, and their cofactors.

357 SGD Update *Mike Cherry*[1] 1) Stanford University.

abstract is not available at the time of print

Saturday, April 25 1:30 PM - 3:30 PM

Disease Models (Zebrafish)

358 Enteroendocrine cells sense gut bacteria and activate a gut-brain pathway *Lihua Ye*[1,2], Justice Lu[1], Jinghu Wang[3], Kenneth Poss[4], Rodger Liddle[2], John Rawls[1] 1) Department of Molecular Genetics and Microbiology, Duke Microbiome Center, Duke University School of Medicine, Durham, NC; 2) Division of Gastroenterology, Department of Medicine, Duke University School of Medicine, Durham, NC; 3) Department of Medicine, Emory University, Atlanta, GA; 4) Department of Cell Biology, Duke University School of Medicine, Durham, NC.

The intestine harbors complex and dynamic microbial communities that contribute significantly to host health and disease. However, mechanisms by which the intestine perceives distinct microbial species and relays that information to the rest of the body remain unresolved. Enteroendocrine cells (EECs) are specialized sensory epithelial cells in the intestine that detect nutrients and other chemicals in the intestinal lumen and respond by releasing hormones via transient increases in intracellular calcium. Recent studies in mice show EECs also synapse directly with vagal neurons and that nutritional stimuli in the intestine can activate this neuroepithelial circuit. Despite these central roles of EECs in intestinal nutrient sensing, it remained unknown if EECs sense bacteria and what the downstream effects may be. To test the impact of bacteria on EECs in vivo, we developed a novel genetic system to record EEC activity in free swimming zebrafish following different bacterial stimuli. In a small screen of zebrafish-associated bacteria, the only strain that significantly elicited EEC activity was the fish pathogen, *Edwardsiella tarda* (*E. tarda*). *E. tarda* stimulation of EEC activity required the transient receptor potential ankyrin 1b (*trpa1b*) gene, which was highly expressed in a subset of zebrafish EECs. Trpa1 is a cation channel that can be activated by a range of noxious chemicals. Optogenetic activation of Trpa1^{+} EECs led to increased peristalsis via signaling to enteric neurons and *trpa1b* mutant zebrafish were unable to clear *E. tarda* from the intestinal lumen. These data suggest *E. tarda* or chemical stimulation of Trpa1 promotes clearance of those noxious luminal stimuli via increased intestinal motility. In addition to these intestinal responses, we found that intraluminal delivery of Trpa1 agonist or *E. tarda* also activated neurons in the vagal ganglia within the central nervous system (CNS). Transgene-mediated targeted ablation of EECs revealed that EECs are required for this vagal response to intraluminal Trpa1 agonist and *E. tarda* stimulation. Collectively, these results indicate that an EEC-CNS signaling pathway is present in zebrafish and that EECs are able to activate this pathway in response to distinct bacterial stimuli. This microbe-gut-brain axis could be manipulated to promote specific CNS activities and behaviors or to treat neurologic disorders which are associated with alterations in the intestinal microbiome.

359 A Novel Model of Retinal Ganglion Cell Death and Regeneration in Zebrafish *Kevin Emmerich*[1], David White[1], Liyun Zhang[1], Tim Mulligan[1], Jeff Mumm[1] 1) Johns Hopkins University School of Medicine, Wilmer Eye Institute, Baltimore, MD.

Glaucoma is the second leading cause of blindness worldwide with 70 million people expected to be diagnosed by 2020. Degeneration of Retinal Ganglion Cells (RGCs), which send visual signals to the brain, is the hallmark feature underlying glaucoma along with optic nerve damage. We hypothesize that studying the cell death and regeneration of RGCs will lead to more understanding of glaucoma and facilitate discovery of therapeutic compounds to slow down cell death or enhance regeneration. To this end, we have developed a transgenic zebrafish model that allows inducible

and selective RGC death. This enables high through-put drug screening and high-resolution imaging in vivo and because zebrafish exhibit robust regeneration of RGCs, also allows interrogation of mechanisms regulating retinal regenerative potential. Our model is based on a newly improved Nitroreductase/prodrug cell ablation system where prodrug interaction with transgenic RGCs induces DNA damage and quantifiable cell death and regeneration. Additionally, we have developed assays to measure vision in larval zebrafish to determine the effects of ablation and drug treatment. Following a 24 hr treatment with a low dose of the prodrug metronidazole ~99.8% of RGCs are ablated. Four days following RGC loss, ~30% of RGCs have regenerated, providing a window to identify factors that either protect RGCs from induced cell death or modulate RGC regeneration rates. In a pilot compound screen, we have identified several neuroprotective compounds. Confocal time series microscopy confirms tectal innervation of regenerating RGCs and is being used to investigate interactions between dying RGCs, Müller glia (the resident stem cells of the zebrafish retina) and microglia (the resident macrophages of the retina). NTR-mediated ablation of RGCs also has a significant impact on visual function. Preliminary results suggest that RGC ablated fish show a marked deficit in adapting to a lighter background, suggesting profound visual deficits bordering on blindness.

360 Building the Vertebrate Codex using the Gene Breaking Protein Trap Library *Noriko Ichino*[1], MaKayla Serres[1], Rhianna Urban[1], Mark Urban[1], Anthony Treichel[1], Kyle Schaefbauer[1], Lauren Greif[1], Gaurav Varshney[2], Kimberly Skuster[1], Melissa McNulty[1], Camden Daby[1], Ying Wang[3], Hsin-kai Liao[3], Suzan El-Rass[4], Yonghe Ding[1,5], Weibin Liu[1,5], Jennifer Anderson[6], Mark Wishman[1], Lisa Schimmenti[1,7,8], Sridhar Sivasubbu[9], Darius Balciunas[10], Matthias Hammerschmidt[11], Steven Farber[6], Xiao-Yan Wen[4], Xiaolei Xu[1,5], Maura McGrail[3], Jeffrey Essner[3], Shawn Burgess[12], Karl Clark[1], Stephen Ekker[1] 1) Department of Biochemistry and Molecular Biology, Mayo Clinic, MN; 2) Functional & Chemical Genomics Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) Department of Genetics, Development and Cell Biology, Iowa State University, IA; 4) Zebrafish Centre for Advanced Drug Discovery & Keenan Research Centre for Biomedical Science, Li Ka Shing Knowledge Institute, St. Michael's Hospital, Unity Health Toronto & University of Toronto, Toronto, Ontario, Canada; 5) Department of Cardiovascular Medicine, Mayo Clinic, MN; 6) Carnegie Institution, Baltimore, Maryland, MD; 7) Department of Clinical Genomics, Mayo Clinic, MN; 8) Department of Otorhinolaryngology, Mayo Clinic, MN; 9) CSIR–Institute of Genomics and Integrative Biology, Delhi, India.; 10) Department of Biology, Temple University, 435 Biology- Life Sciences Building, 1900 North 12th Street, PA; 11) Institute for Developmental Biology, Cologne University, Köln, Germany; 12) Translational and Functional Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Despite having a well annotated human genome for over a decade, 90% of protein coding regions in the genome remain under characterized. This skewed emphasis has multiple potential sources, such as technical bias, unawareness of genes and their gene products, lack of knowledge from orthologues in model organisms, and undefined disease / gene associations. To address the issue, we utilized the power of the zebrafish (*Danio rerio*) as a genetic model organism derives from its amenability to genetic modification, optical transparency in development, and functional conservation of human gene orthologs. To explore the functionally uncharacterized loci in vertebrate genomes, we developed the gene-break protein trap transposon (GBT) to simultaneously report and reversibly knockdown any gene. The GBT system allows the definition of 1) gene expression pattern, 2) functional disruption to create mutant phenotype, and 3) revertibility using Cre recombinase. We employed this GBT system in zebrafish to create and catalog a collection of over 1,200 transgenic strains.
Among 1,200+ zebraifsh mutant lines with expression and 214 lines with identified GBT integrated loci, strong transcriptional knockdown >99% was shown in nearly all investigated alleles. Homozygous GBT animals in *ryr1b*, *fras1*, *tnnt2a*, *edar* and *hmcn1*phenocopied the alternative mutants generated by other mutagenic technologies. In GBT *ryr1b* homozygous animals, skeletal muscle Ca^{2+} transients were severely dampened, highlighting the ability to explore between human and zebrafish orthologs for mechanistic studies. Further, the proportions of protein classes represented reflect nearly unbiased random mutagenesis and include 66 mutants in zebrafish orthologs of human disease loci, including nervous, cardiovascular, endocrine, digestive, musculoskeletal, immune and integument systems as models of human disease and 39 cloned GBT lines are potential new models of genetic disease. The protein trap-associated mRFP expression in GBT-confirmed lines shows previously undocumented expression of 35% and 92% of these genes at 2 and 4 days post-fertilization, respectively. Notably, the screen revealed unpredicted protein-coding sequences in the vertebrate genome. This GBT system facilitates novel functional genome annotation towards understanding cellular and molecular underpinnings of vertebrate biology and human disease.

361 The Reissner Fiber is Highly Dynamic in vivo and Controls Morphogenesis of the Spine Benjamin Troutwine[1], Paul Gontarz[2], Ryoko Minowa[1], Adrian Monstad-Rios[3], Mia Konjikusic[1], Diane Sepich[2], Ronald Kwon[3], Lilianna Solnica-Krezel[2], *Ryan Gray*[1] 1) Univeristy of Texas at Austin - Dell Medical School, Austin, TX; 2) Washington University School of Medicine, Saint Louis, MO; 3) University of Washington, Seattle, WA.

Spine morphogenesis requires the integration of multiple musculoskeletal tissues with the nervous system. Cerebrospinal fluid (CSF) physiology is important for development and homeostasis of the central nervous system and its disruption has been linked to scoliosis in zebrafish. Suspended in the CSF is an enigmatic glycoprotein thread called the Reissner fiber, which is secreted from the subcomissural organ in the brain and extends through the central canal where it terminates at the base of the spinal cord. In zebrafish, *scospondin* null mutants are unable to assemble the Reissner fiber and fail to extend a straight body axis during embryonic development.

Here, we describe zebrafish hypomorphic missense alleles, which allow for the assembly of the Reissner fiber and formation of a straight the body axis during early embryonic development. However, these mutants progressively lose the Reissner fiber, concomitant with the emergence of body curvature, alterations in neuronal gene expression, and scoliosis in adult zebrafish. We engineered a *scospondin-GFP* zebrafish knock-in line, with which we demonstrate several intriguing dynamic properties of the Reissner fiber including dynamic, continuously treadmilling from head-to-tail during normal development; and its disassembly during the progression of scoliosis in multiple independent scoliosis mutant zebrafish strains. This demonstrates that disruption of the Reissner fiber is a common pathology associated with scoliosis. In summary, our study establishes a framework for ongoing investigations of the mechanistic roles of the Reissner fiber including its dynamic properties and molecular interactions, and how these processes are involved in the regulation of neural development, spine morphogenesis, and scoliosis.

362 Analysis of craniosynostosis risk factors in zebrafish *Xuan He*[1], Mary Shannon Fisher [1] 1) Boston University.

Bones of the cranial vault protect the underlying brain, but preserve flexibility to allow brain growth. The edges of skull bones are joined by fibrous sutures, which provide structural support and are the sites of bone growth. Craniosynostosis (CS), premature fusion of bones at the sutures, causes skull deformity and secondary problems in brain development. Known mutations cause some CS cases, but for most, the underlying genetic risks are unclear. Bone morphogenetic protein (BMP) signaling is critical in regulating osteoblast differentiation and ossification. Human genetic data strongly

support a central role for BMP signaling in suture formation and suggest that enhanced BMP signaling increases the risk of CS. The first genome wide association study (GWAS) of CS identified two susceptibility loci, near *BMP2* and *BMPER* (BMP Binding Endothelial Regulator), which encodes an extracellular modulator of BMP signaling. I hypothesize that there are enhancers within the two risk loci, and changes in transcriptional regulation of *BMP2* and *BMPER*, respectively, are associated with the genetic risk of CS. To identify candidate enhancers underlying CS risk, I selected multiple conserved sequences within the two risk loci based on a conservation algorithm PhastCons and additional sequences based on the single nucleotide polymorphism markers used in the replication study of the GWAS. We then used a transgenic assay in zebrafish to test for their enhancer activity. We have found multiple conserved sequences within the CS risk locus near *BMPER* that direct *eGFP* expression consistent with tissue-specific expression of *bmper*. We are establishing stable transgenic lines to further examine activity of the identified enhancers. We are also testing potential enhancers for transcriptional factor interactions via an enhanced yeast one-hybrid (eY1H) assay. Understanding transcriptional regulation of *BMP2* and *BMPER* will provide insight into the upstream signals regulating osteoblast differentiation as well as sequences underlying a major genetic risk factor for CS. Also, identification of genetic risk for CS will help uncover the disease mechanism and advance current treatments.

363 NMDA receptor dependent nervous system functions Josiah Zoodsma[1], Kelvin Chan[1], Ashwin Bhandiwad[2], David Golann[1], Amalia Napoli[1], Guangmei Liu[1], Shoaib Sayed[1], Harold Burgess[2], Lonnie Wollmuth[1], *Howard Sirotkin*[1] 1) Stony Brook University, Stony Brook, NY; 2) National Institute of Child Health and Development, NIH, Bethesda, MD.

Rapid communication between cells in the nervous system depends on ion channels that are directly activated by a chemical neurotransmitter. In the human brain, glutamate is the major excitatory neurotransmitter. One key mediator of glutamatergic signaling is the *N*-methyl-D-aspartate receptor (NMDAR). These receptors impact nearly all forms of nervous system function including circuit development, higher brain functions such as learning and memory, and motor processing. Here, we studied NMDARs in early zebrafish development, by generating CRISPR-mediated lesions in the NMDAR genes, *grin1a* and *grin1b*, which encode the obligatory GluN1 subunits. While receptors containing *grin1a* or *grin1b* show high Ca^{2+} permeability, like their mammalian counterpart, *grin1a* is expressed earlier and more broadly in development than *grin1b*. Both *grin1a* and *grin1b* mutants are viable as adults. Unlike in rodents, where the *grin1* knockout is embryonic lethal, *grin1* double mutant fish, which lack all NMDAR-mediated synaptic transmission, survive until about 10 days post fertilization, providing a unique opportunity to explore NMDAR function during development and in generating behaviors. Many behavioral defects in the *grin1* double mutant larvae, including abnormal evoked responses to light and acoustic stimuli, prey capture deficits and a failure to habituate to acoustic stimuli, are replicated by short-term treatment with the NMDAR antagonist MK-801, suggesting they arise from acute effects of compromised NMDAR-mediated transmission. Other defects, however, such as periods of hyperactivity and alterations in place preference, are not phenocopied by MK-801, suggesting a developmental origin. Taken together, the *grin1* double mutants provide a unique opportunity to study NMDAR function in the developing vertebrate nervous system.

Saturday, April 25 1:30 PM - 3:30 PM

Gene Regulation/Genome Integrity (*Drosophila*)

328 Ecdysone Signaling Shapes Tissue Regeneration in Wing Discs through Regulation of Wingless Expression *Faith Karanja*[1], Brittany Martinez[1], Shuchi Amin[1], Danielle DaCrema[1], Sara Weintraub[1], Subhshri Sahu[1], Adrian Halme[1] 1) University of Virginia.

As tissues develop, their regenerative capacity is often diminished. In *Drosophila melanogaster,* imaginal discs (larval precursors to adult tissues) lose the ability to regenerate near the end of larval development. This loss of regenerative capacity coincides with an increase in systemic levels of the steroid hormone ecdysone, a key coordinator of *Drosophila* developmental progression. Artificially increased systemic ecdysone inhibits regeneration prematurely, as seen in the adult wing. Surprisingly, although regeneration was restricted, the high ecdysone levels increased the activation of regeneration genes, *wingless* (*wg*) and *dilp8*. This result was scalable: feeding the larvae lower concentrations of ecdysone induced more activation of regeneration pathways than higher concentrations. Therefore, we hypothesized that low concentrations of ecdysone, such as those found before regeneration is restricted promote regeneration, while higher levels of ecdysone found at the prepupal stage cause changes in disc epithelia that interferes with regeneration. The activation of regeneration in the wing disc is dependent on the activation of the *wg* enhancer locus known as *wg-RRE* (*Regeneration Response Element*). By locally expressing ecdysone receptor (ec*R*) dominant negative, we determined that ecdysone signaling is necessary for both the activation and suppression of regeneration. However the activation of regeneration by ecdysone seems to be independent of the *wgRRE.* This suggests that ecdysone uses independent mechanisms to activate and suppress regeneration.
We looked at downstream targets of ecdysone and found that expression of *broad (br)* splice variants (*Z1-Z4*), which are early targets of prepupal ecdysone signaling, coincides with regeneration restriction. We determined that expression of *br* splice variants are necessary for only regeneration restriction and not activation. Loss of *br* expression using *br-RNAi* or variant-specific mutants allows for activation of key regeneration genes, past the regeneration restriction timepoint. Through the overexpression of the variants early in imaginal disc development and determined that that *brZ1, brZ2 and brZ4,* suppress activation of the *wg-RRE*. Interestingly, *brZ3,* activates both *dilp8* and *wgRRE*. We are currently investigating the mechanism through which *br* variants regulate regeneration. Preliminary data suggests that *br* variants regulate independent mechanisms. We are exploring how *ecR* and *br* facilitates the epigenetic modifications that lead to suppression of regeneration genes at the end of larval development. Our findings would provide insight into how endocrine signals regulate the regeneration activation, extent and competence of tissues.

329 The temporal transcription factor E93 controls enhancer competency during *Drosophila* wing development *Matthew Niederhuber*[1,2,3,4], Spencer Nystrom[1,2,3,4], Daniel McKay[2,3,4] 1) Curriculum in Genetics and Molecular Biology, UNC, Chapel Hill, NC; 2) Department of Biology, UNC, Chapel Hill, NC; 3) Department of Genetics, UNC, Chapel Hill, NC; 4) Integrative Program for Biological and Genome Sciences, UNC, Chapel Hill, NC.

Animal development requires spatial and temporal information to be integrated in order for cell-type specific gene expression to be progressively specified. How precisely this process of integration works is still an open question. Here we describe how a temporal transcription factor controls the ability of an enhancer to respond to spatial cues (enhancer competency) through the control of chromatin accessibility. Our prior studies have shown that chromatin accessibility, that is the degree of nucleosome occupancy at a locus, is highly dynamic during Drosophila wing development, and that temporally dynamic enhancer activity corresponds to periods of increased chromatin accessibility. We have identified the hormone-induced transcription factor Eip93F (E93) as a key regulator required for nearly half of the chromatin accessibility changes observed genome-wide during pupal wing development. We now show that ectopic expression of E93 at an earlier point in wing development (precocious E93) advances

the developmental program by prematurely triggering changes to chromatin accessibility and enhancer activity that normally occur later in pupal development. Using ChIP-seq and FAIRE-seq we observe that precocious E93 binds to the vast majority of its wild-type endogenous binding sites and produces patterns of chromatin accessibility that largely replicate patterns seen later in pupal wings. Transgenic reporter assays show that the genomic effects of precocious E93 correspond to functional changes in enhancer activity, and that precociously activated enhancers appear to respond to similar spatial inputs as they would normally in later pupal wings. These data suggest a model for temporal and spatial information integration that involves the use of temporal transcription factors to define windows of competency for enhancer sets, restricting the times at which enhancers are able to respond to spatial information by modifying chromatin accessibility.

330 The transcription factor M1BP targets CP190 to chromatin to regulate transcription and chromatin insulator activity *Indira Bag*[1], Shue Chen[1], Chen-Yu Liu[1], Elissa Lei[1] 1) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

Chromatin insulators are DNA-protein complexes that establish distinct higher order transcriptional domains. *Drosophila* CP190 is the only common component of all insulator complexes thus far identified, and its genome-wide chromatin binding profile is highly correlated with topologically associating domain borders. Using mass-spec analysis of immunopurified complexes from embryonic nuclear extracts, we identified the transcription factor Motif 1 Binding Protein (M1BP) associated with CP190 and verified this interaction by coimmunoprecipitation. ChIP-seq analysis of both factors in Kc167 cultured hemocytes revealed extensive overlap of the two factors, particularly at Motif 1 containing promoters. Depletion of M1BP results in extensive loss of CP190 chromatin association, and depletion of CP190 also greatly affects M1BP chromatin association. Furthermore, depletion of M1BP results in loss of both enhancer-blocking and barrier activities of the CP190-dependent *gypsy* insulator *in vivo*. Reduction of M1BP levels also disrupts the nuclear localization of CP190-marked insulator bodies within the developing fly. Moreover, EU-seq analysis after depletion of either CP190 or M1BP results in similar changes in nascent transcript levels genome-wide. Further analysis using reporter assays verifies that CP190-dependent gene expression changes are dependent on the presence of Motif 1. Given that Motif 1 is also enriched at TAD borders, our results suggest an intimate mechanistic relationship between CP190 and M1BP function with respect to transcriptional regulation and higher order chromatin organization.

331 Coordinate regulation of salivary gland form and function by *ribbon* during tubulogenesis *Rajprasad Loganathan*[1], Michael Wells[1], Deborah Andrew[1] 1) Johns Hopkins University, Baltimore, MD.

The Drosophila salivary gland (SG) is an ideal model for understanding how epithelial tubular organs form and specialize. Through changes in cell shape, arrangement, migration, and growth, the two-dimensional SG placode is transformed into an elongated, fully internalized epithelial tube, whose primary function is to synthesize and secrete high levels of protein. Here, we focus on the role of Ribbon (Rib), a BTB-domain containing transcription factor, in coordinating embryonic SG development. Whereas cell number, specification, polarity, and junctional marker localization are entirely normal in *rib* mutant SGs, cell volume is decreased (53% of WT) and the shape of *rib* SG cells is altered—being cuboidal rather than columnar as in WT. To learn how Rib affects SG size and shape, we performed SG-specific *in vivo* ChIP-Seq analysis and discovered 494 genes bound by Rib. Gene ontology classification (DAVID analysis) revealed that many Rib-bound genes correspond to morphogenetic regulators (genes encoding proteins involved in cell shape and movement); the class of genes with the highest rank is, however, the ribosome. Indeed, nearly 90% of ribosomal protein genes (RPGs), and genes encoding several translation factors and chaperones required for protein synthesis, are bound by Rib. Rib both activates and represses many of the morphogenetic regulators it binds in the SG, and it is required for full expression of several RPGs tested. The Rib-bound DNA sequences in RPGs are within promoter-proximal enhancers that are also bound by two other known direct regulators of RPG transcription—M1BP and Dref—and a TATA box Binding Protein-like factor, Trf2, the latter implicated in the initiation of RPG transcription without direct DNA binding. To determine how Rib upregulates RPGs, we tested for physical interactions between Rib and the known RPG regulators. Indeed, when expressed in cultured S2R+ cells, Rib co-precipitates with M1BP, Dref and Trf2. We are now testing if Rib links Trf2 to the DNA sequences required for its regulation of RPGs. We demonstrate that *rib* loss adversely impacts subcellular markers of ribosome biogenesis, translation, and secretion. Altogether, these results suggest that Rib boosts translation—a requirement for cell growth and increased secretory capacity—by transcriptional upregulation of the translation machinery. Thus, Rib coordinates SG growth (size gain), form (morphogenesis), and function (secretion) by simultaneous regulation of morphogenetic and translational machinery genes.

332 Ribosomal DNA-specific retrotransposons maintain unstable ribosomal DNA repeats in the *Drosophila* male germline *Jonathan Nelson*[1], Alyssa Slicko[1], Yukiko Yamashita[1,2] 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) HHMI.

A critical role of the germline is to maintain inherently unstable essential genomic elements, such as telomeres, to ensure the transmission of functional genomes from generation to generation. Among these elements are ribosomal DNA (rDNA) loci, which consist of the hundreds of tandem duplications of the rRNA genes needed to for sufficient ribosomal activity. We recently discovered that these rDNA copies are progressively lost from the *Drosophila* male germline during aging, but that germline stem cells (GSCs) can restore lost rDNA, revealing the rDNA locus is maintained through cyclical rDNA copy loss and re-expansion. We found homology-dependent repair factors are required for this expansion, suggesting that the new rDNA copies are created by the unequal exchange of misaligned sister chromatid rDNA copies during the homology-dependent repair of double stranded breaks (DSB), a well described mechanism of repetitive DNA duplication. However, whether DSB repair itself contributes to this expansion, or the potential source of the DSBs to trigger this activity has been unclear. Here we show that a retrotransposon with rDNA-specific endonuclease activity, R2, initiates rDNA copy number expansion, likely through creating DSBs at the rDNA locus during its transposition.
While the expression of transposable elements is normally suppressed in the germline, we found that R2 is derepressed in GSCs with reduced rDNA copy number, and this R2 expression creates DSBs in these GSCs. Despite the fact that R2 transposition disrupts rRNA genes, we found that inhibition of R2 expression by RNAi surprisingly accelerated rDNA copy loss during aging and compromised GSC maintenance, indicating that R2 expression benefits the host genome. Furthermore, we find that R2 inhibition in GSCs completely blocks rDNA copy number expansion in the germline of animals with few rDNA copies, and over-expression of transgenic R2 is sufficient to induce rDNA copy number expansion in animals with sufficient rDNA, suggesting R2 initiates rDNA copy expansion. We are now investigating whether R2 expression induces unequal sister chromatid exchange between rDNA copies to expand rDNA copy number in GSCs with few rDNA copies. These findings indicate an essential function for this retrotransposon to maintain the host genome within the male germline, and reveal a benefit for these elements to the host despite their reputation for being entirely parasitic.

333 Targeted *de novo* centromere formation in *Drosophila* reveals plasticity and maintenance potential of CENP-A chromatin Jason Palladino[1],

Ankita Chavan[1], Anthony Sposato[1], Timothy Mason[1], *Barbara Mellone*[1] 1) University of Connecticut, Storrs, CT.

Centromeres are essential for accurate chromosome segregation during mitosis and meiosis and are organized into a specialized chromatin containing the histone H3 variant CENP-A. CENP-A chromatin has been proposed to be sufficient to seed centromeres at non-centromeric DNA. However, the requirements for such *de novo* centromere formation and transmission *in vivo* remain unknown. Here, we employ *Drosophila melanogaster* to investigate the ability of *de novo* centromeres to assemble and be inherited through development. We induce *de novo* centromeres on integrated lac operator (lacO) repeat arrays by expressing the CENP-A assembly factor CAL1 fused to the Lac Repressor (LacI). *De novo* centromeres form efficiently at six distinct genomic locations, which include actively transcribed chromatin and heterochromatin, and cause widespread chromosomal instability. During tethering, *de novo*centromeres sometimes prevail, causing the loss of the endogenous centromere through centromeric DNA breaks and HP1-dependent epigenetic inactivation. Transient induction of LacI tagged CAL1 and chromosome healing in early embryogenesis shows that, once established, *de novo* centromeres can be maintained through development, suggesting that centromeric DNA is not required for centromere maintenance in mitosis. These results underpin the ability of CENP-A chromatin to establish and sustain mitotic centromere function in *Drosophila*.

334 Gazing into the CRISPR crystal ball – experimental and computational analysis of Cas9-induced alternative end-joining in Drosophila Terrence Hanscom[1], Gabriel DePinho[1], Ishan Gupta[1], Jacob Zuckerman[1], *Mitch McVey*[1] 1) Tufts University.

Targeted induction of DNA double-strand breaks by CRISPR-Cas9 allows for editing at specific genomic loci. Mutagenic repair of these breaks by end joining can disrupt gene function. While several studies have identified dominant repair products resulting from Cas9-induced editing at different sites, the rules that govern end joining repair at these sites remain largely uncharacterized. This is particularly true for polymerase theta-mediated alternative end joining, a common repair pathway in Drosophila and other metazoans. To fully realize the potential of CRISPR-induced mutagenesis, a better understanding of how sequence context impacts repair is needed.

To address this knowledge gap, we have pioneered a system where we inject plasmids with varied sequence contexts flanking a single sgRNA site into Cas9 expressing Drosophila embryos. Following incubation, we recover inaccurate repair products and use high-throughput amplicon sequencing to catalog all junctions. We have previously shown that sequences with the potential to form loops and hairpins frequently serve as primers for limited DNA synthesis. The resulting nascent strands then anneal with short, microhomologous sequences on the other side of the break, generating repair products with characteristic repeat motifs (Khodaverdian *et al*., 2017).

Our recent studies indicate that high GC content, proximity to the break site, and an optimal primer repeat length all correlate with formation of particular repair junctions. Furthermore, we have shown that DNA polymerase theta promotes the use of secondary structure-forming sequences during repair, while replication protein A inhibits their use. We are now injecting libraries of thousands of different sgRNA flanking-sequence constructs into embryos of various genetic backgrounds. The results of these studies are being used to computationally predict how alternative end-joining repair proteins interact with break-proximal flanking sequences to preferentially promote specific repair products. We anticipate that our findings will be useful for anyone utilizing CRISPR for mutagenesis.

Saturday, April 25 1:30 PM - 3:30 PM

New Technology and Resources (Mammal)

342 Efficient and effective curriculum development and training for biological sciences *Sue McClatchy*[1] 1) The Jackson Laboratory.

Technological advances drive data production in greater quantity and complexity, promoting new ways of interrogating biological systems. At the same time, these advances generate a need for new training in data analysis methods and tools. Brief, relevant, and directly applicable training that delivers both the conceptual knowledge and practical skills for analyzing data and using new methods will enable researchers to keep pace with changes. Absent relevant and immediately applicable training, biological researchers are unable to take advantage of novel methods of inquiry that new technologies present.

To provide relevant up-to-date training for researchers, curriculum development must be carried out efficiently and promptly in order to meet training needs within a reasonable time period. Curriculum development is effortful, so the more time and effort spent on curriculum development, the greater the training lag. The challenge is to rapidly develop curriculum and deploy training that keeps pace with new methods and tools generated by technological change.

The expedient solution is to tailor existing materials to the training needs of researchers. We have developed a process to adapt software tutorials and other materials for efficiency and expediency, such that customized training material can be produced, taught, and delivered without too great a lag time from the identification of a critical training need. Once the adaptation is complete, we generate short applied courses in bioinformatics that successfully bring researchers the skills and knowledge needed to pursue new avenues in research. As an example, Quantitative Trait Mapping (https://smcclatchy.github.io/mapping/) was adapted from a user guide for the R package qtl2 and delivered as a two-day hands-on workshop at the Jackson Laboratory and the University of North Carolina Chapel Hill. We have also employed this process to create and deliver training in experimental design, data analysis, machine learning, and image processing.

343 The mouse Gene Expression Database (GXD): fostering insights into the molecular mechanisms of development and disease *Constance Smith*[1], Jacqueline Finger[1], Terry Hayamizu[1], Ingeborg McCright[1], Jingxia Xu[1], David Shaw[1], Richard Baldarelli[1], Cynthia Smith[1], James Kadin[1], Joel Richardson[1], Martin Ringwald[1] 1) The Jackson Laboratory.

The Gene Expression Database (GXD) is an extensive and freely available community resource of mouse gene expression information (www.informatics.jax.org/expression.shtml). Through curation of the published literature and collaborations with large-scale data providers, GXD has integrated data from RNA *in situ* hybridization, immunohistochemistry, *in situ* knock-in reporter, RT-PCR, northern blot, and western blot experiments. GXD covers all developmental stages and includes data from wild-type and mutant mice. It currently contains ~1.7 million annotated expression results,

with ~350,000 accompanying images, for ~15,000 genes. These data are combined with other genetic, functional, phenotypic, and disease-oriented data in Mouse Genome Informatics (MGI), making them easily searchable by a wide variety of biologically- and biomedically-relevant parameters. Ontology browsers and interactive matrix views enable the anatomical comparison of expression and phenotype data and foster insights into molecular mechanisms of disease. As an initial part of our efforts to expand GXD to include high-throughput expression assays, we have developed an RNA-Seq and Microarray Experiment Search utility that allows users to effectively find specific studies, deposited in the public repositories, which examine gene expression in mouse tissues. To enable this search, GXD curators create standardized sample and experiment metadata annotations using controlled vocabularies for anatomical structure, developmental stage, mutant alleles, sex, and strain, as well as for the experiment study type and key experimental parameters. Integration of these standardized annotations with free text searching of experiment title and description allows for searches that quickly and accurately return experiments of interest. Work to incorporate RNA-seq expression data into GXD is underway and will also be described at the meeting. GXD is supported by NIH/NICHD grant HD062499.

344 Completing the GENCODE gene catalogue for the mouse reference genome *Jane Loveland*[1], Adam Frankish[1] 1) European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge, UK.

The Ensembl-Havana team perform manual gene annotation of vertebrate genomes and provide reference gene annotation for whole genomes (currently human and mouse; previously zebrafish), individual chromosomes (Pig chr X and Y), genes (Rat, Pig) and regions of community interest. As part of the GENCODE project, we are responsible for producing detailed reference annotation of all human and mouse protein-coding genes, pseudogenes and long non-coding RNAs. Small RNA annotation in GENCODE is currently produced by an automated pipeline but will be a target for manual annotation in 2020. Having completed the first pass annotation of mouse, GENCODE are now targeting specific loci, for example to identify unannotated protein-coding, pseudogene and lncRNA genes and alternatively spliced transcripts and also to reassess legacy protein-coding gene annotation in the light of current data and update or remove as necessary.
In order to produce the most complete gene set possible for mouse we are integrating novel transcriptomic data types into our analysis pipeline e.g. Cap analysis gene expression (CAGE) was used to confirm transcriptional start sites while PacBio and ONT data support the annotation of novel transcripts and the extension of currently partial models, with RNAseq data generally used to validate introns and exons identified in orthogonal transcriptomic data and inform extensions to 3' UTR sequence.
The availability of new data, particularly long transcriptomic experiments will drive increases in the numbers of transcripts and genes (mostly lncRNAs) which the abundance of evidence from new transcriptomic and proteomic datasets makes absolutely clear are not yet complete. Alongside the efforts to identify new genes we continue to work with closely with our colleagues at RefSeq, MGI and Uniprot to resolve the remaining biotype conflicts between our gene catalogues.
All Ensembl/GENCODE gene annotation is also accessible via the Ensembl and UCSC browsers and Ensembl FTP site and Perl and REST APIs. Updates to annotation are made continuously. To allow access to inter-release gene annotation changes we have created a track hub that is updated every 24 hours and available at: http://ftp.ebi.ac.uk/pub/databases/gencode/update_trackhub/hub.txt

345 Disease Portals at RGD: Access to Consolidated Disease-Related Data and Tools Across Species Jennifer R Smith[1], G Thomas Hayman[1], Shur-Jen Wang[1], Stanley J Laulederkind[1], Matthew J Hoffman[1,2], Mary L Kaldunski[1], Cody Plasterer[1], Monika Tutaj[1], Logan Lamers[1], Harika S Nalabolu[1], Jyothi Thota[1], Marek A Tutaj[1], Jeffrey L De Pons[1], Anne E Kwitek[2], Melinda R Dwinell[2], Mary E Shimoyama[1] 1) Department of Biomedical Engineering, Medical College of Wisconsin, Milwaukee, WI; 2) Department of Physiology, Medical College of Wisconsin, Milwaukee, WI.

RGD (https://rgd.mcw.edu) is a multi-species knowledgebase which provides a substantial corpus of genomic, genetic, phenotypic and disease-related data and an innovative suite of tools for analyzing these data. For researchers specifically interested in disease-focused data, each of RGD's twelve disease portals facilitate the exploration of data associated with a specific disease category. A recent redesign of the portals has substantially increased both their functionality and the incorporated data. Portals now include data for all RGD species and are populated with both manual annotations assigned at RGD and annotation imported from other databases such as ClinVar, OMIM, OMIA, MGI and the Comparative Toxicogenomics Database (CTD). The updated portals provide a customized ontology browser giving users more flexibility in their selection of specific disease categories. Terms in the browser are limited to those for which annotations exist in RGD. In addition to disease, the portals include seven other vocabularies. The terms included for these other vocabularies are derived from annotations assigned to disease-related genes, QTLs and/or strains. Below the browser are downloadable lists of the objects annotated to the term or terms selected. The integrated Genome Viewer shows the positions of these objects relative to the chromosomes for the species selected. Any changes made to the selected vocabulary in the list at the top of the page and/or to the terms selected in the browser are immediately reflected in the counts of annotated objects for each species in the "Select a species" section, in the object lists and in the GViewer display. Another important upgrade to the disease portal is the addition of an embedded ontology enrichment tool. The list of genes matching the species/vocabulary/term selections made in the portal are automatically submitted to this RGD's multi-ontology enrichment tool (MOET) which provides a list of terms from the vocabulary selected that are over-represented in the annotations for the currently displayed gene list, with corresponding p-values and the lists of genes annotated to each term in the list.

Combined transient ablation and single cell RNA sequencing reveals the development of medullary thymic epithelial cells *Kristen Wells*[1], Corey Miller[2], Andreas Gschwind[1], Wu Wei[4], Jonah Phipps[2], Mark Anderson[2], Lars Steinmetz[1,3,4] 1) Stanford University School of Medicine; 2) University of California, San Francisco; 3) European Molecular Biology Laboratory; 4) Stanford Genome Technology Center.

Medullary thymic epithelial cells (mTECs) play a critical role in central immune tolerance by mediating negative selection of potentially autoreactive T cells through collective expression of genes representing the peripheral self-antigen compartment, including tissue-specific antigens (TSAs). Recent work has shown that gene expression patterns within the mTEC compartment include multiple differentiated cell states. One population of mTECs express the autoimmune regulator (AIRE) protein, which plays a central and nonredundant role in the expression of a subset of TSAs. Considerable effort has been devoted to identifying the progenitors of the *Aire*-expressing population, and some have postulated that the progenitors express high levels of *Ccl21a*. However, loss of these cells does not impact *Aire*-expressing cells, suggesting there is a different progenitor cell.
In this study, we combined bioinformatic and experimental techniques to learn the developmental relationships between mTEC populations. Single cell RNA-sequencing with genetic lineage tracing distinguished between early and late *Aire*-expressing cells. To further define developmental relationships, we applied single cell velocity, a version of pseudotime. The pseudotime predictions corresponded to the relationships identified by lineage tracing and provided further resolution of developmental time. To validate the predicted developmental time, we applied transient ablation of the mTEC compartment and performed single cell RNA-seq throughout the time course of recovery.

Using combined genetic lineage tracing and recovery from transient *in vivo* mTEC ablation with single cell RNA-sequencing, we identified a non-stem progenitor population of cycling mTECs that preceded *Aire* expression and was notable for elevated levels of genes associated with chromatin modification and Aire function. Based on our findings, we propose a branching model of mTEC development wherein this progenitor population gives rise separately to *Aire*- and *Ccl21a*-expressing branches, suggesting that *Ccl21a*-expressing cells are not, as previously proposed, the precursor of *Aire*-expressing mTECs. Further, in the *Aire*-expressing branch, TSA expression peaked as AIRE expression decreased implying *Aire* expression must be established before TSA expression can occur. Collectively, our findings provide a map of mTEC development and demonstrate the power of combinatorial approaches leveraging both *in vivo* models and high-dimensional datasets.

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Neuronal Development (*C. elegans*)

322 Homeobox gene encode neuronal cell type diversity *Oliver Hobert*

abstract is not available at the time of print

323 Embryo to mother signal to clean up molecular garbage-transgenerational proteostasis adjustment via exopher production *Sangeena Salam*[1], Ryan J. Guasp[1], Guoqiang Wang[1], Ilija Melentijevic[1], Girish Harinath[1], David Jimenez[1], Monica Driscoll[1] 1) Rutgers, The state University of New Jersey.

Proteostasis imbalance is a cause of aging and neurodegenerative diseases. In addition to the already-known cellular proteostasis maintenance mechanisms that include chaperone action, proteasome-mediated degradation, and autophagy, our lab recently reported that neuronal protein aggregates and organelles can be extruded from *C. elegans* neurons within a large membrane-bound vesicle (~4µm) called an exopher (Melentijevic et al., Nature 2017). The nearly soma-sized exopher enters the surrounding hypodermis, which acts akin to a glial cell and attempts to degrade its contents.
We do not know what triggers neuronal trash removal via the exopher. We do know that exophers are not produced in larval stages, but show a first peak of production around adult day 2 (shortly after the onset of egg laying), raising the possibility that reproduction is tied to that signal. Our recent study found that the early peak of exopher production is inhibited in mutants defective in germ cells. The germline defective mutant *gld-1(q485)* and germline proliferative mutant *glp-4(bn2)* produces significantly lower exophers than the control in early adult life. We specifically disrupted sperm production (using auxin-inducible degradation of SPE-44, which causes spermatogenesis defects), and observed that the exopher production is specifically inhibited in degron transgene animals exposed to auxin. We examined gametogenesis defective *fem-3(q20)* hermaphrodites which produce only sperm and *fem-1(hc17)* hermaphrodites which produce only oocytes. In both cases, exopher production is inhibited. Further, we looked at worms treated with C22 — a chemical known to perforate the eggshell of developing embryos. C22-treated hermaphrodites produce fewer exophers. These results show that the signal for exopher production depends on fertility and seems to require fertilized eggs/embryos. Overall, we report a signaling system from embryo to mother that induces exopher production, which we consider to be a mechanism of trash disposal. We discuss a trans-generation signaling process that may clean up the parent for successful reproduction.

1) Melentijevic, I, et al. *C. elegans* neurons jettison protein aggregates and mitochondria under neurotoxic stress. Nature 2017; 542(7641): 367-371.

324 Retrograde extension as a general mechanism of sensory dendrite development Elizabeth Cebul[1,2], Ian McLachlan[1,2], *Maxwell Heiman*[1,2] 1) Boston Children's Hospital, Boston MA; 2) Harvard Medical School, Boston MA.

As an organism grows, its cells scale in size to keep pace with the growth of the body. In the nervous system, axons and dendrites increase in length by 10- to 100-fold so that neurons remain attached to their targets throughout life. Embryonic morphogenesis involves rapid changes in organ size that place a special burden on maintaining attachments. We have found that most of the head sensory neurons in *C. elegans* grow by rapid stretch, first attaching at the presumptive nose and then elongating up to 10-fold in as little as 120 min of embryo morphogenesis. We refer to this "anchor and stretch" growth as retrograde extension. Retrograde extension has recently been described in zebrafish olfactory axon development, suggesting it is shared across species. To identify molecular mechanisms of retrograde extension, we performed forward genetic screens for mutants in which dendrites fail to remain attached at the nose. These mutants exhibit severely shortened dendrites in mature animals. Recently, we showed that most anterior sensory neurons develop as part of tube-shaped epithelia formed by glia, with tight junctions anchoring dendrites to glia, and glia to epidermis at the nose. The apical extracellular matrix molecule DYF-7 lines the lumen of this epithelial tube. In its absence, the tube ruptures, leading to loss of dendrite anchoring. The amphid, CEP, IL, and OL sensory neurons require DYF-7. In contrast, the gas-sensor neurons URX and BAG form highly specialized attachments to glia and develop by a different molecular mechanism of retrograde extension. Using unbiased and candidate genetic screens, we identified the adhesion molecule SAX-7 and the cytoplasmic molecules GRDN-1 and MAGI-1 as required for URX and BAG dendrite extension. In mutants, URX and BAG dendrites fail to remain attached at the nose, and are much too short. SAX-7 can act both in neurons and glia, while GRDN-1 and MAGI-1 act in glia to promote URX and BAG dendrite extension. All of these factors associate with adherens junctions, and we find that glia- or neuron-specific depletion of the cadherin HMR-1 also disrupts URX and BAG dendrite extension. Thus, retrograde extension is used by diverse sensory neuron types, and involves at least two genetically distinct mechanisms, both of which share features with epithelial junctions. Our results suggest that the cell attachments themselves mediate cell scaling during organismal growth.

325 FKH-7/FOXP regulates sensory neuron function during developmental decision-making Cynthia M. Chai[1], Carsten H. Tirschbirek[1], Wan-Rong Wong[1], Long Cai[1], Paul W. Sternberg[1] 1) Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, CA.

Under adverse environmental conditions, *Caenorhabditis elegans* larvae can enter an alternate stress-resistant diapause state during which growth and metabolism are suppressed. *C. elegans* constitutively secretes a mixture of dideoxy sugar ascarylose derivatives that comprise diapause-inducing pheromone and serves as a proxy for high conspecific density. This information about local competition is integrated by the worm's nervous system with other inputs regarding temperature, food availability, and the worm's internal state to assess the environment's suitability for future reproductive growth. We found that under pheromone-induced dauer formation conditions, null mutants of the forkhead transcription factor *fkh-7* exhibited a dauer entry-defective (daf-d) phenotype with significantly fewer mutants entering diapause compared to wild-type animals. Using a

transcriptional GFP reporter for the longest isoform *fkh-7*.a, we observed strikingly different expression patterns between reproductive and dia-pause developmental trajectories. *fkh-7*.a expression was confined to a few neurons and the intestine throughout reproductive development but was widespread in several tissues during dauer entry. Interestingly, we found that *fkh-7*.a cDNA expression in a small subset of pheromone-sensing neurons was sufficient to rescue the mutant daf-d phenotype. Our smFISH results further confirmed the presence of *fkh-7*.a mRNA transcripts in these sensory neurons during larval stages. In vertebrates, members of the orthologous forkhead box P (FOXP) subfamily are important regulators of neurodevelopment. Clinical studies have associated human *FOXP1* R465G and R514C variants, each harboring a missense mutation within the highly conserved forkhead DNA-binding domain, with autism spectrum disorder (ASD) symptoms. We applied a CRISPR homology directed repair strategy to generate the corresponding disease-relevant *fkh-7* missense mutants and found that both variants also exhibited daf-d phenotypes. We are conducting single-cell RNA-seq to compare transcriptomes of the *fkh-7.*a-expressing pheromone-sensing neurons between wild-type N2 and ASD-associated missense variants. In summary, FKH-7 regulates the ability of a subset of sensory neurons to accurately process environmental stim-uli in a developmental decision-making context. We further aim to provide insights into the evolutionarily conserved signaling pathways mediated by FKH-7/FOXP during neurodevelopment.

326 Distinct mechanisms regulate presynaptic release of functionally similar insulin/IGF-like proteins in *C. elegans* sensory neurons Lauren Kla-bonski[1], Mingjie Ying[1], Nidhi Patel[1], Yair Argon[2], *Tali Gidalevitz*[1] 1) Drexel University; 2) University of Pennsylvania.

Individual neurons often express multiple neuromodulators that can be either co-localized and released together, or targeted into different vesicles and released in response to different stimuli. Such diversity may allow neurons to match their responses to specific signals, but how it is achieved is largely unknown. In *C. elegans*, sensory neurons regulate the developmental and behavioral responses to environmental signals such as food and crowding, including entrance to the adaptive developmental stage known as dauer. Intriguingly, same amphid sensory neurons signal either continual reproductive development, exit from dauer and return to reproductive development, or entry into dauer, by differentially releasing four distinct members of insulin/IGF-like family of growth factors. One of these neurons, the ASI, expresses all four of these neuromodulators, suggesting an ability to tune their release to the specific environmental signals. Yet, the same dense core vesicle (DCV) mechanism is thought to regulate their release. Here, we test this assumption and find that pro-growth IGF-like proteins DAF-28 and INS-4/INS-6, belonging to the same structural class and known to promote reproductive development, use distinct mechanisms for their regulated release from neurons. We find that the while INS-4/INS-6 proteins require the canonical CAPS/UNC-31-dependent DCV release mechanism to signal exit from dauer and return to development, DAF-28 signaling to prevent dauer is UNC-31 independent. We show that, despite UNC-31-independence, DAF-28 localizes to stereotypical peri-synaptic axonal locations, and does utilize the regulated secretion pathway. Furthermore, we find that two commonly used DCV markers in motor neurons, the neuropeptide NLP-21 and the insulin/IGF protein INS-22, while being UNC-31 dependent, diverge in their requirements for the clathrin adaptor AP3/APB-3. Our data show that different insulin/IGF-like growth factors in *C. elegans* neurons are secreted via regulated mechanisms that are divergent in both the initial targeting to the axon and the release mechanism. Furthermore, the chemosensory neurons demonstrate surprising selectivity of the DCV mechanisms by distinguishing between three structurally similar IGF-like proteins. Such selectivity allows the same group of neurons to distinguish between subtly different sensory inputs - in this case continual presence vs. re-appearance of pro-growth signals – and tailor their responses accordingly.

327 Sensory cilia as the Achilles heel of nematodes when attacked by carnivorous mushrooms Ching-Han Lee[1], Han-Wen Chang[1], Ching-Ting Yang[1], Niaz Wali[2], Jiun-Jie Shie[2], *Yen-Ping Hsueh*[1] 1) Institute of Molecular Biology, Academia Sinica; 2) Institute of Chemistry, Academia Sinica.

Abstract
Fungal predatory behavior on nematodes has evolved independently in all major fungal lineages. The basidiomycete oyster mushroom *Pleurotus ostreatus* is a carnivorous fungus that preys on nematodes to supplement its nitrogen intake under nutrient-limiting conditions. Its hyphae can par-alyze nematodes within a few minutes of contact, but the mechanism had remained unclear. We demonstrate that the predator-prey relationship is highly conserved between multiple *Pleurotus* species and a diversity nematodes. To further investigate the cellular and molecular mechanisms underlying rapid nematode paralysis, we conducted genetic screens in *Caenorhabditis elegans* and isolated mutants that became resistant to *P. ostreatus*. We found that paralysis-resistant mutants all harbored loss-of-function mutations in genes required for ciliogenesis, demonstrating that the fungus induced paralysis via the cilia of nematode sensory neurons. Furthermore, we observed that *P. ostreatus* caused excess calcium influx and hyper-contraction of the head and pharyngeal muscle cells, and ultimately resulting in rapid necrosis of the entire nervous system and muscle cells throughout the entire organism. This cilia-dependent predatory mechanism is evolutionarily conserved in *Pristionchus pacificus*, a nematode species estimated to have diverged from *C. elegans* 280-430 million years ago. Thus, *P. ostreatus* exploits a novel nematode-killing mechanism that is distinct from the widely used anthelmintic drugs such as the ivermectin, levamisole, and aldicarb, representing a potential new route for targeting parasitic nematodes in plants, animals, and humans.

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Intracellular Dynamics (*Drosophila*)

335 Adipose mitochondrial metabolism couples nutrients to systemic insulin signaling and growth *Shrivani Pirahas*[1,2,3], Joel Chahal[1,2,3], Erin Thor-son[1,2,3], Michael Turingan[1,2,3], Savraj Grewal[1,2,3] 1) Arnie Charbonneau Cancer Institute, Calgary, AB; 2) Alberta Children's Hospital Research Institute, Calgary, AB; 3) University of Calgary, Calgary, AB.

The larval fat body is central regulator of whole-body physiology and growth in *Drosophila*. For example, in nutrient-rich food, activation of con-served TOR kinase pathway in the fat body promotes endocrine signaling to the brain leading to the release of insulin-like peptides (ILPs) causing increased systemic insulin and body growth. In contrast, in low nutrients, this endocrine insulin signaling is reduced and growth is decreased. An important question is how nutrients control fat body function to mediate these effects on endocrine signaling and growth. Here we describe a role for mitochondrial metabolism in this process. Mitochondria are classically described as bioenergetic organelles that generate ATP through oxida-tive phosphorylation (OxPhos). However, mitochondria also play an important biosynthetic role by providing TCA cycle products as precursors for generation of amino acids, lipids, and nucleotides required for growth. This role is seen in cells and tissues undergoing rapid growth, including the exponential growth that we see in *Drosophila* larvae. We found that in rich nutrients, fat body mitochondria were large with sparse cristae, and

levels of both TFAM, a transcription factor which controls expression of the mitochondrial genome, and OxPhos activity were low. However, when larvae were switched to poor nutrient food, which delayed larval growth and development, TFAM levels and OxPhos activity were increased and fat body mitochondrial ultrastructure became smaller with dense cristae. To explore the significance of this nutrient-dependent change in mitochondrial metabolism we used *RNAi* to knockdown TFAM, which we found lead to reduced OxPhos activity. Interestingly, we saw that fat body specific TFAM *RNAi* caused an acceleration of larval growth and development in rich nutrients, and was sufficient to reverse the delay in development seen in low nutrient conditions. These effects were accompanied by reduced fat body expression of Eiger/TNF-alpha, a negative regulator of brain ILP expression, and Imp-L2, an inhibitor of insulin signaling, and also reduced whole-body expression of FOXO, a forkhead box O transcription factor target genes. These effects are consistent with an overall increase in systemic insulin signaling. In addition, we found that fat body TFAM *RNAi* increased expression of several key glycolytic genes. Moreover, when we promoted fat body glycolysis by either overexpressing lactate dehydrogenase (LDH) or knocking down the mitochondria pyruvate carrier (MPC), we also found that larval development was accelerated. Based on these findings we propose that a mitochondrial-mediated switch in adipose OxPhos vs glycolytic metabolism can couple nutrient availablity to fat-body mediated changes in systemic insulin signaling and growth.

336 Clu bliss particles respond to nutritional regulation in Drosophila germ cells *Kelsey Sheard*[1,2], Aditya Sen[1], Sarah Thibault-Sennett[3], Rachel Cox[1,2] 1) Uniformed Services University, Bethesda, MD; 2) Molecular and Cell Biology Program, Bethesda, MD; 3) Association for Molecular Pathology, Rockville, MD.

Germ cells have evolved a variety of different responses to adapt to environmental fluctuations and stressors including heat shock, oxidative stress, osmotic stress and nutritional stress. A common immediate mechanism for these responses is localization of mRNA to ribonucleoprotein (RNP) granules for post-transcriptional regulation. Upregulation of RNP granules such as stress granules and P-bodies is well-characterized in cellular stress responses, where they affect transport, translation, and stability of mature mRNAs. We have shown that the nucleus-encoded gene *clueless* (*clu*) is directly necessary for properly functioning mitochondria. Drosophila *clu* mutants are sterile and have systemic mitochondrial dysfunctions. Clu is a ribonucleoprotein which physically associates with mRNA, ribosomal subunit proteins, and the mitochondrial membrane transport proteins TOM20 and Porin. Clu exists in female germ cells as large cytoplasmic, mitochondria-associated particles under healthy cellular conditions. However, in contrast to other RNP granules, Clu particles disperse under mitochondrial, oxidative, or nutritional stress. Short periods of starvation cause Clu particle dispersion and refeeding flies causes them to reform, demonstrating the reversible nature of their dynamics. Using live-imaging, we show that Clu particles are highly dynamic and move processively, requiring intact microtubules. In addition, we have found insulin is both necessary and sufficient for their formation. Since there is no corresponding decrease in Clu protein levels after starvation, Clu particles dynamics is not due to protein degradation. Clu particles do not co-localize with many known cytoplasmic bodies, and thus appear to be unique. Given particle dependence on stress-free, well-fed conditions we have named then bliss particles. Our model is that Clu particle formation ties nutritional stress to mitochondrial function and that bliss particles may be sites regulating mRNAs encoding proteins destined for mitochondrial protein import. To further characterize Clu particles as RNP granules, we are currently determining if these particles are sites of mRNA metabolism and mRNA localization in germ cells using live and fixed imaging and whether the presence or absence of Clu particles affects mRNA translation in stressed and unstressed conditions.

337 A rapidly evolving actin-related protein monitors sperm quality in *Drosophila* *Courtney Schroeder*[1], Sarah Tomlin[1], John Valenzuela[1], Harmit Malik[1,2] 1) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute, Seattle, WA.

Cytoskeletal proteins perform many fundamental biological processes in all eukaryotes and are highly conserved among most species. However, we have found that Actin-related proteins (Arps) have undergone recurrent genetic innovation via gene duplication and positive selection (accelerated amino-acid substitutions) in both *Drosophila* and mammals. Unlike the well-conserved members of the Arp superfamily, divergent Arps are testis-specific in expression, yet the function of a male gametic Arp in any species is unknown. Based on their recurrent emergence and rapid evolution, we hypothesized that testis-specific Arps play important roles in sperm production or fitness. To test this hypothesis, we investigated the function of *Arp53D*, a rapidly-evolving testis-specific Arp, in *D. melanogaster. Arp53D* is present in all *Drosophila* species, suggesting it performs a crucial function. Through cytological analyses, we found that Arp53D localizes to two critical germline-specific cytoskeletal structures in the testis: fusomes and actin cones. The fusome is an actin-enriched membranous organelle that connects all germ cells in a cyst in male meiosis. Actin cones are unique structures that separate syncytial spermatids post-meiosis into individual cells and push excess cytoplasm to the end of the sperm flagellar tail. We showed that Arp53D uniquely localizes to the leading edge of actin cones; this localization is dependent on its non-canonical N-terminal tail. Based on these findings, we expected that loss of *Arp53D* would lead to the loss of male fertility. In contrast to this expectation, we found that knockdown of *Arp53D* surprisingly results in increased male fertility. To address why *Drosophila* species would harbor a gene that appears to lower male fertility, we examined the fitness of progeny resulting from *Arp53D*-deficient males. We found that progeny from *Arp53D*-deficient parents have lower reproductive fitness, which is exacerbated under conditions of stress. We hypothesize that Arp53D monitors the quality of sperm. In the absence of Arp53D, poor quality sperm can bypass quality control in the testis, which ultimately leads to unfit progeny. We hypothesize that recurrent specialization of Arps may serve quality-control functions in the male germline of *Drosophila* species.

338 CDK-regulated phase separation seeded by histone genes ensures precise growth and function of Histone Locus Bodies Woonyung Hur[1], James Kemp[2], Marco Tarzia[3], Victoria Deneke[1], Esteban Terzo[2], William Marzluff[2], *Robert Duronio*[2], Stefano Di Talia[1] 1) Duke University, Durham, NC; 2) University of North Carolina, Chapel Hill, NC; 3) Sorbonne Université, Paris, France .

Many membrane-less organelles form through liquid-liquid phase separation, but how their size is controlled and whether size is linked to function remain poorly understood. The Histone Locus Body (HLB) is an evolutionarily conserved nuclear body that regulates the transcription and processing of histone mRNAs. Here, we show using super-resolution light microscopy and quantitative live imaging of *Drosophila* embryos that HLBs are multi-domain structures that form through phase separation. Individual HLB components involved in the transcription or 3' end processing of histone mRNA are found both in distinct, non-overlapping domains as well as in overlapping domains within the HLB of both fly and mammalian cells. One of these domains is enriched in RNA polymerase II and nascent histone transcripts and thus represents the site of histone mRNA synthesis. In the early *Drosophila* embryo, HLB size is controlled in a precise and dynamic manner that is coupled to the cell cycle and zygotic gene activation. Control of HLB growth is achieved by a mechanism integrating nascent mRNAs at the histone locus, which catalyzes phase separation, and the nuclear concentration of the scaffold protein Mxc, which is controlled by the activity of cyclin-dependent kinases. Reduced Cdk2 activity results in smaller HLBs and the appearance of nascent, misprocessed histone mRNAs. Our experiments thus identify a mechanism linking nuclear body growth and size with normal gene expression.

339 Optogenetic dissection of signaling crosstalk in the early embryo *Sarah McFann*[1,2], Sayantan Dutta[1,2], Stanislav Shvartsman[1,2], Jared Toettcher[3] 1) Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 3) Department of Molecular Biology, Princeton University, Princeton, NJ.

Cell fates and movements in the early *Drosophila* embryo are patterned by maternal patterning signals. Some regions receive conflicting signals, like the ventral posterior of the embryo where both dorsoventral and terminal patterning signals are present. Despite receiving both signals, cells in this region adopt a terminal fate rather than a ventral or mixed fate, participating in posterior midgut invagination but not ventral furrow formation. It is known that *wntD* and *huckebein* expression downstream of terminal signaling play a role in suppressing ventral furrow formation, but it is unclear which features of terminal signaling are most important for switching ventral fates to terminal fates. To determine how the timing and duration of terminal signaling affects the switching of cell fates, we optogenetically altered ERK activation in the center of the embryo and monitored gastrulation movements, Dorsal nuclear localization, and the expression of *wntD*, *huckebein*, and *snail*. We identify a time window where ERK signaling is best able to suppress ventral fates and find that a shorter duration of signaling is required to suppress ventral fates than to induce terminal fates. Furthermore, when WntD signaling is hindered and Huckebein alone is left to translate ERK signaling into ventral fate suppression, we find that longer and earlier periods of ERK signaling are required to abolish the ventral furrow. We show that our observations can be summarized compactly using a computational model that accounts for nuclear division and the dynamics of dorsoventral and terminal patterning signals. Our approach, optogenetic stimulation combined with live imaging in wild type and mutant backgrounds, has allowed us to systematically dissect signaling crosstalk, providing a better understanding of how the embryo decodes signaling dynamics and coordinates gastrulation.

340 Regulation of Mitochondrial Network Organization in Muscles *Prasanna Katti*[1], Brain Glancy[1,2] 1) National Heart, Lung, and Blood institute, National Institutes of Health, Bethesda, MD; 2) National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD.

The organization of mitochondrial networks differs depending on muscle type and is important for muscle function. However, the mechanisms that determine mitochondrial organization in the different muscle types remain uncharacterized. To identify the factors that regulate muscle type-specific organization of mitochondrial networks, we used the fibrillar and tubular muscles of *Drosophila melanogaster* as the model system. We found distinct mitochondrial network organization in the fibrillar indirect flight muscles (IFM), the tubular tergal depressor of trochanter (TDT or jump) muscles and leg muscles of Drosophila. The IFMs possess large, parallelly aligned mitochondrial densely packed between the myofibers, similar to mammalian cardiomyocytes. The tubular TDT muscles exhibit thin, long, reticular mitochondria arranged in parallel to the muscle fibers. Interestingly, we show for the first time that there are two types of mitochondrial networks in the tubular leg muscles; parallelly arranged mitochondria and mitochondria in a grid-like arrangement, similar to mammalian oxidative fibers. To identify the regulatory factors, we misexpressed *spalt major* (*salm)*, which regulates fiber-type specificity in Drosophila muscles. *salm* knockdown in the fibrillar IFM caused fiber-type switching to tubular muscles and changed the mitochondrial organization to a grid-like network. Importantly, the mitochondrial organization in the converted IFM resembled that of the tubular leg muscle and not that of the tubular jump muscle. In order to identify the downstream targets that play a role in mitochondrial network organization, we performed high throughput proteomic analysis of the Drosophila muscles following *salm* knockdown and overexpression. One of the factors that showed altered expression in the *salm* knockdown and overexpression conditions was *H15*, the ortholog of vertebrate *Nmr1*. *H15*, along with midline (ortholog of *Nmr2*), is involved in cell fate specification and morphogenesis of the Drosophila heart. However, its role in muscle specification and in mitochondrial organization is unknown. We knocked down *H15* in Drosophila muscles and found that the fibrillar muscles were converted to tubular muscles. Notably, unlike in *salm* knockdown, the mitochondria thin and elongated and remained parallelly arranged following H15 knockdown, similar to those in tubular jump muscles. Moreover, H15 knockdown in the tubular jump and leg muscles resulted in conversion of the parallel mitochondrial alignment into a grid-like pattern, while the muscle fiber type remained tubular. Thus, our findings indicate that while the determination of mitochondrial organization is in the same pathway as the specification of muscle fiber type, players like H15 regulate the mitochondrial network organization independent of fiber type specification, downstream of *salm*.

341 A CSN-SDR-PLIN2 axis regulates lipid droplet size via affecting Brummer ATGL lipase *Xun Huang*[1], Xuefan Zhao[1] 1) IGDB, CAS.

Lipid droplet (LD) buffers the fluctuation of cellular lipids and changes its size and numbers accordingly. The molecular mechanisms underlying these dynamic changes are not fully clear. We discovered that knocking down components of CSN complex, which mediates protein degradation, dramatically increase LD size in *Drosophila* fat body under TAG overloading condition. The levels of several short chain dehydrogenase (SDR) proteins are up-regulated in *CSN* RNAi, and importantly, RNAi of SDR rescues large LD phenotype caused by *CSN* RNAi. The SDR is located in the endoplasmic reticulum (ER) and the elevation of SDR reduces the LD localization of *Drosophila* adipocyte triglyceride lipase (ATGL), Brummer/BMM. Mechanistically, SDR binds to LD-associated protein PLIN2 and its overexpression relocates PLIN2 from peripheral LDs to perinuclear LDs and competes for LD localization of BMM, which facilitates LD growth under TAG overloading condition. Together, these results reveal a CSN-SDR-PLIN2 axis regulates LD size by affecting BMM LD localization.

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Future Visions of Population, Evolutionary, and Quantitative Genetics (PEQG)

346 Announcement of the James F Crow Early Career Researcher Award *Bret Payseur*

abstract is not available at the time of print

347 Selection against archaic DNA in human regulatory regions Natalie Telis[3], Robin Aguilar[1], *Kelley Harris*[1,2,3] 1) University of Washington; 2) Fred Hutchinson Cancer Research Center; 3) Stanford University.

Although traces of archaic hominin DNA persist in the genomes of living humans, these traces have been systematically depleted from genes and other functionally important regions. This depletion suggests that many Neandertal and Denisovan alleles had harmful effects on the fitness of human hybrids, but the nature of these harmful effects is poorly understood. Some archaic alleles may have been neutral or beneficial in the populations where they arose, but have caused harmful epistatic interactions in human hybrids. Any such epistatic effects were likely qualitatively different from the fitness effects of new mutations. Another possibility is that Neandertal and Denisovan alleles were detrimental in their populations of ori-

gin, but drifted to high frequency because of inbreeding in these dying archaic species. In this second case, fitness effects of archaic alleles are more likely to have been qualitatively similar to the effects of new mutations. Motivated by the contrast between these predictions, we measured the depletion of archaic DNA from human enhancers and exons and asked whether the regions most depleted of introgression also showed the strongest evidence selection against new mutations. We found that enhancers as well as exons showed evidence of selection against archaic DNA, suggesting that Neandertal and Denisovan alleles likely dysregulated gene expression in specific human tissues, causing systematic depletion of archaic DNA from enhancer regions annotated by the ENCODE RoadMap project. We also show that enhancers are modestly depleted of common variation that is not of archaic origin, suggesting a pattern of selection against new mutations in regulatory regions as well. These landscapes of constraint have many commonalities; for example, highly pleiotropic enhancers consistently appear more constrained than enhancers that appear active in only a few tissues. In other respects, however, selection against introgression appears qualitatively different from selection against new mutations. Fetal brain and fetal muscle are the tissues most depleted of archaic SNPs in their regulatory regions, but brain enhancers appear to have high deleterious mutation rates and experience stronger selection against new mutations than other enhancers do. In contrast, fetal muscle enhancers show no evidence of high deleterious mutation rates, leading us to hypothesize that their depletion of archaic DNA resulted from divergent selection for higher muscle mass in Neandertals and Denisovans compared to humans.

348 Natural hybridization reveals incompatible alleles causing melanoma in swordtail fish Powell Daniel[1], Garcia Mateo[2], Keegan Mackenzie[3], Rosenthal Gil[2], Schartl Manfred[4], *Molly Schumer*[1] 1) Stanford; 2) Texas A&M University; 3) Northeastern University; 4) University of Wurzberg.

The establishment of reproductive barriers between populations is the key process that fuels the evolution of new species. A genetic framework for this process was proposed decades ago which posits "incompatible" interactions between genes that result in reduced survival or reproduction in hybrids. Despite this foundational work, progress has been slow in identifying individual genes that underlie hybrid incompatibilities, with only a handful known to date. Here, we use a combination of approaches, including naturally occurring hybrid populations, to precisely map the genes that drive the development of a melanoma incompatibility in swordtail fish hybrids. We find that one of the genes involved in this incompatibility also causes melanoma in hybrids between distantly related species. Moreover, we show that this melanoma reduces survival in the wild, likely due to progressive degradation of the fin. The frequency of the alleles underlying melanoma in some hybrid populations suggest possible fitness tradeoffs underlying its maintenance.

349 The role of local adaptation in shaping GxE *Emily Josephs*[1], Robert Williamson[2], Jeremy Berg[3] 1) Michigan State University; 2) Rose-Hulman Institute of Technology; 3) University of Chicago.

Determining the evolutionary processes maintaining variation for quantitative traits has long been a goal of evolutionary biology. Despite numerous examples which show that genotype-by-environment interactions (GxE) are important for the maintenance of variation, we lack a clear understanding of how GxE shapes genetic variation and of the evolutionary processes that shape GxE. Here, we investigate the role that local adaptation for specific plastic responses could play in shaping GxE. We extend Qst-Fst based methods that detect local adaptation in quantitative traits to look for evidence of adaptation for specific environmental responses. In particular, we develop a conditional approach that allows us to tease apart selection on a specific response from correlated evolution between responses to different environments. We then use these methods to identify local adaptation in a plastic response in Arabidopsis thaliana. This work provides both evidence that selection on plastic responses shapes GxE and a method that could be used in additional systems that have genomic data and phenotypes measured in multiple environments.

350 Evolution of the essential gap gene *giant* causes hybrid inviability in *Drosophila* *Daniel Matute*[1], Wenhan Chang[2] 1) University of North Carolina, Chapel Hill; 2) University of CHicago.

It has been widely observed that hybrid organisms—which have parents from different species—are less healthy than pure-species organisms. Hybrid inviability is an extreme example of this. Defects result from incompatibilities between genes that have diverged in the parental species. Few genotype-phenotype relationships are better understood than those of the *Drosophila* embryogenesis genes. This makes them ideal candidates for evolutionary inquiry, though they are widely assumed to be evolutionarily conserved. We identified the *Drosophila melanogaster* allele of the highly conserved and essential gap gene *giant* (*gt*) as a key genetic determinant of hybrid inviability in crosses with *D. santomea*. The presence of this allele in *D. melanogaster/D. santomea* hybrids causes an abdominal ablation not seen in either pure species. *giant* is a key developmental regulator whose role in anterior-posterior specification of the dipteran embryo is conserved over 100 million years. The interaction of *giant* with a second allele, *tailless¾tll¾*is involved in the hybrid defects observed in these *Drosophila* hybrids. These results indicate that even genes with crucial developmental roles that are conserved over vast evolutionary time scales can experience functional evolution that leads to inviability of hybrids.

351 The epistatic norm of reaction *Brandon Ogbunu*[1] 1) Brown University.

New technologies and large data sets have facilitated the study of epistasis at an increasing level of rigor. Among many characteristics, these modern studies have revealed how the magnitude of non-linear effects of mutations are not only driven by the presence/absence of other mutations, but also by environments. These are particularly notable observations when cast in light of provocative topics such as phantom heritability and genetic modification, two topics where the unpredictable, environmentally-modulated effects of mutations are front and center. In this seminar, I impose the structure of the *norm of reaction*—a central abstraction in ecology for summarizing the performance of organisms across sets of environments—on calculations of higher-order epistasis (from *in silico* and biological empirical data sets) to introduce the *epistatic norm of reaction*, which depicts the phenotypic interactions between mutations as a function of varying contexts (genetic and environmental). I will attempt to demonstrate how the *epistatic norm of reaction* applies to different kinds of environmental gradients (discrete, continuous; chemical and genotypic, etc.) and can even communicate how epistasis manifests pleiotropically across related traits.

367 Chromatin and epigenomic variation reveals the gene regulatory landscape of adaptive divergence in sticklebacks Stanley Neufeld[1], Jukka-Pekka Verta[1], Jonas Schwickert[1], Muhua Wang[1], Domenico Scionti[1], Malina John[1], Elena Avdievich[1], *Felicity Jones*[1] 1) Friedrich Miescher Laboratory of Max Planck Society, Tuebingen, Germany.

The adaptation of natural populations to changing environments is often driven by numerous genetic loci predominantly found in non-coding regions with likely gene regulatory roles. Using adaptively diverging marine and freshwater stickleback fish ecotypes as a model, we performed comparative epigenomics, chromatin profiling, transcriptomics and genetics to identify thousands of regulatory elements with divergent epigenom-

ic profiles between the ecotypes. Divergent elements are enriched at the promoter and 5'UTR of genes, are proximal to genes showing differential expression, and vary across tissues, with the liver showing considerably higher regulatory divergence than kidney or gills. Allele-specific analyses in F1 hybrids reveals that divergence in chromatin accessibility is mostly cis-regulated and these elements show molecular signatures of natural selection. Additionally, divergent epigenomic marks cluster into 'islands' of genetic differentiation and low recombination, including chromosomal inversions. We show through functional transgenic assays how these cassettes act as hubs to cause concerted changes in gene expression between adaptively diverging populations. The high resolution maps of the chromatin and epigenomic lanscape in diverging stickleback ecotypes provides functional annotation of regulatory elements within adaptive loci. Our study shows how cis-regulated chromatin variation and epigenomic marks at regulatory elements is associated with adaptive divergence and the early stages of speciation, and links their co-inheritance as adaptive regulatory cassettes to the fast and repeated adaptive radiation of sticklebacks.

Saturday, April 25 4:00 PM - 6:00 PM

Keynote Session 3

364 The generation of neural diversity *Claude Desplan*[1] 1) New York University.

abstract is not available at the time of print

365 Selective interference and the evolution of sex *Sarah Otto*[1] 1) University of British Columbia.

abstract is not available at the time of print

366 Spatial patterning of meiotic recombination *Abby Dernburg*[1] 1) University of CA, Berkeley.

abstract is not available at the time of print

Sunday, April 26 12:00 AM - 11:59 PM

Posters without Q&A 450C The Genomics Education Partnership: Exploring best practices in implementation of a genomics CURE *Matthew Wawersik*[1], Anna Allen[4], Cindy Arrigo[5], Andrew Arsham[6], Daron Barnard[7], Rebecca Burgess[8], Justin DiAngelo[9], Jennifer Jemc[10], Christopher Jones[11], Lisa Kadlec[12], Adam Kleinschmit[13], Mollie Manier[14], Hemlata Mistry[15], Alexis Nagengast[15], Susan Parish[16], Anne Rosenwald[17], Joyce Stamm[18], Sarah Elgin[3], Laura Reed[2] 1) William & Mary, Williamsburg, VA; 2) University of Alabama, Tuscaloosa, AL; 3) Washington University in St. Louis, St. Louis, MO; 4) Howard University, Washington, DC; 5) New Jersey City University, Jersey City, NJ; 6) Bemidji State University, Bemidji, MN; 7) Worcester State University, Worcester, MA; 8) Stevenson University, Owings Mills, MD; 9) Penn State Berks, Reading, PA; 10) Loyola University Chicago, Chicago, IL; 11) Moravian College, Bethlehem, PA; 12) Wilkes University, Wilkes-Barre, PA; 13) University of Dubuque, Dubuque, IA; 14) George Washington University, Washington, DC; 15) Widener University, Chester, PA; 16) McDaniel College, Westminster, MD; 17) Georgetown University, Washington, DC; 18) University of Evansville, Evansville, IN.

Since 2006, the Genomics Education Partnership (GEP) has incorporated authentic genomics research experiences into the undergraduate curriculum, introducing thousands of students to eukaryotic gene annotation, comparative genomics, and the evolution of contrasting genome domains. Our 100+ participating institutions include community colleges as well as primarily undergraduate, minority-serving, and research-intensive PhD-granting institutions. Using our shared resources and publicly-accessible databases, students contribute meaningfully to scientific investigations coordinated through the GEP. A large consortium of faculty implementing a course-based undergraduate research experience (CURE) in a variety of ways also provides opportunities to examine best practices for lab course design and implementation. Through use of faculty logs, assessment of student learning gains, and responses to surveys and focus groups, we have examined faculty actions and student attitudes that impact student learning. The data indicate that our research format encourages use of active learning strategies while promoting dialogue about science; student responses overall are positive, but those students with positive attitudes toward science benefit the most. We also looked for particular aspects of the GEP CURE that promote student learning. Our data suggest that students experience a beneficial process of "formative frustration" where they are initially allowed to fail in their analyses, followed by exploration, re-evaluation, adjustment, and re-analysis. Additionally, the relatively low cost, low stakes structure of a genomics CURE may encourage faculty to let their students experience this formative process. To support the GEP's ongoing mission of conducting genomics research with undergraduates, we have partnered with the developers of Galaxy to create G-On Ramp, a web-based platform for constructing genome browsers from novel genome assemblies. This is enabling us to initiate new collaborative annotation projects that harness the research power of the GEP collective. We are looking for additional "science partners" who have interesting projects for collaboration, and for new faculty members interested in incorporating GEP curriculum and projects into their classroom. We provide fully-supported training for new GEP faculty members through online mentoring and in-person workshops. Those interested in joining can contact us at http://gep.wustl.edu/contact_us. Supported by NSF IUSE-1915544 and NIH IPERT-1R25GM130517-01 to LKR.

459C Integration of Bioinformatics into Life Science Curricula: Community Development, Dissemination, and Assessment of a NIBLSE Learning Resource *Adam Kleinschmit*[1], Rachel Cook[2], Barbara Murdoch[3], Elizabeth Ryder[4], William Tapprich[5] 1) University of Dubuque, Dubuque, IA; 2) Fairmont State University, Fairmont, WV; 3) Eastern Connecticut State University, Willimantic, CT; 4) Worcester Polytechnic Institute, Worcester, MA; 5) University of Nebraska at Omaha, Omaha, NE.

Big data and computational tools have transformed the way we address biological questions. To prepare undergraduates for tomorrow's challenges in the biological sciences, life science curricula should integrate the understanding and use of these tools at all levels. The Network for Integrating Bioinformatics into Life Science Education (NIBLSE) has developed a framework to facilitate achieving this goal. Introductory bioinformatics exercises typically walk students through the use of computational tools, but often provide little understanding of what a tool does "under the hood." A solid understanding of how computational algorithms function, including their limitations, is essential for interpreting the output in a biologically relevant context. Here we describe the development, assessment, and dissemination of an introductory learning resource that focuses on the core concept of sequence similarity and its biological applications, using the NIBLSE framework. The resource was built upon the bioinformatics core competen-

cies developed by NIBLSE. An initial version of the resource was converted into modular format and expanded for a wider audience by a community of faculty operating within the collaborative NIBLSE Incubator model. After multiple pilot rounds of classroom implementation and refinement, a polished version of the resource was published in the journal CourseSource. The resource was further disseminated using the QUBES Faculty Mentoring Network (FMN) model. In addition to implementing the resource into classrooms across the nation, a subset of FMN participants produced adaptations of the original resource that are linked as part of the collection on QUBES. To test the effectiveness of the resource, five institutions piloted an assessment instrument. Matched pre-/post-quiz scores (n=175) showed an improvement of 40% ($p<0.00001$), indicative of objective learning gains in both understanding and utilizing computational tools. A retrospective attitudinal survey indicated that students self-reported perception of competence in performing learning outcomes was also significantly higher ($p<0.0001$), with medians on all questions shifting from negative to positive responses after module completion. This resource and its developmental process serves as an exemplar for the ability of the NIBLSE collaborative model to address barriers for the integration of bioinformatics into undergraduate curricula and harness community intellectual capital.

460A The Network for Integrating Bioinformatics into Life Sciences (NIBLSE): Barriers to integration *Anne Rosenwald*[1], Elizabeth Dinsdale[2], William Morgan[3], Mark Pauley[4], William Tapprich[5], Eric Triplett[6], Jason Williams [7] 1) Georgetown University ; 2) San Diego State University ; 3) College of Wooster ; 4) National Science Foundation; 5) University of Nebraska at Omaha; 6) University of Florida ; 7) Cold Spring Harbor Laboratory .

The Network for Integrating Bioinformatics into Life Sciences Education (NIBLSE) seeks to promote the use of bioinformatics and data science as a way to teach biology to life sciences students in the US. Just as molecular biology revolutionized the way faculty and students viewed biology in the 1980s, we believe the time is ripe for a similar revolution with respect to bioinformatics. Thus far, the network has contributed two significant publications to the literature on bioinformatics education, one detailing a set of bioinformatics core competencies for undergraduate biologists (Wilson Sayres et al., 2018, PLoS One, https://doi.org/10.1371/journal.pone.0196878), and a second documenting barriers to integrating bioinformatics into life sciences education (Williams et al., 2019, PLoS One https://doi.org/10.1371/journal.pone.0224288).

In order to explore the extent to which bioinformatics is currently taught in the US, we conducted a study of US life sciences faculty. A survey was deployed to more than 10,000 life sciences faculty in the US. From the more than 1,200 responses we were able to document the barriers that limit bioinformatics instruction. Faculty training was cited as the most common barrier. Two ways in which NIBLSE is attempting to alleviate this issue is through online training (see the parallel poster by Kleinschmit et al.) and the development of a collection of bioinformatics learning resources (see the parallel poster by Morgan et al.). In addition to lack of faculty training in the discipline, there were a number of other interesting findings that we plan to explore more thoroughly, including the observation that junior faculty members, who have the most formal training in bioinformatics, teach bioinformatics less frequently than more senior faculty. In addition, faculty who are members of recognized minorities themselves report barriers at higher frequencies than majority faculty. We are currently conducting focused interviews with faculty, chairs, and administrators based on these findings in efforts to develop strategies to alleviate these barriers.

470B The Triad Approach: Stimulating learning in the undergraduate classroom *Melissa Eslinger*[1] 1) US Military Academy.

National education efforts emphasize the need to practice the process skills of science rather than strictly focus on discipline-specific content. Developing these skills early within undergraduate education promotes cognitive progression and conceptual application; however, they require deliberate cultivation, particularly with early-stage scientists. We developed the Triad Approach for use in the genetics classroom as a departure from traditional lecture. The Triad leverages active-learning through iterative cycling of lectures, journal clubs, and case studies on specific genetics course related themes. Thus, students observe, analyze, and apply content three times from unique perspectives, resonating with multiple learning styles. Students were directly assessed on comprehensive problem sets, analysis of case studies, and primary literature interpretations. Direct and indirect measures, self-assessments and student surveys, suggest a marked improvement in learning and scientific literacy. Students' averaged 0.55 normalized gains in quantitative knowledge (modeling); 0.33 gains in critical thinking; 0.40 quantitative reasoning; and a 0.63 gain in analytical genetic skills (-omics). Similarly, comparison of cumulative pre-course GPA to overall course performance identified students who over or under performed in the Triad Approach compared to traditional learning strategies. Notably, compared to students across the entire university, we see higher reported means for critical thinking and learning. Together, we present the Triad Approach as an alternative teaching strategy, which leverages contemporary tools, to stimulate student learning and skill development.

472A Characterizing the soil microbiome in the James River Park in Richmond, VA: a research based instructional design *Dianne Jennings*[1], Fernando Tenjo[1] 1) Virginia Commonwealth University, Richmond, VA.

The soil microbiome plays a vital role in the species composition and richness of plant communities in various ecosystems. (Van der Putten, 2017). A small amount of soil may contain thousands of microbial species that can drive plant community diversity via plant-microbial interactions (Bardgett and Van der Putten, 2014; O' Brien et al., 2005). A research based instructional design (REIL) was implemented to characterize the fungal community from samples obtained at the James River State Park System in Richmond, VA. This course is an introductory laboratory class for first-semester transfer students. One of our goals was to determine the feasibility of implementing a reliable molecular approach to identify fungi using DNA barcoding using the ITS rRNA region (O'Brien et al., 2005). In addition to student generated data, preliminary data was collected to assess the knowledge and technical skills that students gained during the course. Data that students generated, data on student knowledge and skills gains and potential research questions that students can generate based on this REIL instructional design will be discussed.

478A Structured Discovery: Learning about Gene Structure and Function through Analysis of *Arabidopsis* Genes *Andrew Woodward*[1,2] 1) University of Mary Hardin-Baylor, Belton, TX; 2) Rice University, Houston, TX.

Undergraduate biology majors must memorize basic aspects of gene structure and function. However, students often have a shallow understanding of basic gene and protein features. I developed activities in which students analyze DNA and protein sequences to look for patterns. Students use NCBI and The *Arabidopsis* Information Resource (TAIR) to search for sequences. Students summarize intron/exon boundaries in an *Arabidopsis* gene to look for patterns. In their own words, students write an apparent rule for intron/exon boundary sequences based on data from one large gene. Then, they examine other genes from *Arabidopsis* and other creatures to determine whether their rule seems generally correct. Next, students read about snRNP complexes to learn how the splicing process relates to the sequence rule they devised. In a related protein module, students align protein sequences and make hypotheses about active-site and mutant amino acids. Finally, they observe the three-dimensional shape of the protein using UCSF Chimera software, and they evaluate the accuracy of their hypotheses. Through data collection, hypothesis development, and model

refinement, these *in silico* activities offer students a deeper experience of gene structure and function than is often possible in the classroom.

479B Active Learning in Medical School: A Comparison of Approaches for Interdisciplinary Teaching of Genetics and Pharmacology *Linda Siracusa*[1], Vicki Coffin[1] 1) Hackensack Meridian School of Medicine at Seton Hall University.

Active learning is an approach to instruction that helps students stay engaged during class by applying their knowledge for higher order thinking and problem solving. Studies have shown that an outcome of active learning is increased retention of knowledge compared to traditional lectures. The Liaison Committee on Medical Education (LCME) has embraced active learning and medical schools across the United States are changing their curriculum to incorporate different forms of active learning for all (or some percentage) of total classroom time for pre-clinical materials. To work successfully, active learning requires that students: 1) study materials by learning *de novo* for the session, or 2) recall knowledge learned previously in earlier sessions, or 3) a combination of both studying *de novo* and recall. When problem solving occurs in groups, students have the added benefit of brainstorming together to solve problems while teaching each other. We delivered a two-hour session entitled "Targeted Cancer Therapies"; this topic covers cutting-edge concepts and essential principles of interdisciplinary work in genetics and pharmacology. We designed and compiled pre-work to be read prior to class that jointly integrated knowledge from these disciplines. The "Targeted Cancer Therapies" session was taught by two methods: 1) standard Team-Based Learning (TBL), and 2) mini-lectures interspersed with group problem solving, called Large Group Active Learning (LGAL). The multiple choice and application questions posed to medical students were similar for both sessions, regardless of the methodology used in the classroom. A significant difference between these methods was that for TBL, resulting scores counted towards students' final grades whereas for LGAL, answering questions successfully in class was its own reward. We present highlights of the materials used and a compilation of students' comments as well as faculty conclusions from comparison of these active learning strategies.

490A Detection of genetic manipulation in thoroughbred racehorses – a new frontier in doping control Jillian Maniego[1], James Scarth[1], *Edward Ryder*[1] 1) LGC, Fordham, UK.

Throughout the history of horse racing, doping techniques used to suppress or enhance performance have expanded to match the technology available. Examples include simple chemicals such as caffeine, to misuse of steroids and peptides used in human and veterinary medicine. The next frontier in doping, both in the equine and human sports areas, is predicted to be genetic manipulation, due to recent advances in genome editing and gene therapy.
As part of a new research programme recently established at LGC Sport and Specialised Analytical Services, in conjunction with the British Horseracing Authority, we are investigating methods in which prohibited and unethical genetic manipulation in racehorses can be detected. This includes the direct and heritable alteration of genes implicated in performance, or inappropriate use of gene therapy to reduce recovery time after injury. We highlight here our research to identify the presence of exogenous transgenes by use of automated assay design, PCR and next generation sequencing.
Detection of such manipulation will not only ensure fairness in racing, but also act as a deterrent against initialising such activities, contributing to the health status and continued welfare of the animals.
This work is funded by the British Horseracing Authority

498C Wrapping culture plates with Parafilm M® increases *Caenorhabditis elegans* growth *Jessica Shinn-Thomas*[1], Sara Scanga[1], Patrick Spica[1], Hardik Nariya[1], Emra Klempic[2], Mary Brockett[3] 1) Utica College, Utica, NY; 2) University of Rochester Medical Center, Rochester, NY ; 3) Uniformed Services University, Bethesda, MD.

Parafilm M® is a moisture-resistant thermoplastic commonly used to seal agar media plates for a variety of model organisms. Minimal research has evaluated the effects Parafilm wrapping could have on these organisms. Parafilm is used to seal Nematode Growth Media (NGM) agar plates on which *Caenorhabditis elegans* is cultured to reduce media dehydration and microbial contamination. For *C. elegans* individuals, the effects of placing this barrier between the external environment and the interior of the NGM plate are currently unknown. Our research aims to determine if this common practice engenders developmental changes, such as growth, that could subsequently and unintentionally alter experimental data. We compared the larval growth over 48 hours of animals cultured on Parafilm-wrapped and unwrapped control NGM plates.

Wrapping culture plates with Parafilm significantly accelerated and increased larval growth. Recent research in *Arabidopsis thaliana* cultures also demonstrated that growth was affected by Parafilm wrapping and gas exchange may be affected as well (Banerjee *et al.,* 2019, PLOS ONE). Therefore, investigators of all organisms should be aware that wrapping their experimental cultures with Parafilm may result in statistically detectable changes, such as in growth and possibly other developmental processes (adapted from Shinn-Thomas *et al.,* Submitted to *BMC Research Notes)*.

509B Large-scale transgenic *Drosophila* resource collections for loss- and gain-of-function studies *Jonathan Zirin*[1], Yanhui Hu[1], Luping Liu[1], Donghui Yang-Zhou[1], Ryan Colbeth[1], Dong Yan[2], Ben Ewen-Campen[1], Eric Vogt[1], Sara VanNest[1], Cooper Cavers[1], Christians Villalta[1], Aram Comjean[1], Jin Sun[5], Xia Wang[5], Yu Jia[5], Ruiba Zhu[5], Pin Peng[5], Jinchao Yu[5], Da Shen[5], Yuhao Qiu[5], Henna Ragoowansi[1], Ethan Fenton[1], Senait Ephrem[1], Annette Parks[3], Kuniaki Saito[4], Shu Kondo[4], Liz Perkins[1], Stephanie Mohr[1], Jianquan Ni[5], Norbert Perrimon[1,6] 1) Harvard Medical School, Boston, Massachusetts, USA; 2) Chinese Academy of Sciences, Shanghai, China; 3) Bloomington Drosophila Stock Center, Bloomington, Indiana; 4) National Institute of Genetics, Mishima, Shizuoka, Japan; 5) Tsinghua University, Beijing, China; 6) Howard Hughes Medical Institute, Boston, Massachusetts.

The Transgenic RNAi Project (TRiP), a *Drosophila* functional genomics platform at Harvard Medical School, was initiated in 2008 to generate and distribute a genome-scale collection of RNAi fly stocks. To date, the TRiP has generated >15,000 RNAi fly stocks. As this covers most *Drosophila* genes, we have largely transitioned to development of new resources based on CRISPR technology. Here, we present an update on our libraries of publicly available RNAi and CRISPR fly stocks focusing on the TRiP-CRISPR knockout (TRiP-KO) and TRiP-CRISPR overexpression (TRiP-OE) collections. TRiP-KO stocks express one or two sgRNAs targeting the coding sequence of a gene or genes, allowing for generation of indels in both germline and somatic tissue. TRiP-OE stocks express sgRNAs targeting upstream of a gene transcription start site. Gene activation is triggered by co-expression of catalytically dead Cas9 (dCas9) fused to an activator domain, either VP64-p65-Rta (VPR) or Synergistic Activation Mediator (SAM). We show that these reagents are potent and specific activators of target gene expression *in vivo* and describe the libraries of TRiP-OE and TRiP-KO sgRNA stocks we have generated. These resources provide versatile, transformative tools for gene activation, gene repression, and genome engineering.

515B Engineering biosafe gene drives in *Drosophila suzukii* for population suppression *Amarish Yadav*[1], Fang Li[1], Esther Belikoff[1], Maxwell Scott[1] 1)

North Carolina State University, Raleigh, NC, USA.

Drosophila suzukii, also known as spotted wing drosophila (SWD), is an invasive pest of soft skinned fruits like peach, cherries, blueberries, and strawberries etc. It is largely controlled through multiple annual applications of broad-spectrum insecticides. We have been developing species-specific genetic methods for control of this pest include Cas9-based homing gene drive. Firstly, to develop a split gene drive system, we made transgenic lines that express Cas9 in the germline and evaluated by crossing with strains that express gRNAs for *white* and *Sex lethal*. The crosses with *white* gRNA produced male offspring with white eyes. Female offspring with variegated eyes were obtained from crosses with vasa-Cas9 but not nos-Cas9 lines, indicating significant somatic expression of Cas9 from the *vasa* promoter. From the crosses of *Sxl* gRNA and *vasa*-Cas9 lines, female offspring had deformed ovaries and ovipositors. From crosses between nanos-Cas9 and Sxl-gRNA lines, all female offspring were sterile. Upon dissection and examination by confocal microscopy, the ovaries revealed the development of small ovaries filled with a large number of cells, similar to the "bag of marbles" phenotype described in *D. melanogaster*. Therefore, this study suggested a conserved function for *Sxl* in female germline and the suitability of these Cas9 lines to be used in homing gene drive constructs evaluation. Homing constructs have been made that target genes required for female development or fertility such as *doublesex*. The potential for these homing systems for genetic suppression will be evaluated in small cage experiments.

521B Single cell transcriptomic atlas of *Drosophila* oogenesis *Deeptiman Chatterjee*[1], Allison Jevitt[2], Taylor Otwell[2], Xian-Feng Wang[1], Gengqiang Xie[2], Yi-Chun Huang[1], Wu-Min Deng[1,2] 1) Tulane University; 2) Florida State University.

Oogenesis is a complex developmental process that involves spatiotemporally-regulated coordination between the germline and supporting somatic-cell populations. This process has been modelled extensively using *Drosophila* ovary. While different ovarian cell types have been identified through traditional means, the underlying fate-specific transcriptional signatures and how they change over time are unknown. In this study, we have used single-cell RNA sequencing to survey the expression profiles of cells from the adult *Drosophila* ovary and assign transcriptomic identity to known cell types. We have described a cost-effective protocol to build a high-quality dataset without requiring expensive replication. The key to this approach is our all-inclusive tissue sampling method which enabled us to remove multiplets and ambient RNA contaminants, that goes beyond the practices described in conventional quality-control pipelines. This previously unreported approach allowed us to recover high-quality cells through removing contaminants with conflicting marker expression and experimentally validating the identity of clusters using new markers. We have further used this approach to observe the transcriptional changes that occur in the follicular epithelia upon disruption of apicobasal cell polarity which causes neoplastic overgrowth. Altogether, our findings provide a broad perspective of oogenesis at a single-cell resolution, while also revealing new transcriptional programs in less-studied tissues and transcriptomic convergence among and between distant tissues.

527B The 'etc' in the GAL4 etc QuickSearch tab: using Experimental tools to find everything *Sian Gramates*[1], Gillian Millburn[2], Victor Strelets[3], Josh Goodman[3], Jim Thurmond[3] 1) Harvard University, Cambridge MA; 2) Cambridge University, Cambridge UK; 3) Indiana University, Bloominton IN.

Drosophila melanogaster has an exceptionally rich and ever-growing genetic toolkit, making it a powerful model organism in which to address a wide range of biological questions. Many of these genetic tools are expressed in specific temporal-spatial patterns. Several years ago, FlyBase introduced the 'GAL4 etc' QuickSearch tab, allowing our users to search for a defined set of transgenic binary drivers (GAL4, QF, and lexA) and non-binary reporters (lacZ and GFP) by expression pattern. We have recently incorporated the Experimental Tools module into the 'GAL4 etc' QS hitlist. This enhancement supports filtering the results of an expression pattern search to select any pattern-specific genetic tool with a desired set of features, including split drivers, fluorescent tags, regulatory regions, or conditional expression.

535A Introducing GenFisher, a rapid, effective and uniform target enrichment technology, enabled by semi-conductor technologies and novel chemistry Long Fan[1], Jianpeng Wang[1], Lumeng Ye[1], Jiashi Wang[2], *Irene Song*[2], Cedric Wu[1,2] 1) GenScript Inc., Nanjing; 2) GenScript Inc., Piscataway.

Next-gen sequencing (NGS) has been instrumental to empowering new scientific discoveries and clinical diagnostic technologies. Despite declining sequencing costs, whole genome sequencing for a desirable coverage depth remains prohibitive. Targeted sequencing therefore has continued to gain popularity as more applications benefit more from higher sequencing depths than from broader genome coverage, such as detecting low frequency disease alleles, tracking minimal residual diseases, etc. Here we present a novel target enrichment technology, the GenFisher capture probes and the associated kit, a hybrid capture method at its core. Uniquely, GenFisher capture probes, 120bp biotinylated dsDNA, are created by using proprietary semi-conductor driven DNA synthesis technology. Computer-controlled electrodes on the array chip are able to electrochemically synthesize DNA rapidly, evenly, consistently and scale-agnostic. >20 GenFisher probe panels, designed for human, mouse, fly and bacteria genomes, have been generated to date with target sizes ranging from 50K to 6M bp. The production time of these panels averaged at 2 weeks from design to receiving, significantly faster than conventional methods of making capture probe pools, whose turnaround time scales with probe numbers. To evaluate the effectiveness of GenFisher technology, whole-genome NGS libraries were prepared by a commercially available kit with TruSeq style adapters, with various but common sample index lengths of 6nt, 8nt and 10nt. For each capture evaluation, 500 ng of total library was used, with which two other widely accepted capture solutions were also tested in parallel according to their respective manual. We compared key targeted-NGS metrics, namely on-target base %, average target coverage and its uniformity, target dropout %, duplicate ratio, and multiplexing capacity. GenFisher consistently produced superior results at different panel size levels. For example, a 100 human oncogene GenFisher panel consisting of ~8,000 probes was able to consistently deliver >90% on-target rate, >99% bases at 30X coverage depth with only 4 million subsampled reads with 0 dropout and 16-plex capture ability without impacting NGS results. Additionally, proprietarily designed GenFisher universal blocker, part of the GenFisher, was the only true "universal" solution that efficiently blocked off-target binding for all index lengths, yielding >80% captured reads on-target, whereas all others require a separate blocker mix.

537C Novel and truly universal hybrid capture blockers solution enables greater targeted sequencing throughput Jianpeng Wang[1], Long Fan[1], Lumeng Ye[1], *Jiashi Wang*[2], Irene Song[2], Cedric Wu Wu[1,2] 1) GenScript Inc., Nanjing; 2) GenScript Inc., Piscataway.

Hybrid capture is a popular and widely accepted method to select targeted genomic regions for subsequent next generation sequencing (NGS). Though getting increasing utility in research and clinical diagnostics, hybrid capture NGS is challenged by the rapid adoption of the NovaSeq, Illumina's flagship platform. NovaSeq creates up to terabytes of DNA information per run, allowing unprecedented level of sample multiplexing, especially for targeted NGS. To enable the maximum pooling potential of targeted NGS libraries, here we introduce a novel design and chemistry to hybrid capture blockers, an essential component in the experimental workflow to block the permissive interactions between library adapters and target

probes, and adapters themselves, both of which lead to wasteful sequencing. Selection of conventional blockers, whose sample index-matching portion uses either degenerate bases or universal pairing bases, depends on the length of the sample index for the libraries. For example, if an NGS library has 8nt-index, the blockers for its hybrid capture need to have a matching 8nt stretch, dramatically impeding the flexibility of index-blockers pairing. Our new invention, GenFisher Universal Blockers, create a novel solution to the duplexing between sample indexes on libraries and blockers, enabling blockers to be agnostic to the index length. NGS libraries are created with a commercially available kit and TruSeq style adapters that contain sample indexes with 3 different lengths, 6ng, 8nt and 10nt. Hybrid captures were then carried out with GenFisher Hybridization and Wash kit, Universal Blockers and a custom 8,000-probe GenFisher panel, for each library individually and for combined libraries with different indexes. Sequencing data showed that the on-target base percentage was consistent at >75% for all captures and libraries, whereas hybrid capture performed without blockers yielded <30% on-target. We compared GenFisher Universal Blockers to the blockers from two other providers, which are also claimed to be 'universal', using each provider's recommended reagents and protocol. Our data showed that GenFisher Universal Blockers was the only truly universal solution that worked with all 3 lengths while the other two struggled to work with 10nt indexes (on-target ~50%, compared to >65% with 6/8nt). With its versatility, GenFisher Universal Blockers permit higher multiplexing capacity, as well as optimized index selection freedom to hybrid capture NGS users.

539B The personal genome: everyone has one *Adelaide Rhodes*[1], Matthew Bolden[1], Sunnie Southern[1] 1) Onix Health & Life Sciences.

The advent of the "personal genome" is revolutionizing how we derive medical insight from whole-genome DNA sequencing for individuals. Sequencing millions of personal genomes integrated with phenotype (observable (BMI) versus inherited (eye color) characteristics) and personal medical data creates data storage and analytical challenges on an unprecedented scale. Researchers are developing a new generation of algorithms to overcome limitations of approaches that rely on mapping sequences to one consensus human reference genome, which is not a "one-size-fits-all" solution to medical research, as it was built from few individuals - resulting in missing data and large discrepancies when applied to diverse populations. Having reference genomes that are customized for ancestry, phenotype and disease state can provide more specific insights that lead to effective treatments. The ability to store and analyze multiple reference genomes on the cloud will be a fundamental building block in this new research paradigm.

This demonstration takes attendees on a journey from the familiar to the possible by deep-diving into some of the latest tools available in the cloud stratosphere to support data-driven discovery for "personal genomics" by optimizing the use of new and existing analytics tools for researchers. This demonstration provides best practices to get the most out of databases for genomics applications and simple ML to examine cohorts and populations using pan-genomes, a compilation of a representative genome for a given population. We'll also explore the potential of AI and ML high-throughput data analysis tools to speed up research insights from personal genomes.

550A Development of CRISPR knockout screening in mosquito cell lines *Raghuvir Viswanatha*[1], Enzo Mameli[1,2], Jonathan Rodiger[1], Yanhui Hu[1], Nobert Perrimon[1,3] 1) Dept. of Genetics, Harvard Medical School, Boston, MA; 2) Dept. of Microbiology, Boston University School of Medicine, Boston, MA; 3) Howard Hughes Medical Institute, Boston, MA.

In a pooled-format genetic screen in cultured cells, each cell receives one reagent (e.g. CRISPR sgRNA) at random from a complex library of reagents. We previously developed a delivery method for pooled reagents compatible with insect cells, and used this approach to conduct the first genome-wide CRISPR knockout screens in *Drosophila* cells, identifying essential genes and new components of the ecdysone response pathway. We now present evidence that similar approaches can be applied to mosquito cells, in particular from species that are vectors of human disease in which genome-wide perturbation platforms are currently unavailable. We first identified optimal U6 promoters and confirmed that we can observe CRISPR knockout-induced phenotypes in diverse mosquito cell lines. We next created an online portal for semi-automated design of focused CRISPR libraries for two mosquito species. Finally, we modified *Anopheles gambiae* Sua-5B cells for our recombination-based screening approach and used the modified cell line and focused libraries to perform proof-of-concept CRISPR knockout screens. The results presented demonstrate the wide applicability of pooled screening in insect cells. Future directions will include genome-wide fitness, host-virus, and drug interaction screens in mosquito cell lines.

552C Deciphering glial evolution: genetic and functional characterization of ancestral glia *Larisa Sheloukhova*[1], Hiroshi Watanabe[1] 1) Okinawa Institute of Science and Technology (OIST).

Central nervous systems of bilaterian animals generally consist of two cell types – neurons and glial cells. Glia participates in almost every process taking place in the nervous system of bilaterian animals. Therefore, tracing back the first glia and elucidating its ancestral function is important for understanding the evolution of the nervous system. The ultimate goal of this study is to understand the evolutionary processes of glial cells at the molecular level. Histological examinations have not so far revealed any morphological sign of glial cells in *Cnidaria*, the closest outgroup to *Bilateria*. It is thus believed that glial cells appeared after the common bilaterian ancestor had branched off from *Cnidaria*. However, this view has not been examined well at the genetic level. Therefore, we used *Nematostella vectensis* (*Cnidaria*), an emerging cnidarian model organism amenable to gene function analysis, to identify evolutionarily conserved glial gene sets and explore their functions. According to our analysis, *GCM* (glial cells missing) is the most conserved glial transcription factor (TF) that may have a pivotal and evolutionary conserved function to instruct cells to become glial-like. Based on the preliminary results obtained from *GCM* expression and knockdown experiments, it is not clear if the function of *GCM* in *Nematostella* is to guide neuronal differentiation or control the expression of glial functional genes, or both. Thus, we performed genome-wide transcriptomic analysis of *GCM* knockdown animals to identify its target genes and pathways. Further experiments focused on functional analysis of *GCM* target genes will clarify conserved functions of *GCM.* Morphological assessment of *GCM*-expressing cells will help identify if these cells are indeed glial-like. This study is the first to challenge the dogma of glial absence in *Cnidaria*. With omics data and molecular techniques in non-model and phylogenetically privileged animal lineages such as *Cnidaria*, it is now possible to experimentally address this important biological question at the molecular level.

556A Enhancing the sensitivity and power of transcript detection across species: A customizable, selective, and precise approach for the removal of abundant RNA species *Kaylinnette Pinet*[1], Deyra Rodríguez[1], Bradley Langhorst[1], Keerthana Krishnan[1], Lynne Apone[1], Fiona Stewart[1], Eileen Dimalanta[1], Theodore Davis[1] 1) New England Biolabs, Ipswich, MA.

RNA-seq technology has been pushed to extremes of very low and degraded sample inputs but still battles with the challenge of having a large

dynamic range of transcript expression. Highly expressed transcripts with minimal biological interest can dominate readouts, masking detection of more informative lower abundant transcripts. Here, we present a customizable approach to enrich for RNAs of interest by eliminating highly abundant and unwanted RNAs. This method is based on hybridization of probes to targeted RNA and subsequent enzymatic degradation of the selected RNAs.

We developed a user-friendly web tool to enable custom probe design for targeted RNA depletion. The custom probe sequences confer RNA removal specificity and can be designed to deplete unwanted RNA from any organism. Furthermore, these probes can be designed to target RNA in single species, multiple species, or to supplement existing probe sets. We used this web tool and depletion method to remove rRNA from total RNA of various species, such as mosquitoes (*Aedes aegypti*) and archaebacteria (*Thermococcus kodakarensis* and *Pyrococcus furiosus*). Additionally, we used this approach to target highly abundant coding RNAs in human total RNA, as a supplement to an existing anti-rRNA probe set, to achieve depletion of both rRNA and the selected coding RNAs.

Using strand-specific RNA sequencing we measured depletion efficiency and transcript expression. We achieved high depletion efficiency (up to 99%) for all targeted RNAs across species, while maintaining transcript abundance of non-targeted RNA. This translated into an enrichment of RNAs of interest and an increased depth of sequencing coverage.

The method and tool described here are a simple and reliable solution that greatly improves the sensitivity and power of RNA-seq studies. Importantly, the targeted RNA depletion and subsequent library construction method are amenable to high throughput sample preparation.

583A The Development of a Yeast-based Paper Analytical Device (Bio PAD) for the Detection of Prednisone and Prednisolone in Nepali Pharmaceutical Samples *Don Paetkau*[1], Jennifer Hartman[1], Heather Shepherd[1,2] 1) Saint Mary's College; 2) University of Note Dame.

The sale of substandard and falsified medical products, including allopathic medicines, is a worldwide problem. Ethnobotanical, traditional, or ayurvedic medicines can also be falsified by the addition of allopathic pharmaceuticals, such as steroid hormones. Cost-effective, easy to use, scalable detection methods must be developed. To address the specific problem of monitoring prednisone/prednisolone presence in allopathic and ayurvedic medicines, a *Saccharomyces cerevisiae* biological sensor was created by modifying an existing tetracycline-responsive promoter/*LacZ* reporter gene system (AH109::pCM176). The two new yeast strains consisted of a sensor (a glucocorticoid binding receptor/transcription factor) and a blue-color indicator (a glucocorticoid receptor responsive promoter connected to either a *LacZ* or *MEL1* reporter gene) incorporated into CML282 yeast cells. Yeast-based Paper Analytical Devices (Bio PADs) were created by resuspending the two yeast strains separately in a sodium algenate/trehalose hydrogel and spotting onto paper. Prednisone standard was detected at a concentration of 3.5mg/ml (28 mg total). In order to test the use of the Bio PADs in the field, 128 ayurvedic and 152 allopathic pharmaceuticals were collected in four different regions of Nepal following the World Health Organization (WHO) standard of at least 30 units of medicine per region. Liquid Chromatography-Mass Spectrometry (LC-MS) and Raman spectrometry suggested that a number of the allopathic medicines lie outside the US standards (<90% or >110% of marketed active pharmaceutical ingredient), indicating the samples obtained can be used to test the effective real-world detection limits of the Bio PAD in the field. Challenges of working in resource limited conditions indicated the need for improvements in Bio PAD loading and storage. These improvements are being incorporated into the newest versions of these devices, which could then be used as a first-line inexpensive screening method to direct evidence-based focused screening with more expensive devices in resource limited settings.

584B SGD and the Alliance of Genome Resources Stacia R. Engel[1], Edith D. Wong[1], Robert S. Nash[1], Felix Gondwe[1], Patrick Ng[1], Suzi Aleksander[1], Stuart Miyasato[1], J. Michael Cherry[1] 1) Stanford University.

The yeast research community has long enjoyed the support provided by the *Saccharomyces* Genome Database (SGD), and has flourished because of its existence, making great breakthroughs and technological advances, and contributing countless key insights to the fields of genetics and genomics over the past decades. SGD has recently joined forces with five other model organism databases (MODs) - WormBase, FlyBase, ZFIN, RGD, and MGI - plus the Gene Ontology Consortium (GOC) to form the Alliance of Genome Resources (the Alliance; alliancegenome.org). The Alliance website integrates expertly-curated information on model organisms and the functioning of cellular systems, and enables unified access to comparative genomics and genetics data, facilitating cross-species analyses. The site is undergoing rapid development as we work to harmonize various datatypes across the various organisms. Explore your favorite genes in the Alliance to find information regarding orthology sets, gene function, mutant phenotypes, alleles, disease associations, gene expression and more!

585C *Saccharomyces* Genome Database (SGD) through the years. *Joanna Argasinska*[1], Suzi Aleksander[1], Micheal Alexander[1], Barbara Dunn[1], Felix Gondwe[1], Sage Hellerstedt[1], Sagar Jha[1], Kalpana Karra[1], Stuart Miyasto[1], Robert Nash[1], Patrick Ng[1], Matt Simison[1], Marek Skrzypek[1], Shuai Weng[1], Edith Wong[1], Stacia Engel[1], Michael Cherry[1] 1) Stanford University.

Since 1993, the *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) has been supporting the yeast research community, serving as a comprehensive resource for biological information related to budding yeast. Recently, the database formed the Alliance of Genome Resources with five other major model organism databases in order to create a mega-resource to help understand the genetic and genomic bases of human biology and disease. Here we present the evolution of SGD from its beginnings in 1993, through the times of the completion of the first complete eukaryotic sequence, expression DNA microarray assays, synthetic lethal screens, genomics and epigenomics, and into the future. SGD has a long history of service to yeast researchers and to the broader genetics community as a whole. Throughout its existence, SGD has maintained its high level of dedication to quality and remains the primary annotation resource for *S. cerevisiae*. We continue in our mission of educating students, enabling bench researchers, and facilitating scientific discovery.

586A The Use of the Gene Ontology to Describe Biological Function at *Saccharomyces* Genome Database Suzanne A. Aleksander[1], Stacia R. Engel[1], Kevin A. MacPherson[1], Robert S. Nash[1], Patrick Ng[1], Marek S. Skrzypek[1], Edith D. Wong[1], J. Michael Cherry[1] 1) Stanford University.

The *Saccharomyces* Genome Database (SGD) is a comprehensive resource of curated molecular and genetic information on the genes and proteins of *Saccharomyces cerevisiae*. Since 2001, SGD has used the Gene Ontology (GO) to annotate the functions of gene products in budding yeast. The GO comprises three sets of structured vocabularies, or “ontologies”: the Molecular Function ontology describes activities of gene products; the Biological Process ontology places these molecular functions in a biological context; and the Cellular Component ontology indicates the subcellular

localizations of gene products. Expert curators select GO terms to apply to gene products based on published scientific literature. At SGD, results from traditional experimental methods are the primary sources of evidence used to support GO annotations. In addition, results from comparative sequence and genomic studies, as well as analyses of functional genomic and proteomic data, have provided valuable insights into the biological roles of gene products, and these data are incorporated into SGD as well. SGD has several web interfaces and analysis tools that display and use these data. The **Locus Summary** briefly lists each GO annotation. The **GO Term Finder** aids in discovery of potential gene similarities. The **GO Slim Mapper** maps annotations of a group of genes to more general terms and/or bins them into broad categories, also known as "GO Slim" terms. Gene Ontology annotations are also incorporated into **YeastMine**, SGD's multifaceted search and retrieval environment that provides access to diverse data types. These interfaces and tools are important as part of SGD's ongoing mission facilitate research, education, and discovery using the Gene Ontology.

587B Yeast-Human Cross-Species Complementation and Associations with Disease-related Genes Robert Nash[1], Stacia Engel[1], Kevin MacPherson[2], Kalpana Karra[1], Edith Wong[1], J. Michael Cherry[1] 1) Stanford University; 2) OHSU.

The Saccharomyces Genome Database (SGD; http://www.yeastgenome.org) is a comprehensive resource for curated, molecular and genetic information on the genes and proteins of *S. cerevisiae*. Model organism genetics holds great promise for advancing our understanding of human gene function and involvement in disease. Elucidating the biology of yeast genes has in many cases provided valuable insight into the function of their homologous human counterparts. With the goal of making connections between yeast genes, human homologs and associated diseases, we have undertaken a project to collect and display this information at SGD.

At the start of this project, yeast-human cross-species functional complementation results were collected from the literature and stored in the YeastMine data warehouse where the data can be accessed using preformed template queries. Relevant information was also added to the respective Locus Summary Page descriptions. These functional complementation relationships including some where genes also share homology and the corresponding relationship types have been stored in the database and are now displayed on SGD pages. A subset of these human homologs have been determined to be disease associated. For this subset, the corresponding disease ontology (DO) terms were identified and associated with both the human gene and the corresponding yeast homolog, along with supporting information. Diseases associated with human genes that have a computationally determined yeast homolog have also been included in this set. Disease pages have been designed that include the following pieces of information: disease name, ID and definition from DO, yeast systematic and ORF names, human HGNC-approved gene names (https://www.genenames.org), annotation type (manual vs HTP), evidence code, reference, source and relevant links. A disease summary that has been generated at SGD is included on relevant Locus Summary pages with a link to the browsable Disease page. It is our hope that making this information available to our users will facilitate studies aimed at understanding the biological functions of these genes and the role these genes play in the pathology of disease.

594C stop-PRISM integration allele in the *rb1* tumor suppressor recapitulates developmental and cellular neural progenitor loss of function phenotypes *Austin Winker*[1,2], Wes Wierson[1], Sekhar Kambakam[1,4], Steve Ekker[3,4], Karl Clark[3,4], Jeff Essner[1,4], Maura McGrail[1,4] 1) Iowa State University; 2) Undergraduate Major in Genetics, Iowa State University; 3) Mayo Clinic; 4) Iowa State/Mayo Clinic Alliance for Genome Engineering.

To simplify screening and genotyping of mutant embryos we generated a set of vectors for CRISPR/Cas9 targeted integration called pPRISM, which contain secondary markers that allow for simple visual genotyping (plasmids for Precise Integration with Secondary Markers). The plasmids are designed for integration using short homology arms as we reported previously (Wierson et al., 2019 bioRxiv). The stop-PRISM vectors have a cassette for transcriptional termination followed by a secondary marker for heart, lens or hatching gland-specific fluorescent reporter expression. Targeted integration into an early exon is predicted to cause early transcription termination and create a loss of function mutation. We isolated an *rb1-stop-PRISM-cmlc2:GFP* line and tested whether the integration allele behaved as a loss of function mutation in trans with our previously published 7 bp indel allele *rb1D7is54*. The gross morphology of *rb1-stop-PRISM-cmlc2:GFP/rb1D7is54* trans heterozygotes is relatively normal, however the larvae fail to develop a swim bladder and are lethal, similar to *rb1D7is54/D7is54* homozygotes. To examine if the *rb1-stop-PRISM-cmlc2:GFP* allele disrupts *rb1* function at the cellular level we used live embryo confocal imaging of H2A.F/Z:GFP labeled chromatin in the developing optic tectum to follow neural progenitor cell cycle dynamics. At 5 dpf, *rb1-stop-PRISM-cmlc2:GFP/rb1D7is54* mutant neural progenitors re-enter the cell cycle and condense their chromatin. The cells then appear to arrest in prophase, and fail to align the chromosomes at the metaphase plate and progress through the cell cycle, as we previously found for *rb1D7is54/D7is54* homozygotes (Schultz et al., 2018). PCR genotyping of the imaged embryos revealed the expected genotypes matched the observed phenotypes. Quantification of the neural progenitor mutant phenotype is in progress. Future analysis will compare phenotype and quantification data between our *rb1-stop-PRISM-cmlc2:GFP/rb1D7is54* trans heterozygotes and homozygous *rb1-stop-PRISM-cmlc2:GFP/stop-PRISM-cmlc2:GFP* embryos. Our results confirm integration of the pPRISM cassette can create loss of function mutations that recapitulate the phenotype of standard indels in the tumor suppressor *rb1*, both at the morphological and cellular level. stop-PRISM loss of function alleles will be useful genetic tools for rapid genotyping in combination with more complex edited alleles to study tissue or cell type specific gene function.

599B Chemical Phenomics Initiative to Drive Therapeutic Target Discovery Charles C. Hong[1], Charles H. Williams[1] 1) Division of Cardiovascular Medicine, Departments of Biochemistry & Molecular Biology, and Physiology University of Maryland School of Medicine, Baltimore, MD .

Chemical genetics involves the discovery, development and use of chemical probes for interrogation of biological processes and for translational discoveries. In a manner analogous to classic forward mutagenesis screens, the Hong lab has conducted an unbiased, high-throughput chemical screen for small molecules that specifically modulate early embryonic development in zebrafish, and has carried out the follow-up task of identifying the pharmacological targets of a number of developmental modulators. Using this target-agnostic, "high content" phenotypic screening platform, we have discovered novel BMP, Wnt and hedgehog inhibitors, as well as first-in-class modulators of cell signaling components. Moreover, since disturbances in developmental pathways play central role in the pathogenesis of many human illnesses, small molecules that selectively target them have significant translational potential. Yet the complexity and unbiased nature of phenotypic screens make the crucial follow-up task of identifying the biologically relevant target of each hit very challenging. Leveraging the available molecular genetic information on early zebrafish embryogenesis, we have developed ZePASS (Zebrafish Phenotypic Anatomical Similarity System), an unbiased deep learning method to map the actions of small molecules and accelerate target identification. Finally, we are leveraging UK Biobank phenotype-genotype database to identify the clinical phenotypes associated with naturally occurring human genetic variations in the target genes, and then utilize these associations to ultimately guide

therapeutic development for important unmet clinical needs. Finally, digitally annotated results of chemical screens and target deconvolution will be made available to the broader scientific community via a searchable, online database named Chemical Phenomics Initiative.

618C Javelin - a novel protein, is essential in Drosophila bristle actin bundles formation *Ramesh kumar Krishnan*[1], Uri Abdu[1], Anna Bakrhat[1] 1) Ben-Gurion University of the Negev, Beer-Sheva 8410501 Israel.

Parallel actin bundles are composed of loosely packed parallel filaments that all have the same polarity and are cross-linked by an actin-bundling protein. These actin bundles are key components of eukaryotic cytoskeleton structures such as the brush border of intestinal epithelial cells, stereocilia of hair cells of the vertebrate ear and Drosophila bristle, where they appear to function in part as scaffolds that help support or stabilize cellular protrusions. In the present study, the Drosophila bristle cells were used to study actin bundle formation since alterations in bristle morphology are simple to follow during their formation using live imaging. Moreover, small changes in the cytoskeleton induced by drugs or mutations often result in easily detectable bristle phenotypes in adult flies. In Drosophila, actin bundling requires the sequential action of at least two different actin-bundling proteins during bristle formation: Forked and Fascin.
In this present study, using RNA sequence analysis a new gene, Javelin (Jv), was identified that directly affect actin bundles formation. *jv* mutant bristles do not taper like bristles in wild-type flies, instead presenting a small enlargement before the tip. In *vitro* and in *vivo* studies were conducted using Javelin in the presence of Forked and Fascin and we found that different combination of these proteins displayed varying thickness of well-established actin bundles.
Although Forked and Fascin were found to be the major actin bundling proteins in bristle formation, we still know little about how the utilization of this set of proteins in a time- and space- dependent manner influences the properties of an actin bundle within the cellular cytoskeleton structures with which it is associated. It was also suggested that there is at least one actin filament-membrane connector and possibly even a third unknown actin-bundling protein which remains to be identified. Could Javelin be that missing link?

624C Early transcriptome responses to mating in the lower reproductive tract of *Drosophila melanogaster* females *Sofie Delbare*[1], Andrew Clark[1], Mariana Wolfner[1] 1) Cornell University.

The female's lower reproductive tract is exposed directly to the male's ejaculate, making it a hotspot for mating-induced responses shortly after mating. In *Drosophila melanogaster,* mating induces physiological changes in preparation for fertilization events. Microarray studies have detected modest transcription-level changes at early time points after mating, but no study has examined immediate transcriptome changes of both coding and noncoding RNAs in the female reproductive tract. To more precisely detect the earliest events in the female's reproductive tract, we measured transcript abundance in lower reproductive tracts of virgin females and females collected within 10-15 minutes after the end of mating. We observed mating-induced transcriptional changes in several functional categories, including 13 long noncoding RNAs, 15 genes involved in remodeling of the nervous system and synaptic transmission and 30 genes associated with activation of wound healing. Several additional genes involved in the stress response, including components of the innate immune Toll and Imd signaling pathways, were up-regulated. In addition, using SNPs, we identified transcripts in female samples that had been transferred by their mates. Many of these transcripts likely originate from the male accessory gland, but whether they have a physiological effect in females remains to be determined. Our results shed light on the molecular changes that accompany very early responses to mating and are useful for further examination of the crosstalk that occurs between female and male molecules inside the reproductive tract.

625A Model organism Encyclopedia of Regulatory Networks (modERN) validation using *Drosophila* transcription factor RNAi and RNA-seq *Bill Fisher*[1], Ann Hammonds[1], Richard Weiszmann[1], Ben Booth[1], Alec Victorsen[2], Jaeda Patton[3], Connor Kubo[3], Mark Gerstein[4], Kevin White[2], Valerie Reinke[4], Robert Waterston[3], Susan Celniker[1] 1) Lawrence Berkeley National Laboratory; 2) University of Chicago; 3) University of Washington; 4) Yale University.

Our goal is to create comprehensive maps of transcription factor (TF) binding sites in *D. melanogaster* and *C. elegans*. To validate these maps we are conducting RNAi knockdowns of embryonically-expressed Transcription Factors (TFs) followed by RNA-seq to identify potential targets of each TF. We used the TRiP lines from the Drosophila RNAi Screening Center to knock down TF expression measured in biological replicate at three time points: 0-1.5 hrs, 16-18 hrs and the 2 hr period of maximal embryonic expression for each TF. To date we have examined 50 TFs, generating over 450 samples for RNA-seq. About one third of the RNAi TF knockdowns resulted in lethal phenotypes, either as embryos (4), larvae (10), or pupae (3). In the RNAi knockdown of the transcription factor *Estrogen Related Receptor (ERR)* almost all animals died during the larval period. Of the 20 genes whose expression was most reduced (log2fold change > -1) after knockdown of *ERR* expression, ten genes encode enzymes required for glycolysis and their transcripts are expressed primarily in muscle. Of the remainder, six are required for carbohydrate metabolism and four appear to be part of a small operon of which two are known peroxidases and two are yet to be fully characterized. Our ChIP-seq experiments show the location of *ERR* binding sites near the promoters or within the first introns of the target genes we have identified by RNAi. In the case of the target gene *Lactate dehydrogenase (Ldh)* ERR binding sites are clustered with *Ecdysone receptor (EcR)* binding sites. RNA-seq data are available at encodeproject.org for access by the research community.

633C *Har-P*, a short *P*-element variant, weaponizes *P*-transposase to severely impair *Drosophila* development. Satyam Srivastav[1], Reazur Rahman[2], Qicheng Ma[1], Jasmine Pierre[1], Saptaparni Bandyopadhyay[1], *Nelson Lau*[1,2,3] 1) Department of Biochemistry, Boston University School of Medicine, Boston, MA; 2) Department of Biology, Brandeis University, Waltham, MA; 3) Genome Science Institute, Boston University School of Medicine, Boston, MA.

Without transposon-silencing Piwi-interacting RNAs (piRNAs), transposition causes an ovarian atrophy syndrome in *Drosophila* called gonadal dysgenesis (GD). *Harwich* (*Har*) strains with *P*-elements cause severe GD in F1 daughters when *Har* fathers mate with mothers lacking *P*-element-piRNAs (i.e. *ISO1* strain). To address the mystery of why *Har* induces severe GD, we bred hybrid *Drosophila* with *Har* genomic fragments into the *ISO1* background to create *HISR-D* or *HISR-N* lines that still cause Dysgenesis or are Non-dysgenic, respectively. In these lines, we discovered a highly truncated *P*-element variant we named "*Har-P*" as the most frequent *de novo* insertion. Although *HISR-D* lines still contain full-length *P*-elements, *HISR-N* lines lost functional *P*-transposase but retained *Har-P*'s that when crossed back to *P*-transposase restores GD induction. Finally, we uncovered *P*-element-piRNA-directed repression on *Har-P*'s transmitted paternally to suppress somatic transposition. The *Drosophila* short *Har-P*'s and full-length *P*-elements relationship parallels the MITEs/DNA-transposase in plants and SINEs/LINEs in mammals.

634A The moving target of transposon landscape changes in aging *Drosophila*. *Nachen Yang*[1], Satyam Srivastav[1], Qicheng Ma[1], Reazur Rahman[2], Michael Rosbash[2], Madoka Chinen[3], Elissa Lei[3], Nelson Lau[1,2,4] 1) Department of Biochemistry, Boston University School of Medicine; 2) Department of Biology, Brandeis University; 3) Nuclear Organization and Gene Expression Section, NIDDK, NIH; 4) Genome Science Institute, Boston University School of Medicine.

Genetic mechanisms that strongly repress transposable elements (TEs) in young animals decline during aging because TE transcripts become re-activated. Does TE transcriptional reactivation during aging then alter and damage the genome? To test this hypothesis, we quantified Transposon Landscape (TLs) via deeply sequencing genomes of young and aged *Drosophila* strains of wild-type and mutant backgrounds. We quantified TLs in aging whole flies as well as dissected brains, and we validated the feasibility of our approach in detecting increases in new TE insertions in aging *Drosophila* genomes when RNAi and Piwi pathways are compromised.

By also incorporating droplet digital PCR as an important validation methodology for measuring genomic TE loads, we also show that genetic mutations that strongly reactivate TE RNA expression only exhibit modest genomic TL changes. Additionally, we examined extra-chromosomal DNA circles (eccDNAs) as a source of accumulating TE copies and describe improved sequencing methods to quantify eccDNAs in *Drosophila*. Our analysis suggests that small RNA surveillance mechanisms still prevent genomic TL expansion despite the increase in transposon transcripts during aging. However, to combat the natural progression of increased TE expression during animal aging we show that knocking down the PAF1 complex that regulates RNA Pol II elongation and transcription termination can reduce aging related TE expression increases.

635B Defining the role of Retrotransposon Insertion and Expression in Aging in *Drosophila melanogaster* *Blair Schneider*[1], Shannon Lightcap[1], Wenge Li[1,2], Nicholas Skvir[3], Nicola Neretti[3], Jan Vijg[1,4], Julie Secombe[1,5] 1) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Department of Medicine, Albert Einstein College of Medicine, Bronx, NY; 3) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI; 4) Department of Ophthalmology and Visual Sciences, Albert Einstein College of Medicine, Bronx, NY; 5) Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Across all organisms, aging correlates with functional decline. One potential contributor to decreased health is the expression and mobilization of transposons (RTs), insertion of which are likely to alter cellular functioning. RTs are mobile genetic elements that replicate using an RNA intermediate to form double stranded DNA, capable of inserting into new genomic locations. RT expression and copy number have been shown to increase with age within multiple organisms. However, it remains unknown which specific RTs are most active in different aging somatic cells and where in the genome *de novo* insertions occur. Additionally, the cellular and organismal consequences of insertions have not been investigated. Our goal is to determine *de novo* RT insertion number and genomic location (insertional hot spots) in different cell types implicated in aging. Another goal is to quantify the effects of altered RT expression/insertion on health and lifespan. We utilize *Drosophila melanogaster* to dissect evolutionarily conserved mechanisms that mediate aging. Using a single cell whole genome sequencing approach, we have shown that RT insertions increase with age in female indirect flight muscle, with the most active element being 412. To determine the biological importance of 412 insertions, we ubiquitously knocked down the expression of this RT. This significantly extended lifespan in females and improved their performance in several assays of health. Flies show improved climbing ability with age in a negative geotaxis assay when 412 was knocked down. Additionally, 412 knockdown flies demonstrated increased oxidative stress resistance with age, correlating with our sequencing data. RTs are a source of DNA damage themselves as they induce double stranded breaks. We predict that knocking down 412 allows the organism to better cope with the reactive oxygen species (ROS) induced DNA damaged that occurs with paraquat exposure. To complement these studies, we have generated transgenic flies able to overexpress 412 using the dCas9·VPR transcriptional activator system to test whether increased 412 expression impacts health and/or lifespan. Together these data highlight the significance of age onset 412 and RT activation in general. Importantly, our data are consistent with the hypothesis that the expression/mobilization of RTs is a key driver of the health decline that occurs with age.

639C The persistence of low-titer *Wolbachia pipientis* infection in antibiotic-treated *Drosophila* *Cynthia Ulbing*[1], Miwa Wenzel[1], Paula Fernandez Begne[1], Jaclyn Bubnell[1], Charles Aquadro[1] 1) Cornell University.

Wolbachia is an endosymbiotic bacterium common to insects and nematodes. It has been shown to have a range of effects the host reproductive system, leading to a growing interest in its molecular interactions. Previous research in our lab has shown that *Wolbachia* infection rescues the mutant loss-of-function phenotype of the *Drosophila bag of marbles* (*bam*) germline stem cell gene. In order to analyze the functional consequences of *Wolbachia* infections in *Drosophila* it is critical to establish a negative control for endosymbiont infection. Our research group therefore has sought to obtain specific *Drosophila* fly lines without *Wolbachia* infections, cleared by the standard methods of treating the flies with the antibiotic tetracycline for three generations. We have received tetracycline-treated flies from other laboratories as well as treated our own fly stocks with the antibiotic. Despite these efforts, endpoint PCR for *Wolbachia* on these lines reveal intermittent, yet persistent, positive results. We initially considered these *Wolbachia*-positive results to be a consequence of PCR amplicon contamination. However, further experiments have suggested, that these flies are infected with a low-titer strain of Wolbachia. To more closely investigate the *Wolbachia* status of these lines we have used a range of experimental techniques to detect its presence. We have employed endpoint PCR, touchdown-PCR, quantitative PCR, and cytological assays on our fly lines. All of these methods have shown a very low, nearly undetectable, presence of *Wolbachia* in tetracycline-treated flies. We are currently using fluorescent in-situ hybridization to perform targeted imaging of *Wolbachia* in host cells. We are also characterizing the low-titer *Wolbachia* found in our flies to identify the specific strain that is present. *Wolbachia* has a diverse phylogeny across its host species. We are using multilocus sequence typing (MLST) to sequence five loci in the *Wolbachia* genome that appear in strain-specific combinations. These sequences will be compared against a database that contains sequences across the *Wolbachia* phylogeny to closely differentiate between strains. The persistence of *Wolbachia* infections in antibiotic treated flies raises questions about the effectiveness of tetracycline to completely eliminate this bacterium from its host. Despite its persistence, we find that low-titer *Wolbachia* infection does not appear to be sufficient to functionally rescue host *bam* function.

640A A multiplex F1 RNAi screen for defects in *Drosophila* female meiosis *William Gilliland*[1], Kelly Conger[1], Doreen Elrad[1], Olivia Johnson[1], Marcin Marciniak[1], Denny May[1], Gabrielle Presbitero[1] 1) DePaul University.

Traditional forward genetic screens identify genes required for female meiosis by inducing mutations, making the mutated chromosomes homozygous, and then examining the progeny of mutant flies for evidence of chromosome segregation errors. This approach requires that mutant females be viable and fertile, and will therefore miss any meiotic genes that are lethal when homozygous or required to produce viable offspring. Our lab is

currently screening the VALIUM22 collection that was produced by the Harvard TRiP Project, which contains RNAi constructs targeting genes known to be expressed in the germline. By driving RNAi with a germline-specific promoter, we can test genes that would be lethal if knocked down in all cells, and by examining unfertilized metaphase-arrested mature oocytes by confocal microscopy, we can identify genes even if their knockdown causes sterility.

We are screening this collection to identify genes that disrupt either of two phenotypes: the ability of meiotic chromosomes to undergo congression to a single mass at the end of prometaphase, and the structural components of enigmatic cytoplasmic filaments that Mps1-GFP becomes sequestered to during acute hypoxia. At the time of this meeting, we will be close to finishing the primary screening of the ~1500 lines in the collection, and have obtained multiple hits for both phenotypes, including several genes that were not known to play a role in meiosis as well as finding the first phenotypes to be associated with multiple previously uncharacterized genes. In addition to describing the results of the screen, the challenges and limitations in doing this kind of genetic screening at a primarily undergraduate institution will be presented.

642C collaborative cross graphical genome *Hang Su*[1,2], Ziwei Chen[1], Jaytheert Rao[1], Maya Najarian[1], John Shorter[3], Fernando Pardo Manuel de Villena[3,4], Leonard McMillan[1] 1) Department of Computer Science, University of North Carolina, Chapel Hill; 2) Curriculum of Bioinformatics and Computational Biology, University of North Carolina, Chapel Hill; 3) Department of Genetics, University of North Carolina, Chapel Hill; 4) Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill.

The mouse reference is one of the most widely used and accurately assembled mammalian genomes and is the foundation for a wide range of bioinformatics and genetics tools. However it represents the genome of a single inbred mouse strain, C57BL/6J. Recently, inexpensive and fast genome sequencing has enabled the assembly of other laboratory mouse strains at a quality approaching that of the reference but using these alternative assemblies in standard genomics analysis pipelines presents significant challenges. Moreover, new genetic resource populations are being developed, *e.g.* Collaborative Cross (CC), whose genomes are a mosaic of multiple inbred mouse strains. This presents a dire need to integrate multiple genomes into the standard sequence analysis. It has been suggested that a pangenome reference assembly, which incorporates multiple genomes into a single representation, are the path forward, but there are few standards for, or instances of practical pangenome representations suitable for large eukaryotic genomes. We present a pragmatic graph-based pangenome representation as a genomic resource for the widely-used recombinant-inbred CC mouse strains and its eight founder genomes. The pangenome representation leverages existing standards for genomic sequence representations with backward-compatible extensions to describe graph topology and genome-specific annotations along paths. It packs 83 mouse genomes (8 founders and 75 CC strains) into a single graph representation that captures important notions related to genomes such as identity-by-descent and highly variable genomic regions. The introduction of special anchor nodes with sequence content provides a valid coordinate framework that divides large eukaryotic genomes into homologous segments and addresses most of the graph-based position reference issues. Parallel edges between anchors place variants within a context that facilitates orthogonal genome comparison and visualization. Furthermore, the graph structure allows annotations to be placed in multiple genomic contexts and simplifies their maintenance as the assembly improves. The CC reference pangenome provides an open framework for graph-based tool chain development and analysis.

661A Zika virus transiently altering host chromatin accessibility during infection *Brandon Buck*[1], Sarah Ogden[1], Lauren Cole[1], Athanasios Vouzas[1], Jane Benoit[1], Peiyao Zhao[1], Hengli Tang[1], Jonathan Dennis[1,2] 1) Department of Biological Science, The Florida State University, Tallahassee, Florida, USA; 2) The Center for Genomics and Personalized Medicine, The Florida State University, Tallahassee, Florida, USA.

The accessibility of DNA in chromatin is an important factor in all DNA-templated processes, and chromatin remodelers are responsible for regulating this accessibility. Our group has previously described a Genomic Transient Intermediate State (GTIS), a widespread chromatin remodeling event that occurs during viral reactivation. Here we describe the regulation of chromatin response during the infection of neuronal cells with Zika virus. Zika presents a threat to the health of pregnant women and developing fetuses. The role of the virus in the developing brain is particularly important as Zika infection in pregnant women may cause microcephaly and other severe fetal brain defects. There is a current gap in understanding of how Zika affects the host chromatin and genome accessibility. We utilized the SNB-19 glial cell, and PRVABC59 Puerto Rican strain of Zika virus to observe genome wide chromatin alterations measured at 0, 12, and 24 hours after infection. We observed a GTIS occurring 12 hours post Zika infection. The host cell chromatin demonstrated increased sensitivity to micrococcal nuclease at 1647 genes (7.5% of all genes). Genes that gained nucleosomal sensitivity were associated with telencephalon regionalization, and cerebral cortex cell migration. Additionally, 2287 genes (10.4% of all genes) demonstrated a decreased resistance to micrococcal nuclease. Genes in this group were enriched for ATP-dependent chromatin remodeling, and nucleosome organization. These results suggest that studies of chromatin structure dynamics and consequent access to the underlying genome during viral infection will give important insights into the genomic regulatory landscape that leads to the pathologies associated with Zika infection.

667A Functional Testing of a Stress Adaptation Biosignature Observed in the Devil Worm *Megan Guerin*[1], Deborah Weinstein[1], John Bracht[1] 1) American University.

Halicephalobus mephisto was discovered nearly 1.3 kilometers below Earth's surface in the Beatrix Gold Mine of South Africa. When underground, the organism was found to be residing in extreme environmental conditions that could prove to be very strenuous on the organism, such as heated (37 °C), methane-rich, hypoxic water. Genome sequencing of the nematode indicated that there were expansions of the 70 kilodalton heat shock protein (Hsp70) and *avrRpt2* -induced genes (AIG1), with both gene families having been previously identified as cellular survival genes. This expansion of both Hsp70 and AIG1 gene families were also found to be convergently expanded in distantly related Mollusks, with expansion specifically present in bivalves. The presence of these convergently expanded gene families within two distantly related animal phylum that both routinely endure environmental stressors such as hypoxia and elevated temperatures can indicate a biosignature response to environmental stress. However, to identify if the unique gene family expansion is truly a biosignature of environmental stress, the survival of the organism must be tested with the inactivation of the expanded gene families. In this work we are exploring the function of these genes, starting with a proposed master regulator gene: ARMET/MANF (Arginine-Rich Mutated in Early State Tumors / Mesencephalic Astrocyte Derived Neurotrophic Factor). RNA interference will allow the inactivation of suspected intermediate regulator proteins such as ARMET, which could then cause a change in either AIG1 or Hsp70 expression and ultimately lead to organismal impairment or death.

678C Sex peptide can associate with rival as well self sperm and function with both in polyandrous females *Snigdha Misra*[1], Mariana Wolfner[1] 1)

Cornell University.

In populations where females mate with more than one male, sperm-sperm competition and cryptic female choice can occur, biasing sperm use and influencing males' paternity share outcome of the mating. There has been much focus on the strategies that are used, and selected for, by males to win the competition and females to influence the outcome. But, molecules and cells derived from males and females can work together towards the common goal of optimal fertilization. A male's seminal fluid molecules modify the female's physiology to increase reproductive success. But since some of these modifications induce permanent changes in female physiology that can indirectly benefit rival males, rival males can and do tailor their ejaculate accordingly, minimizing the energy cost of mating. Here we show that ejaculate from one male can also directly benefit a rival. We show that sex peptide that a female receives from one male can bind to sperm from previous male, that were already in storage in the female. Moreover, the second male's SP can restore fertility and sperm use/ release efficiency in female whose first mate did not provide SP to induce these effects. Our results show that sex peptide from one male can benefit another and as such is a key molecular component in the process of "copulation complementation".

679A *Drosophila* models of pathogenic copy-number variant genes show global and non-neuronal defects during development *Tanzeen Yusuff*[1], Matthew Jensen[1], Sneha Yennawar[1], Lucilla Pizzo[1], Siddharth Karthikeyan[1], Dagny Gould[1], Avik Sarker[1], Yurika Matsui[1], Janani Iyer[1], Zhi-Chun Lai[1], Santhosh Girirajan[1] 1) Pennsylvania State University.

While rare pathogenic copy-number variants (CNVs) are associated with both neuronal and non-neuronal phenotypes, functional studies evaluating these regions have focused on the molecular basis of neuronal defects. We report a systematic functional analysis of non-neuronal defects for homologs of 59 genes within ten CNVs and 20 neurodevelopmental genes in *Drosophila melanogaster*. Using wing-specific knockdown of 136 RNA interference lines, we identified qualitative and quantitative phenotypes in 72/79 homologs, including 21 lines with severe wing defects and six lines with lethality. In fact, we found that 10/31 homologs of CNV genes also showed complete or partial lethality at larval or pupal stages with ubiquitous knockdown. Comparisons between eye and wing-specific knockdown of 37/45 homologs showed both neuronal and non-neuronal defects, but with no correlation in the severity of defects. We further observed disruptions in cell proliferation and apoptosis in larval wing discs for 23/27 homologs, and altered Wnt, Hedgehog and Notch signaling for 9/14 homologs, including *AATF/Aatf*, *PPP4C/Pp4-19C*, and *KIF11/Klp61F*. These findings were further supported by tissue-specific differences in expression patterns of human CNV genes, as well as network connectivity of CNV genes to signaling pathway genes in brain, heart and kidney-specific networks. Our findings suggest that multiple genes within each CNV differentially affect both global and tissue-specific developmental processes within conserved pathways, and that their roles are not restricted to neuronal functions.

683B Systems Genetics of Aging in *Drosophila melanogaster* Maria E. Adonay[1], Shanshan Zhou[2], Logan Everett[3], Terry Campbell[4], Robert R. H. Anholt[1,5], Trudy F. C. Mackay[1,5] 1) Center for Human Genetics, Clemson University, Greenwood, SC; 2) Covance Companion Diagnostics, Covance Inc., Research Triangle Park, NC; 3) US Environmental Protection Agency, Research Triangle Park, NC; 4) WM Keck Center for Behavioral Biology and Department of Genetics, NC State University, Raleigh, NC; 5) Department of Genetics and Biochemistry, Clemson University, Greenwood, SC.

Determining the effect of genetic variation on gene expression as the human body ages will improve our understanding of how diseases progress and what it means to preserve health with age. Model organisms such as *Drosophila melanogaster* present unique advantages to study such phenomena: they avoid confounding from environmental perturbations and are easily maintained in the laboratory setting. Several studies have shown that aged flies display a reduction in mitochondrial turnover and that thousands of transcripts have altered expression with increasing age. However, a thorough investigation of the changes in genome-wide gene expression across different genetic backgrounds due to aging is still lacking. To fill this gap, we performed deep RNA sequencing for 193 Drosophila Genetic Reference Panel lines for young flies and 3 week old flies. We estimated expression levels for both annotated genes and novel transcribed regions, mapped expression quantitative loci, and constructed sex-specific transcriptional regulatory networks for both young and older flies. These data identify transcriptional biomarkers of aging and identify key network hubs that are genetically variable and change with age.

684C Identification of mating-type specific genes in the methylotrophic yeast *Ogataea polymorpha* Kristie M. Shirley[1], Sara J. Hanson[1] 1) Molecular Biology, Colorado College, Colorado Springs, CO.

Processes of cellular differentiation give rise to a variety of cellular forms and functions. In budding yeast, haploid cells of opposite mating-type (a and alpha) will fuse through the process of mating to become diploid cells capable of meiosis and sporulation. Haploid a and alpha cells are distinguished primarily by the particular pheromone they secrete and the receptor to the pheromone of the opposite cell type they possess on their cell surface. In diplontic yeast, such as *Saccharomyces cerevisiae*, the cell-type specific expression of the transcription factors *MATa2* or *MATalpha1* drives the constitutive expression of these genes in a and alpha-type cells, respectively. This allows these cells to readily mate, even in nutrient-rich conditions. In contrast, the methylotrophic yeast *Ogataea polymorpha* is haplontic and requires nitrogen starvation in order to mate. Although the expression of *MATa2* and *MATalpha1* are cell-type specific and essential for determining mating-type identity in *O. polymorpha*, their regulatory targets (a- and alpha-specific genes) have been challenging to determine, as the target genes are not constitutively expressed in their cell types. To identify a- and alpha-specific genes in *O. polymorpha*, the overexpression of the transcription factor S*TE12* was induced in strains lacking *MATa2* or *MATalpha1*. Using transcriptome analysis, we identified a- and alpha-specific genes, including genes involved in the pheromone response pathway. In comparing our dataset with previously generated data identifying *STE12* target genes in *O. polymorpha*, we have also identified the genes likely to be haploid-specific. These findings further our understanding of the genetic basis for cell-type specification in *O. polymorpha*.

690C Analysis of reticulate evolutionary relationships using PhyloNet *Zhen Cao*[1], Zhi Yan[1], Luay Nakhleh[1] 1) Rice University, Houston, TX.

Reticulate evolutionary relationships arise when processes such as gene flow or hybridization occur. In the presence of such processes, evolutionary relationships cannot be represented adequately using a tree structure; instead, a more general graph structure, known as phylogenetic networks, is necessary. PhyloNet is a software package that includes a wide array of methods for inferring phylogenetic networks from data sets of unlinked loci while accounting for both reticulation (e.g., hybridization) and incomplete lineage sorting. In particular, PhyloNet now allows for maximum parsimony, maximum likelihood, and Bayesian inference of phylogenetic networks from gene tree estimates. Furthermore, Bayesian inference directly from sequence data (sequence alignments or bi-allelic markers) is implemented. Maximum parsimony is based on an extension of the "minimizing deep coalescences" criterion to phylogenetic networks, whereas maximum likelihood and Bayesian inference are based on the multispecies network coalescent. All methods allow for multiple individuals per species. As computing the likelihood of a phylogenetic network is computationally hard,

PhyloNet allows for evaluation and inference of networks using a pseudo-likelihood measure, as well as inference using a divide-and-conquer technique. PhyloNet summarizes the results of the various analyses, and generates phylogenetic networks in the extended Newick format that is readily viewable by existing visualization software. Last but not least, PhyloNet has a wide variety of other tools for comparing networks.

692B Transposable elements dynamics in the face of hybridization: insights from the wild yeast *Saccharomyces paradoxus* *Mathieu Henault*[1,2,3], Souhir Marsit[1,2,3], Guillaume Charron[1,2,3], Christian R Landry[1,2,3] 1) Institute of Integrative and Systems Biology (IBIS), Université Laval, QC, Canada; 2) Département de Biochimie, Microbiologie et Bio-informatique, Université Laval, QC, Canada; 3) Département de Biologie, Université Laval, QC, Canada.

One of the key aspects of hybridization is the generation of transgressive phenotypes, which can both favor or hinder the maintenance of hybrids as independent lineages. In this regard, one important class of traits is mutation rates. Moderate changes in mutation rates can affect the adaptability of hybrids. On the other hand, extremely high mutation rates can be a source of postzygotic reproductive isolation as hybrids may not survive high levels of deleterious mutations. Among the diverse types of mutations, transposable elements (TEs) are thought to be especially responsive to genomic perturbations like hybridization. Many examples show TEs reactivation in hybrids and illustrate how they impact hybrid phenotypes. However, the determinants of TE activity levels in hybrids are not fully understood. We investigate the accumulation of TEs in hybrid genomes using a mutation accumulation experiment with artificial hybrids of the undomesticated yeast *Saccharomyces paradoxus.* We chose *S. paradoxus* and *S. cerevisiae* strains that span various levels of genetic divergence and TE abundance levels. We find strong evidence for the effect of individual genotypes on TE accumulation, with many hybrids of similar divergence levels having contrasting TE abundances after evolution. We find that overall, parental TE abundance at the onset of the experiment is a better predictor of TE accumulation in hybrids than parental genetic divergence. Using data from natural lineages of *S. paradoxus*, we show that although lineages may have important current variation in TE abundance, lineages that have a history of hybridization do not exhibit TE reactivation. This is consistent with TE abundance levels at the time of hybridization being the main determinant of TE accumulation in hybrids. Overall, our results suggest a major effect of genetic background on TE accumulation in this model system, with modest contributions from population genetic and environmental factors.

695B Host response to an invading TE: extinction vs repression *Luyang Wang*[1], Farnaz Naeemikia[1], Shuo Zhang[1], Erin Kelleher[1] 1) University of Houston.

Transposable elements (TEs) are genomic parasites that burden their host genome with deleterious mutations and incite genome instability. To avoid these costs, host genomes control the mobilization of TEs through piRNA-mediated silencing. However the mutational and epigenetic processes that give rise to piRNA-mediated silencing when new TEs invade the host remain poorly understood. P-elements have invaded the genomes of three Drosophila species within the last century, providing unique opportunities to study the evolution of piRNA mediated repression. We introduced P-elements into naive D. melanogaster strains through germline transformation, and chronicled their effects in 10 laboratory populations at two different temperatures. At high-temperatures we observed that elevated transposition rates drove population extinction before the evolution of repression, whereas at low temperatures repression evolved in some populations. We are reconstructing the evolution of repression through genome resequencing and deep sequencing of small RNAs in evolved populations.

700A Shifts in the Microbial Community of the Anacostia River in Washington, DC due to the implementation of the Anacostia River Tunnel *Gaurav Arora*[1], Skyler Officer[1], Tyler Mitchell[1], Caroline Solomon[1] 1) Department of Science, Technology and Math, Gallaudet University. Washington DC..

DC Water built a storage tunnel beneath the Anacostia River that was implemented in March 2018 to address environmental and pollution concerns.. The aim of the tunnel is to divert sewage and storm water and its associated nutrients and *E. coli* from entering the river. Samples were collected pre-tunnel (2017) and post-tunnel (2018) implementation to evaluate the effectiveness of the tunnel and examine relationships between nutrient dynamics and microbial communities. Based on previous work, we hypothesized that in 2018 there would be decreases in concentrations of NO_3^- and NH_4^+ and the NH_4^+: NO_3^- ratio that would lead to a shift to more diatoms and less cyanobacteria. Our data shows a shift from 2017 to 2018 with lower counts for certain bacterial (16S) groups such as *Burkholderiales, Cerasicoccales, and Cytophagales*. Overall, the results show that there was a decrease in the composition of the microbial (16S & 18S) communities from pre- to post-tunnel implementation despite changes in nitrogen concentrations and the NH_4^+:NO_3^- ratio. These changes in microbial community composition may be due to the increase in rainfall during 2018 and cannot be attributed to the tunnel implementation.

703A *Wolbachia* and *Bag of Marbles* (*bam)* Dynamics in *Drosophila melanogaster* *Catherine Kagemann*[1], Charles Aquadro[1] 1) Cornell University, Ithaca, NY .

Wolbachia, a maternally inherited bacterial endosymbiont, is known to rescue the reduced fecundity phenotype of a *bag of marbles (bam) Drosophila melanogaster* partial loss of function mutant (hypomorph). We wish to understand the functional mechanisms behind the rescue of the *bam* phenotype in Drosophila. In *D. melanogaster*, genetically diverse substrains of Wolbachia have been identified and characterized. Wmel-like Wolbachia substrains are characterized by a longer life span and low Wolbachia titer while WmelCS-like Wolbachia substrains are characterized by a shorter life span and a high Wolbachia titer. We are testing whether different Wolbachia genotypes and different Wolbachia titer levels influence *bam* function (and resulting fecundity) in *D. melanogaster*. Our preliminary results show that wildtype female *D. melanogaster* infected with a Wmel-like *Wolbachia* have a higher titer of Wolbachia present in the ovaries compared to the carcass over the fly's life span. Conversely, wildtype female Drosophila infected with a WmelCS-like Wolbachia substrain have a stable low titer in the ovaries but a higher titer of Wolbachia present in the carcass. In the Drosophila *bam* hypomorph infected with Wmel-like and WmelCS-like substrains, Wolbachia titer is stable at a low titer in the ovaries but increases in titer in the carcass over the fly's life span. These results suggest that the partial loss of function of *bam* can impact Wolbachia titer and that the potential for differential rescue between Drosophila of different substrains is due to Wolbachia strain genotype rather than Wolbachia titer alone. Follow up experiments will test for differential rescue between Drosophila of different Wolbachia substrains.

704B Verifying polymorphisms associated with long and short sleep using polycistronic CRISPR coupled with extreme QTL mapping *Akanksha Singh*[1], Terese R Tansey[1], Carey Fagerstrom[2], Nasser Rusan[2], Susan T Harbison[1] 1) Laboratory of System Genetics, National heart Lung and Blood Institute, NIH, Bethesda, MD; 2) Laboratory of Molecular Machines and Tissue Architecture, National Heart Lung and Blood Institute, Bethesda, MD.

Artificial selection and genome-wide association mapping have isolated unprecedented numbers of candidate polymorphisms putatively involved in sleep. One of the greatest challenges in functional genomics is to understand how these polymorphic variants affect sleep. CRISPR technology offers the possibility of replacing alleles to directly observe their effects; however, the task can be daunting for traits having large numbers of predicted polymorphic targets. Previously, we identified 126 polymorphisms for long and short night sleep using artificial selection. Here we apply a new approach to verify these polymorphisms. Using a polycistronic CRISPR gRNA design, we expressed multiple gRNAs per polycistronic construct to create indels near target polymorphisms in a long-sleeping line of the Sleep Inbred Panel, SIP_L1_9. We cloned four gRNAs into a pCFD5 plasmid and injected the plasmid into SIP_L1_9. We allowed the resulting transformants to mate randomly for two generations in order to recombine the transformed chromosomes. We then measured sleep in the transformed populations. Night sleep ranged from 136.7 min. ± 25.2 to 706.3 min. ± 7.4 for progeny from one polycistronic construct and from 266.9 min. ± 103.0 to 696.9 min. ± 9.7 for a second polycistronic construct, exceeding the range of night sleep in the unperturbed SIP_L1_9 background. This suggests that efficient transformation occurred in both populations. We collected the 10% shortest-sleeping and 10% longest-sleeping flies for each construct and extracted their DNA. We are currently sequencing the genomic DNA from each of the high/low 10% and will associate the sleep phenotypes with the number and combination of genomic breaks. In principle, more breaks should be present in the short sleeping flies than in the long sleepers as long sleepers represent the unperturbed background of the injected strain. In this way, we can quickly verify sleep-relevant polymorphisms with the greatest effects, screening out any false positives.

715A The Effect of MCHM on Stress Response Pathway Regulators, Med15 and Snf1 *Suk Lan Ser*[1], Jennifer Gallagher [1], Casey Nassif[1], Amaury Pupo[1] 1) West Virginia University.

Living cells respond by changing molecular and cellular pathways when they are exposed to stressful environmental conditions. Damage to intracellular molecules depends on the duration of stress exposure and the severity of the stressor. In 2014, large volumes of MCHM (4-methylcyclohexanemethanol) spilled into the Elk River of West Virginia contaminating drinking-water supplies. By studying cellular stress response through the Mediator complex in *Saccharomyces cerevisiae*, the molecular effects of the industrial coal-cleaning chemical can be determined. Mediator, a highly conserved multi subunit complex, relays signals from DNA binding transcription factors to RNA polymerase II and can both positively and negatively regulate transcription. Med15, a subunit found within the tail domain of the Mediator complex, works with stress-induced transcription factors and is regulated by many kinases including CDKs and the yeast homolog of AMP kinase, Snf1. Snf1 interacts with the Mediator complex and is required for many cellular activities such as glucose-repressed gene transcription and pH tolerance. *snf1Δ* yeasts are much more sensitive to MCHM compared to wildtype. Med15 contains a KIX domain, and two polyQ tracts separated by a polyQA tract. Due to the difference in polyQ tracts, genetic variation of the Med15 subunit between yeast strains (YJM789 and S288c) leads to varying levels of MCHM tolerance. Cells containing $Med15^{YJM789}$ tagged with Myc were more sensitive to MCHM due to the polyQ expansion. Under adverse conditions, both Med15 and Snf1 are essential for the regulation of stress response pathways.Another interesting discovery was that the 13xMyc tag that was used throughout the study, have shown to have an impact on Med15 and in turn affects its sensitivity towards MCHM and other chemical stressors. There are multiple isoforms of Med15-Myc tag protein detected on a western blot. The protein levels of the Med15 differ based on the alleles and the presence of Snf1 and Reg1 (negative regulator of Snf1). Although $Med15^{S288C}$ is much bigger than $Med15^{YJM789}$, it ran much smaller on the blot suggesting that perhaps post-translational modifications may have slowed down the migration of the protein. Snf1 regulates energy homeostasis and is often misregulated in cancer and other metabolic diseases, while patients DiGeorge Syndrome, who multiple developmental defects and are often sick are missing one copy of Med15. Understanding how energy homeostasis and stress response are related will improve understanding of exposure risks to MCHM and how conserved pathways interact.

720C Genomic profiling of budding yeast resistance to food carcinogens underscore the importance of DNA damage tolerance pathways in avoiding mutations *Michael Fasullo*[1], Nick St. John [1], Michael Dolan [1], Faizan Zaidi[1] 1) SUNY Polytechnic Institute.

The human response to food carcinogens is highly variable. Mycotoxins, such as aflatoxin B1, are potent liver carcinogens while heterocyclic aromatic amines are correlated with colon cancer. Risk factors include low penetrant genes, lifestyle, and diet, but genetic factors that increase susceptibility to food carcinogens are still unknown. To identify low penetrant genes that confer carcinogen resistance we used *Saccharomyces cerevisiae* (budding yeast) as a model organism. Since many carcinogens require cytochrome P450 and phase II-mediated activation, we introduced the human CYP1A2 and NAT2 genes into the diploid yeast deletion collection. We screened both the yeast deletion collection containing the human genes and the original yeast deletion collection for resistance to aflatoxin B1 and the heterocyclic aromatic amines, 2-amino-3-methylimidazo[4,5-*f*] quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline (MeIQx). We performed BarSeq using the HiSeq Illumina platform and individual growth curves to confirm results. 85 aflatoxin resistance genes were identified that included those in nucleotide excision repair (NER, *RAD1*), recombinational repair (*RAD54, RAD57,CSM2*), DNA damage tolerance (*RAD18, RAD5*), cell cycle checkpoints, and protein degradation (*CUE1*). We observed that the SHU complex was important in conferring aflatoxin resistant and that *CSM2* was required for suppressing aflatoxin-associated mutations at the *CAN1 l*ocus. Only one aflatoxin resistant gene was identified from the library that did not express any CYP gene. The screen for IQ resistance identified 135 genes, including recombinational repair (*RAD54, RAD57*), base excision repair (*NTG*1), and DNA damage tolerance (*RAD18*). Double mutant combinations of NER and recombinational repair mutants, and NER and DNA damage tolerance genes, demonstrate synergistic sensitivity to aflatoxin. These studies thus underscore the importance of DNA damage tolerance pathways in conferring resistance to multiple carcinogenic chemicals. The libraries that we constructed will also be valuable in screening additional CYP-activated pharmaceuticals and carcinogens.

723C A genome-wide screen in the yeast *Saccharomyces cerevisiae* pinpoints protein kinase A as a major determinant of resistance to visible light *Anders Blomberg*[1] 1) Univ Gothenburg.

To provide mechanistic insight into cellular responses to visible light in the range 400 to 700 nm, we introduce a methodology for high-throughput screening of gene-to-light interactions using the yeast gene deletion collection. Detecting gene-light interactions on yeast fitness is challenging because of a strong dependence between the cell density in a yeast colony and the growth during light exposure. We identified 490 genes required for light resistance at physiologically relevant conditions using a semi-high throughput method and more than 20 genes were also validated to be sensitive to visible light in individual validation assays. Light-sensitive mutants were involved in a wide array of functions including MAPK signaling, protein translation and protein modification (in particular diphthamide biosynthesis) and there was a high degree of overlap with genes required for oxidative stress resistance. On the contrary, genes required for mitochondrial functions were strongly depleted in the sensitive set pointing to mitochondria being responsible for light-induced oxidative stress. Classification of mutants based on the nucleocytoplasmic shuttling of the stress responsive transcription factor Msn2 as well as on glycogen accumulation, indicated enhanced protein kinase A activity to be a common denomina-

tor in several of the light-sensitive mutants. We confirmed by use of a fluorescent PKA-reporter that PKA repression was essential for cells to grow upon illumination, and that a mutant with constantly high PKA was high sensitive to visible light. Taken together, our data links yeast light resistance to a pathway governing many metabolic and morphological outputs suggesting that light may well have shaped the evolution of baker's yeast and other non-photosynthetic organisms.

726C Abundances of transcripts, proteins, and metabolites in the cell cycle of budding yeast reveals coordinate control of lipid metabolism
Heidi Blank[1], Ophelia Papoulas[2,3], Narita Maitra[1], Riddhiman Garge[2,3], Brian Kennedy[4,5,6], Birgit Schilling[6], Edward Marcotte[2,3], Michael Polymenis[1] 1) Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX; 2) Center for Systems and Synthetic Biology, University of Texas, Austin, TX; 3) Department of Molecular Biosciences, University of Texas, Austin, TX; 4) Departments of Biochemistry and Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 5) Centre for Healthy Ageing, National University of Singapore, National University Health System, Singapore; 6) Buck Institute for Research on Aging, Novato, CA.

Establishing the pattern of abundance of molecules of interest during cell division has been a long-standing goal of cell cycle studies. In several systems, including the budding yeast *Saccharomyces cerevisiae*, cell cycle-dependent changes in the transcriptome are well studied. In contrast, few studies queried the proteome during cell division, and they are often plagued by low agreement with each other and with previous transcriptomic datasets. There is also little information about dynamic changes in the levels of metabolites and lipids in the cell cycle. Here, for the first time in any system, we present experiment-matched datasets of the levels of RNAs, proteins, metabolites, and lipids from un-arrested, growing, and synchronously dividing yeast cells. Overall, transcript and protein levels were correlated, but specific processes that appeared to change at the RNA level (e.g., ribosome biogenesis), did not so at the protein level, and vice versa. We also found no significant changes in codon usage or the ribosome content during the cell cycle. We describe an unexpected mitotic peak in the abundance of ergosterol and thiamine biosynthesis enzymes. Although the levels of several metabolites changed in the cell cycle, by far the most significant changes were in the lipid repertoire, with phospholipids and triglycerides peaking strongly late in the cell cycle. Our findings provide an integrated view of the abundance of biomolecules in the eukaryotic cell cycle and point to a coordinate mitotic control of lipid metabolism.

729C Investigation of helicases and TERRA non-coding RNAs in telomere maintenance *Taizina Momtareen*[1] 1) West Virginia University.

Yeast (*Saccharomyces cerevisiae*) telomeres are characterized as T(G_{1-3}) repeats coated with a variety of proteins. There are replication, processing, and recombination proteins among which are helicases Sgs1 and Y'-Help1, and the long noncoding TERRA RNAs. Telomeres progressively shorten with every cell division. Cells with critically short telomeres and no telomerase undergo senescence and cell death. However, a subset of these telomerase null mutants can escape senescence by elongating their telomeres using recombination and they are classified into types I and II survivors. This project aims to investigate the processes that generate and maintain these survivor yeast cells. Sgs1 is crucial in telomerase independent maintenance of telomeres. It is the yeast homolog of the bacterial RecQ helicase and the human WRN protein, the mutation of which causes Werner's syndrome. Sgs1 takes part in telomere processing by causing C-strand resection of telomeres. In contrast, TERRAs are long non-coding RNAs transcribed from the telomeres and implicated in several functions such as promoting the formation of heterochromatin, G-quadruplexes, and R-loops. Based on the needs of the cell, TERRAs protect telomeres from the DNA repair machinery or promote homologous recombination. Both Sgs1 and TERRAs are essential for the survival of the type II telomerase mutant cells. Thus, one of my aims is to determine how Sgs1 and TERRAs regulate telomere biology in type II survivors. On the other hand, type I survivors require the subtelomeric Y' elements for telomere elongation via recombination. The *YRF1* genes of the Y' elements presumably encode a helicase that according to my hypothesis, aid the telomere elongation process. To explore the level of expression of *YRF1*, qRT-PCR was carried out for all seven of the *YRF1* paralogs. However, expression could not be detected for any of them. Moreover, overexpressing *YRF1-4* paralog from a plasmid could not rescue growth of cells with hydroxyurea induced DNA damage. From this, I have learned that *YRF1* is not expressed in detectable levels in WT and sgs1 mutants and thus further experiments should be conducted in type I telomerase null mutants where increased Y' element copy numbers are observed. Furthermore, I want to discover the underlying mechanisms that cause a cell to become either type I or type II. Results will lead to a deeper understanding of the workings of cells that do not express telomerase.

730A A yeast DNA replication chaperone regulates cellular location of a retrotransposon Gag protein. Jill Keeney[1], *Haley Smith*[1] 1) Juniata College.

The Rtt105 protein (Regulator of Ty1 transposition 105) of *Saccharomyces cerevisiae* has been shown to function as a chaperone for Replication Protein A (RPA), which protects ssDNA regions at replication forks during DNA replication. Rtt105 is thought to escort RPA to the nucleus and facilitate loading at replication forks. RTT105 was first identified in a screen for host regulators of Ty1 transposition and thus named Regulator of Ty Transposition. Ty1 is a retroelement that is transcribed to RNA and then reversed transcribed to DNA and integrated into the host genome. Our lab has found that in wild type yeast cells, the Ty1 Gag protein is located in cytoplasmic foci; however, when cells are stressed with hydroxyurea (HU), the Ty1 Gag is transported to the vacuole. In the absence of Rtt105 protein, Ty1 Gag protein remains in cytoplasmic foci under stress, suggesting that Rtt105 may also be a chaperone for Ty1 Gag. The goal of this project has been to mutate Rtt105 in multiple positions and to determine if the transport of the Tyl Gag protein to the vacuole is inhibited by these mutations. Microscopy was used to localize fluorescently tagged Gag protein within the yeast cell. With many of these mutant Rtt105 proteins, Ty1 Gag stayed in the cytoplasmic foci in both stressed and non-stressed conditions. This data supports the hypothesis that Rtt105 plays a role in transporting the Ty1 Gag protein within the cell.

731B Molecular determinants of Ty1 reverse transcription initiation in yeast Jill Keeney[1], *Aine Boudreau*[1] 1) Juniata College.

The *S. cerevisiae* genome contains several Ty retroelements, segments of DNA which produce virus-like particles (VLPs) containing mRNA that can be reverse-transcribed into cDNA and integrated into the genome. The Ty element has two products analogous to retroviral proteins: Gag and a Gag-Pol. Pol is cleaved into three separate proteins: protease, reverse transcriptase (RT), and integrase, which along with the mRNA transcript and a tRNA, are packaged into a VLP made of Gag. In the first step of reverse transcription, $tRNA_i^{Met}$ binds to the Ty1 mRNA primer-binding site (PBS), which has complementarity to the $tRNA_i^{Met}$ acceptor stem, providing a primer for reverse transcriptase. This DNA strand is subsequently used as a template for the complementary strand, generating a double-stranded cDNA that is integrated into the genome. Previous work has shown that Ty1 transposition is substantially reduced by mutations outside of the $tRNA_i^{Met}$ acceptor stem, suggesting that the tertiary structure of $tRNA_i^{Met}$ has a significant role in the first priming step. Potential interaction sites can be explored by characterizing the interaction of Ty1 mutants that rescue transposition with mutant tRNA. We screened a library of mutations directed to distinct Gag and Pol regions of Ty1 for variations that rescue transposition using a mutant $tRNA_i^{Met}$. Ty1 transposition in yeast is assayed using a galactose-inducible plasmid-borne Ty1 element. The Ty1 element also contains

a marker gene (*his3AI*) to select for transposition on media lacking histidine. Cells are patched to selective media to hold the plasmid, replica-plated to galactose-containing media to induce Ty1 transcription, and replica-plated to media lacking histidine to select for transposition. The first step in this project is to rescue selected Ty1 mutants and characterize their compatibility with wild type and mutant $tRNA_i^{Met}$ primer at permissive and non-permissive temperatures. Transposition assays have identified Ty1 mutants with compromised transposition at an elevated temperature and mutants that do not transpose with wild type $tRNA_i^{Met}$ at any temperature. Selected Ty1 mutants will be sequenced and also be tested for protein stability at the temperatures used in transposition assays.

745A Localizing *tra-2* mRNA in germ cells *Lauren Skelly*[1,2], Melissa Davis[1], Eric Haag[1,2] 1) Department of Biology, University of Maryland, College Park; 2) Biological Sciences Graduate Program, University of Maryland, College Park.

C. elegans hermaphrodites are essentially female worms that have achieved self-fertility. Making sperm in an otherwise female body requires precise regulation of the feminizing gene, *tra-2*. While TRA-2 is ubiquitously expressed in the soma, its regulation is of special interest in the germ line where sperm are specified. There are two major mRNA isoforms of *tra-2*, *tra-2a* (4.7 kb) and *tra-2b* (1.8kb) [1]. The 1.8 kb transcript is specific to the hermaphrodite germline [1, 2]. The 4.7 kb transcript is predicted to be a transmembrane receptor for the male-specific protein HER-1 [3]. HER-1 represses *tra-2* activity to allow male cell fates in XO animals, but is not expressed in XX hermaphrodites [4]. Instead, available evidence suggests that hermaphrodites repress *tra-2* by the binding of a GLD-1/FOG-2 heterodimer to its 3' UTR [5, 6]. While *tra-2* mRNA is abundant in the germline, epitope-tagged TRA-2 (TRA-2::HA) was undetectable in the presence of GLD-1 and FOG-2, and only slightly elevated in their absence [7]. We are now investigating the regulation of *tra-2* at the mRNA level using single-molecule fluorescence in-situ hybridization (sm-FISH). We hypothesized that the 4.7 kb version of *tra-2* is not present in the hermaphrodite germline, as the 1.8 kb transcript contains all the domains necessary for interaction with both *fem-3* and *tra-1* [8, 9]. The data suggest *tra-2a* is expressed in the germ line, but at lower levels than *tra-2b*. This indicates some TRA-2 membrane protein makes it to the ER, and perhaps even to the surface of hermaphrodite germ cells. Full-length TRA-2 could then be processed by TRA-3 as it is in the soma. *tra-2b* is consistently expressed in the rachis and oocytes of adult worms. We will further investigate *tra-2* regulation by using sm-FISH in a mutant that lacks GLD-1 binding sites on the 3'UTR (*tra-2(e2020)*). We expect this RNA to be de-regulated and potentially localize differently than wild type. We are also investigating the mechanism of FOG-2 action, with an emphasis on identification of additional protein-protein interactions.

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747C Regulation of the oxidative stress response by ZTF-17 Cindy Tran[1], *Terry Kubiseski*[1] 1) York Univ.

Reactive oxygen species (ROS) are common by-products of normal cellular metabolism and have important physiological roles in cell signaling and homeostasis. However, when there is an excess production of ROS, a dangerous condition known as oxidative stress occurs whereby the body is overwhelmed and unable to detoxify these free radicals. ROS readily react with other macromolecules causing damage to DNA, lipids and proteins, severely compromising cell health and contributes to the onset of age-associated diseases. Many organisms have devised antioxidant systems to protect themselves and in *Caenorhabditis elegans,* two important transcription factors, SKN-1/Nrf2 and DAF-16/FOXO promote the expression of stress resistance genes. Phase II detoxifying genes such as *gst-4* is expressed through SKN-1, while *sod-3* is under DAF-16 control, and both confer stress resistance when activated. When an RNAi against transcription factor ZTF-17 was used, enhanced *gst-4p::gfp* expression was observed suggesting that ZTF-17 possessed repressor like functions. ZTF-17 is uncharacterized but its mammalian homolog, ZFP42/REX1, is a pluripotency factor that represses transcription of the *Xist* gene during X-chromosome inactivation. We observed that *ztf-17(tm963)* deletion mutants had increased *gst-4p::gfp* and *sod-3p::gfp* expression and confirmed by qRT-PCR that mRNA levels of both genes were significantly enhanced when compared to wild type. Although the detoxification process exists, the mechanism by which the levels of free radicals are regulated, and the molecular players involved in maintaining proper function under oxidative stress remains unclear. Our lab aims to investigate ZTF-17's function along with characterizing its role as a potential negative regulator of SKN-1 and DAF-16 target genes implicated in the oxidative stress response, lifespan and longevity.

757A GFP Tagging interferes with myosin assembly and function *Michael Russell*[1], Savannah Shaw[1], Jasmine Vo[1], Kia Skipper[1], Ryan Littlefield[1] 1) University of South Alabama.

In animals, myosin heavy chain (MHC) II motors undergo rapid attachment/detachment cycles on actin filaments to generate the mechanical forces and movement responsible for muscle and non-muscle contraction. To investigate MHC II assembly into muscle thick filaments within an intact organism, we used CRISPR-based homologous repair to generate GFP-MyoB, an N-terminal fusion of GFP and unc-54, the major MHC II isoform in C. elegans body wall muscle (BWM) and vulval muscle (VM). GFP-myoB heterozygous strains (GFP/+) display normal sarcomere organization and function, however GFP-myoB homozygous strains (GFP/GFP) display abnormal assembly and function in the BMW compared to the wildtype (+/+). To assess BWM assembly, organization and function, we took live images of developing embryos using single plane illumination microscopy (SPIM), confocal microscopy of live adults, swimming assays. In swimming assays, movement of GFP/GFP worms was reduced by ~50% compared to +/+ and GFP/+ worms. Defects in muscle function and structure appeared at initial myofibril assembly in developing GFP/GFP embryos, Surprisingly, while GFP/+ worms began twitching and moving within the egg, many GFP/GFP worms displayed a hypercontracted appearance with reduced twitching and moving and we observed a significant reduction in hatching. Within GFP/GFP adults, BWM thick filaments appeared as non-uniform clusters instead of broad, well-aligned oblique striations found in +/+ and GFP/+ adults. In addition, egg laying assays showed that GFP/GFP and GFP/+ worms laid fewer eggs than +/+ worms, which indicates that vulva muscle function is also impaired by GFP tagging of myoB. We suggest that GFP sterically interferes with the efficient detachment of myosin motors from actin filaments and that this process is important for myofibril assembly, organiza-

tion, and function in C. elegans.

758B Visualizing the conformation of titin in live C. elegans body wall muscle *Gabrielle Prince*[1], Ryan Littlefield[1] 1) University of South Alabama.

Titin is a giant, modular protein that connects the myosin – containing thick filament to the Z-line of the sarcomere (mammalian cells) or dense bodies (Z-line equivalent in the nematode worm *C. elegans*). In vertebrates, the assembly, organization, and mechanical properties of Titin have been studied extensively, but little is known about how the TTN-1 ortholog functions in *C. elegans*. To investigate the functional role of TTN-1 in *C. elegans* muscles, we used nested CRISPR gene editing to generate translational fusions of TTN-1 with a fluorescent tag (wrmScarlet) at either the N-terminus (exon 1) or in the middle (exon 25) of the molecule. By confocal microscopy, wrmScarlet appeared as narrow, continuous striations within body wall muscle (BWM) in all stages from late embryos to adults for both modifications. By super-resolution instant Structured Illumination Microscopy (iSIM), wrmScarlet appeared as non-uniform, yet relatively continuous striations, distinct from the punctate appearance of alpha-actinin or integrins located within dense bodies. Our data indicates that the N-terminus of TTN-1 is not restricted to dense bodies and suggests that TTN-1 is organized into a semi-continuous structure that extends between dense bodies may help sarcomeres form oblique striations. Our gene edited TTN-1 strains will be useful for further investigating how BWM sarcomere lengths respond during movement and growth.

761B Regulation and function of the *odd-skipped 2* transcription factor in *C. elegans* Jonathan Rappi[1], Gregory Carlson[1], *Amy Groth*[1] 1) Eastern Connecticut State University.

Zinc-finger transcription factors from the conserved *odd-skipped* (*odd*) family play roles in a variety of developmental signaling pathways. There are two *odd* homologs in *C. elegans*, *odd-1* and *odd-2*. Both genes are expressed in the intestine, and *odd-2* is also expressed in the rectal gland cells. An *odd-2* mutation is larval lethal, while an *odd-1* mutant has less than 10% combined embryonic and larval lethality. The zinc finger region of *odd-2* is more closely related to both human homologs (*odd-skipped related (OSR) 1* and *2*). Further, because they have the same number of zinc fingers, *odd-2* in *C. elegans* is structurally more similar to the mammalian *OSR1* than *OSR2*. Experiments were conducted to identify genes that regulate *odd-2* expression and to identify potential regulatory targets of *odd-2.* Twenty-one candidate transcription factors were screened by RNAi in an ODD-2::GFP reporter (JR2005). Knockdown of *fos-1* by RNAi led to ectopic expression of ODD-2::GFP in the germline. Experiments are being conducted to determine which cells ectopically express ODD-2. RNAi experiments were also conducted to identify possible downstream targets of ODD-2. *Odd* genes from Xenopus and mammals have been shown to affect the expression of genes in the Wnt signaling pathway. Here, studies were conducted to explore the relationship between *odd-2* and the Wnt signaling pathway in *C. elegans*. Knocking down *odd-2* by RNAi in L4 worms in an *rrf-3(pk1426)* background had no effect on the expression of a GSK-3 reporter (LP538) or an EGL-20 (canonical Wnt ligand) reporter (CF1045) in early larvae. The other four Wnt ligands in *C. elegans* have not yet been tested. Early larvae were chosen for analysis because of the larval lethality caused by knockdown of *odd*-2, but later stage experiments can potentially be done by RNAi feeding of L1 larvae.

773B Functional screen identifies factors that coordinate with miRNAs to regulate gene expression in *C.elegans* *Shilpa Hebbar*[1], Isana Veksler-Lublinsky[2], Anna Zinovyeva[1] 1) Kansas State University, Kansas; 2) Ben-Gurion University of the Negev, Israel.

Regulation of gene expression is essential for normal physiology and development. One of the ways cells regulate gene expression is through a class of non-coding RNAs called microRNAs (miRNAs). miRNAs associate with Argonaute proteins to form miRNA Induced Silencing Complexes (miRISCs), which post-transcriptionally repress gene expression by targeting mRNA 3'UTRs through partial sequence complementarity. We have previously identified proteins that co-precipitate with the miRISC component, Argonaute ALG-1. In addition, we also identified potential physical interactors of specific miRNAs. Gene ontology (GO) places these factors into multiple functional groups, including factors in the ribonucleoprotein complex and mRNA processing factors. To determine which ALG-1 and miRNA interactors are important for miRNA activity and/or processing, we carried out functional assays in several genetically sensitized backgrounds in *C. elegans*. Specifically, we used RNAi to knock down 56 factors in animals with compromised miRNA activity. We found that RNAi of approximately 30 genes modified the phenotypes associated with the reduction of miRNA activity in three distinct miRNA sensitized backgrounds. One such factor that strongly enhanced miRNA mutant phenotypes was *hrp-1,* a homolog of human hnRNP A1 protein. We are currently identifying whether *hrp-1* modulates miRNA gene-regulatory activity through affecting miRNA biogenesis or miRISC function or acts indirectly by regulating mRNA targets. We ultimately aim to characterize the mechanisms through which this and other factors co-ordinate with miRNA-mediated gene regulation.

779B Comparative proteomics reveal Me31B's interactome dynamics, expression regulation, and assembly mechanism into germ granules during *Drosophila* germline development *Aidan McCambridge*[1], Dhruv Solanki[1], Nicholas Olchawa[1], Neal Govani[1], Carol Dilts[1], Jonathan Trinidad[2], Ming Gao[1] 1) Indiana University Northwest, Gary, IN; 2) Indiana University, Bloomington, IN.

Me31B is a protein component of *Drosophila* germ granules and plays an important role in germline development by interacting with other proteins and RNAs. To understand the dynamic changes that the Me31B interactome undergoes from oogenesis to early embryogenesis, we characterized the early embryo Me31B interactome and compared it to the known ovary interactome. The two interactomes shared RNA regulation proteins, glycolytic enzymes, and cytoskeleton/motor proteins, but the core germ plasm proteins Vas, Tud, and Aub were significantly decreased in the embryo interactome. Our follow-up on two proteins, Tral and Cup, present in both interactomes revealed that they likely colocalize with Me31B in different types of ribonucleoprotein (RNP) granules throughout oogenesis. We further show that Tral and Cup are both needed for maintaining Me31B protein and mRNA stability, with Tral's effect being more specific. In addition, we provide evidence that Me31B likely colocalizes and interacts with germ plasm marker Vas in the ovaries and early embryo germ granules. Finally, we show that Me31B's localization in the germ plasm is likely independent of the Osk-Vas-Tud-Aub germ plasm assembly pathway, although its proper enrichment in the germ plasm may still rely on conserved germ plasm proteins such as Aub.

783C The early transcription factors CLAMP and Zelda cooperate to regulate the maternal-to-zygotic transition in *Drosophila* early embryos *Jingyue Duan*[1], Leila Rieder[2], Erica Larschan[1] 1) Brown University; 2) Emory University.

After fertilization, compact germ cell genomes undergo dramatic reprogramming to remove the epigenetic makeup from the previous generation, producing a totipotent embryo. This process is largely driven by maternally deposited proteins and mRNAs since the zygotic genome remains transcriptionally quiescent. Zygotic genome activation (ZGA) occurs when transcription factors (TFs) activate transcription globally. However, only a few TFs, called pioneer TFs, can bind directly to chromatin as remodelers prior to ZGA. In *Drosophila*, the ZGA protein Zelda plays an essential role

as a pioneer TF to increase the binding accessibility of DNA to other TFs. Moreover, there are likely to be other pioneer TFs in *Drosophila* that have not yet been identified. Chromatin-linked adaptor for Male-specific lethal (MSL) proteins (CLAMP) regulates dosage compensation in males, but also binds throughout the early embryonic genome and is required for embryos to progress through ZGA. However, the functional relationship between CLAMP and Zelda, two key early TFs, has not been investigated.
Here, we depleted maternally deposited Zelda or CLAMP and used ChIP-seq to define the relationship between CLAMP and Zelda before ZGA (0-2hr) and after ZGA (2-4hr) in the early *Drosophila* embryo. We found synergistic binding between CLAMP and Zelda, suggesting a cooperative interaction largely at promoters of genes regulated by both proteins. Depletion of maternally-deposited CLAMP reduces Zelda binding before and after ZGA. However, depletion of maternally-deposited Zelda mainly influences CLAMP binding before ZGA. Moreover, we characterized the similarity and differences between sites that CLAMP and Zelda bind either dependently or independently of each other. The CLAMP and Zelda motifs are both present at dependent and independent sites which both regulate target genes with TF activity. Interestingly, we found that dependent sites are enriched for the motif of the required protein in a broad peak binding pattern at promoter regions, while independent sites are narrow peaks that are mainly located in introns. Furthermore, maternal depletion of Zelda or CLAMP also significantly decreased the transcription level of genes at dependent sites more than independent sites. Taken together, our results reveal that CLAMP and Zelda work individually at intronic sites and cooperatively at promoters to facilitate the genomic remodeling that drives transcriptional activation during ZGA.

790A Roles of Hippo and Ecdysone Receptor Signaling in the regulation of *dronc* *Karishma Gangwani*[1], Amit Singh[1,2,3], Madhuri Kango-Singh[1,2,3,4] 1) Department of Biology; 2) Center for Tissue Regeneration and Engineering at Dayton (TREND); 3) Pre-medical program; 4) Integrative Science and Engineering, University of Dayton OH 45469 .

The Hippo pathway is an evolutionarily conserved pathway that regulates organ size and tissue homeostasis in *Drosophila* and mammals. The pathway functions by regulating the nuclear availability of transcriptional cofactor Yorkie (Yki), mammalian YAP, which is regulated by the activity of a core kinase cascade comprising the serine-threonine kinases Hippo (Hpo) and Warts (Wts) and their accessory proteins. Yki binds with transcription factors like Scalloped (Sd) or Homothorax (Hth) to regulate target genes involved in cell proliferation and survival. Downregulation of the Hpo pathway causes increased cell proliferation and overgrowth, whereas hyperactivation of this pathway leads to cell death due to the activation of caspases. Caspase proteins are cysteine aspartic proteases which play essential roles in cellular signaling and development via apoptosis. We showed that the initiator caspase *dronc* (mammalian Caspase 9) is a transcriptional target of Yki. We found that loss of Hippo signaling leads to the downregulation of *dronc* expression, whereas downregulation of Sd resulted in upregulation of *dronc* expression. We also found that known binding partner of Sd like E2F1 is also involved in regulating *dronc* expression. Earlier studies have shown that *dronc* expression is regulated by the Ecdysone receptor (EcR) signaling pathway and an EcR regulatory element has been identified on the *dronc* promoter. We hypothesize that *dronc* expression is regulated by the Hippo and EcR signaling pathways. Using genetic interaction and epistasis approaches, we found that depletion of EcR or its corepressors like Smrter caused upregulation of *dronc* expression. Regulation of *dronc* expression may also involve Taiman (Tai) a binding partner of both EcR and Yki. Here, we present our work on the regulation of *dronc* by the Hippo and EcR signaling pathways, and its implications on development.

795C Regulation of repeat-induced silencing and position-effect variegation by genomic position, developmental timing and the TOR pathway *Emily Holmquist*[1,2], Adam Smiley[1], Nhi Vuong[1], Spencer Tye[3], Andrew Arsham[1] 1) Bemidji State University; 2) North Hennepin Community College; 3) University of Kansas.

Large percentages of eukaryotic genomes are composed of repetitive DNA sequences, including tandem repeats and interspersed repeats. The transcriptional silencing of repetitive sequence elements by heterochromatin is integral for the maintenance of gene expression and genomic integrity. It is unknown if the silencing triggered by different types of repetitive elements occurs through distinct or similar mechanisms. A 256-copy tandem array of the E. coli lac operator sequence (LacO) can trigger variegation of a downstream white+ reporter gene in Drosophila melanogaster when inserted into certain euchromatic genomic locations proximal to large blocks of heterochromatin. This stochastic silencing of the reporter gene is dependent on heterochromatin protein 1a and histone deacetylation machinery but appears to be independent of histone H3K9 methyltransferases and the piRNA pathway. While variegation is dependent on a combination of cis-acting sequences and local chromatin landscape, it is also modified by environmental conditions. Both inhibition of the TOR pathway with rapamycin and rearing the organisms at 18°C suppress variegation, leading to increased area and intensity of pigmentation in the eye. Concentrations of rapamycin that are insufficient to delay development are nonetheless sufficient to suppress variegation suggesting that the mechanism is independent from developmental timing and that temperature and rapamycin-induced suppression of variegation may operate through distinct molecular mechanisms converging on the establishment and/or maintenance of heterochromatin.

822C -Understanding Molecular Mechanisms Controlling Fiber Type-specific Expression of Muscle Genes *Max Andrews*[1], Anton Bryantsev[1] 1) Kennesaw State University.

Our lab studies how distinct complex traits arise during differentiation of muscle fibers. Adult skeletal muscles are represented by several muscle fiber types, each having unique molecular properties. Notably, embryonic muscles are homogeneously made of the same fiber type, but acquire distinct traits during subsequent differentiation. The regulation of muscle fiber diversity is not clear, although it may be critical in treating muscle-related diseases and enhancing physical performance.

In this study, we searched for critical genes and mechanisms that control expression of muscle-specific genes, using *Drosophila* muscle actin *Act57B* as a model. *Act57B* is expressed pan-specifically in embryonic and larval musculature, but becomes excluded from many specialized adult muscles. One example of such muscles is the flight muscles (IFMs) that express a different actin gene. Activity of *Act57B* in embryonic muscles strongly depends on the myogenic factor MEF2, which, however, continues its expression in IFMs and other adult muscles after metamorphosis. To understand such disconnect between MEF2 and *Act57B* expression, we quantified activity of *Act57B LacZ*-based genetic reporter in IFMs which expressed altered levels of MEF2. Our data indicate that MEF2 switches roles and becomes a repressor of *Act57* in IFMs.

To get an insight into MEF2 reprogramming, we carried out an RNAi screen, looking for outcomes where repressive action of MEF2 is reversed and *Act57B* expression re-activated in IFMs. After testing over 200 genes, we identified several hit-producing genes among chromatin remodelers and structural chromatin components. We speculate that MEF2 binds a repressive co-factor (e.g. Gug), which subsequently brings histone-modifying factors (e.g. G9a) to the *Act57* enhancer/promoter. Methylated histones attract structural components (e.g. Chro) to induce chromatin condensa-

tion and shut down expression of *Act57B*. Our future efforts will be to validate my findings by alternative methods, including testing for the physical interaction between *Mef2* and *Gug* and other hit-producing gene, their levels of expression in different muscles.

823A Developmental and functional analysis of *kelch* mRNA stop codon readthrough in Drosophila *Nicholas Szabo*[1], Andrew Hudson[1], Norma Wills[2], Gary Loughran[3], John Atkins[2, 3], Lynn Cooley[1] 1) Yale University School of Medicine, New Haven, CT; 2) University of Utah, Salt Lake City, UT; 3) University College Cork, Cork, Ireland.

Expanding evidence shows that stop codon readthrough during translation occurs in many eukaryotes, including Drosophila, yeast, and humans. Recoding of one of three stop codons to a sense codon allows the ribosome to produce C-terminally extended proteins. We are investigating the biological significance of tissue-specific stop codon readthrough during translation of Drosophila *kelch*. This gene is unusual in that it produces two protein products: a 76 kDa (ORF1) protein as well as a larger 160 kDa full-length (ORF1 + ORF2) protein resulting from readthrough of a natural UGA stop codon. The ORF1 product has a well-defined role in oogenesis, where it functions as a component of an E3 ubiquitin ligase; the function of ORF2 in full-length Kelch is unknown. Immunoblot analysis using an antibody against Kelch ORF1 revealed high levels of readthrough in the central nervous system. To further investigate Kelch readthrough expression during Drosophila development, we used CRISPR/Cas9-mediated homology-directed repair (HDR) to insert sensitive reporters of *kelch* readthrough translation. Western analysis of these reporters confirmed high levels of readthrough in the CNS compared to other tissues, and we determined the pattern of readthrough expression within the CNS using fluorescence microscopy. We also established a dual-luciferase/GFP reporter system to analyze *cis*-acting sequences surrounding the ORF1 stop codon. We defined a ~200 bp minimal fragment flanking the stop codon that promotes readthrough in both transfected human cells and transgenic Drosophila. Expression of these reporters using cell type-specific Gal4 drivers revealed that within the CNS, neurons, and not glia, support readthrough. Finally, we created mutations in *kelch* that result in either constitutive readthrough and full-length protein, or truncated Kelch lacking ORF2. Constitutive expression of full-length protein in the ovary impairs Kelch function, leading to altered ring canal structure. Analysis of phenotypes caused by mutations that eliminate ORF2 is underway.

831C Investigating the binding mechanism of germ plasm protein Me31B and Tudor *Neal Govani*[1], Aidan McCambridge[1], Ming Gao[1] 1) Indiana University Northwest.

Drosophila germ plasm proteins Me31B and Tudor are important for germ cell development. The helicase Me31B and the conserved germ plasm protein Tudor have been previously showed to physically interact. To unravel the interaction mechanism, we aim to investigate the Tudor-binding motifs of Me31B. Full length, N-terminal, and C-terminal Me31B expression plasmids were constructed and used to express the protein domains in S2 cell culture. HA-tagged Tudor proteins were purified from fly ovaries and used in an *in vitro*binding assay with the Me31B protein domains above. Preliminary results showed that C-terminal has the highest binding affinity for Tudor, however, some binding was noticed on N-terminal Me31B. To further validate our results, we are conducting the binding assay in reverse: use HA-Tud as “bait” protein and add purified Me31B domains to the bait. Our study further elucidates the interaction mechanism of germ plasm proteins and how they contribute to germ plasm assembly.

840C Integration of BMP, JAK/STAT and EGFR signaling in the Drosophila egg chamber during anterior-posterior fate determination *Kelvin Ip*[1], Scott De Vito[1], Baptiste Rafanel[1], Laura Nilson[1] 1) McGill University, Montreal, Canada.

Each *Drosophila* oocyte is surrounded by an overlying follicular epithelium which eventually produces the eggshell. The *Drosophila* eggshell displays an anterior-posterior and a dorsal-ventral axis. The establishment of these axes involves the localized secretion of Gurken (Grk) around the oocyte nucleus which induces EGFR signaling in the overlying follicle epithelium.

Depending on the location, Grk induces one of two different transcription factorsthat determine follicle cell fate: *midline* (*mid*) in posterior follicle cells during early oogenesis and *mirror* (*mirr*) in dorsal-anterior follicle cells in mid-oogenesis. Two additional secreted ligands – Unpaired (Upd), a JAK/STAT signaling ligand, and Decapentaplegic (Dpp), a BMP signaling ligand– were previously found to determine the choice between these alternative EGFR signaling outcomes. At the anterior, Dpp is required for *mirr* expression and also independently represses *mid*; the posterior Upd is required for *mid* expression and also independently represses *mirr*. In addition, this choice between *mid* and *mirr* expression is also regulated by their mutual repression.

To ask how *mid* and *mirr* integrate multiple signaling inputs from Grk, Upd, and Dpp to give a final binary output, we identified *cis*-regulatory modules (CRMs) from the *mid/H15* and *mirr* loci that generate patterned reporter expression in this tissue and assessed their response to the different known signaling pathway inputs.

We focused first on the *mid* locus, for which we identified two putative CRMs driving posterior expression. While both CRMs are active in posterior follicle cells, they differ in expression boundary and their response to signaling inputs. One of the *mid* CRMs is responsive to BMP signaling but not *mirr*. Its expression boundary is expanded towards anterior. Deletion of the BMP responsive site resulted in a further expansion of the expression domain. These observations are consistent with our model in which both *mirr* and Dpp inputs at the anterior are important in restricting *mid* expression at the posterior follicle cells. By identifying and mutating responsive elements to the signaling inputs in the CRMs, we hope to dissect the regulatory network behind the AP patterning of follicular epithelium and determine the role of different regulatory inputs (i.e. Grk, Upd and Dpp) in establishing the boundary between *mid* and *mirr* expression domains, and/or the robustness of fate choice.

846C Investigating the Regulation of Position-Effect Variegation by Cis-Acting Repetitive Elements and Transgene Expression *Nhi Vuong*[1,2], Adam Smiley[1], Christina Yang[1,2], Emily Holmquist[1,2], Brandon Tran[1], Luke Ziegler[1], Melinda Natysin[1,2], Heidi Pipkin[1,2], Andrew Arsham[1] 1) Bemidji State University, Bemidji, MN 56601-2699; 2) North Hennepin Community College, Brooklyn Park, MN 55445.

Investigating the Regulation of Position-Effect Variegation by Cis-Acting Repetitive Elements and Transgene Expression

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Eukaryotic genomes contain two basic types of chromatin: euchromatin, which is gene-rich and general transcriptionally active, and heterochromatin, which is typically gene-poor and transcriptionally silent. Heterochromatin plays several vital roles in the eukaryotic chromosome, including the regulation of gene expression through transcriptional silencing. Genes placed in proximity to heterochromatin by transposition or chromosomal rearrangement are incompletely silenced, a phenomenon called position-effect variegation first observed by Hermann Muller in the 1920’s. We use

a transposable eye pigmentation reporter construct to study the formation and maintenance of heterochromatin as a function of genomic position in *Drosophila melanogaster*. The transgene includes an FRT-flanked 256-copy tandem array of the *E. coli* lac operator (LacO), a *white+* reporter gene, and a loxP-flanked *yellow+* secondary reporter gene. Eye color-variegating insertions of this reporter were isolated from a transposition screen and the repeat-dependence of silencing in each line was characterized by removing the LacO array with FLP recombinase. For each insertion we also tested whether a nearby highly expressed (and presumably euchromatic) *yellow+* transgene had any effect on variegation in cis by removing it with Cre recombinase. Some insertions appear prone to allelic trans-silencing or transvection, but the mechanism and prevalence of this phenotype are not clear due to the high rate of homozygous lethality of transposon insertions. Our findings show that LacO repeats which are not likely to be present in existing piRNA clusters can nonetheless trigger heterochromatin formation in gene-rich euchromatic regions.

848B Epigenetic Mediated Neuroprotection by Tip60 in a Drosophila Model of ALS *Visha Parmar*[1], Mariah Beaver[1], Akanksha Bhatnagar[1], Felice Elefant[1] 1) Drexel University.

Histone acetylation is a type of epigenetic modification that is regulated by histone acetyltransferases (HAT) and histone deacetylases (HDAC). This antagonistic activity under homeostasis controls chromatin accessibility that mediates gene expression critical for proper neuronal and cognitive function. Disruption of this fine-tuned HAT/HDAC balance is believed to contribute to early deficits in synaptic and cognitive function found in Alzheimer's disease (AD). Although attempts have been made to restore histone acetylation homeostasis using general HDAC inhibitors, efforts to target and manipulate HAT activity as a means for therapeutic specificity remains to be explored. Our more recent studies revealed that alteration of Tip60/HDAC2 balance is an early common feature in *Drosophila* models of Parkinson's disease (PD), Huntington's Disease (HD), and Amyotrophic Lateral Sclerosis (ALS). We showed that increasing Tip60 HAT levels in the brain protects against cognitive deficits in *Drosophila* models of PD and HD. Here, we ask whether cognitive deficits in a *Drosophila* model of ALS can also be protected against by increasing Tip60 in the brain.

ALS is a progressive neurodegenerative disease that results in degeneration of motor neurons in the brain and spinal cord, leading to paralysis, locomotive defects, and cognitive impairments. Genetic studies have shown that decreased HAT and increased HDAC activities result in repression of critical genes, contributing to the onset of ALS. The *Drosophila* ALS model used overexpresses Vap-33-1, a vesicle-associated membrane protein B (VAPB) protein homolog that normally regulates neuronal and neuromuscular junction (NMJ) growth, protein trafficking, and ER homeostasis. **I hypothesized that increasing Tip60 HAT levels will rescue cognitive, synaptic, and NMJ defects in the ALS model.** Cognitive defects were assessed by conducting a single odor paradigm for olfactory associative learning using sucrose as the gustatory reinforcer and Linalool as the associative odor. NMJ area relative to muscle 6/7 area, total, Type-Ib, Type-Is, and satellite boutons were quantified by visualizing NMJ located between muscles 6/7 of segment A3 of third instar larvae to assess for defects in synaptic plasticity. We show that ALS larvae exhibited defects in learning and short-term memory which were partially rescued by increasing Tip60 HAT levels. An increase in Type-Ib, Type-Is, satellite, and total boutons per NMJ were observed with no increase in NMJ area. Together, our findings reveal that Tip60 imbalance is a general early feature in ALS and support a neuroprotective role for Tip60 in cognitive defects associated with ALS.

862A Characterization of HP1 Double-Mutants in *Drosophila melanogaster* *Sarah Sims*[1], Mina Momeni[1], Nicole Riddle[1] 1) University of Alabama at Birmingham.

Chromatin, a complex of DNA wound tightly around histone proteins, occurs in two forms: euchromatin, which is typically transcriptionally active, and heterochromatin, which is typically transcriptionally silent. Many proteins are involved in maintaining chromatin states, including the Heterochromatin Protein 1 (HP1) family. Drosophila melanogaster has three somatic homologs of the HP1 family: HP1a, HP1B, and HP1C. The effects of removing individual HP1 homologs has been investigated; however, little research has been performed on the loss of multiple HP1 proteins. We generated double-mutant fly strains lacking pairwise combinations of the HP1 proteins and investigated the impact of this manipulation. HP1B/HP1C double-mutant third instar larvae had a 7.8% and 15% survival rate, depending on the HP1b allele used. Of these surviving double-mutant larvae, 2.4% and 8.3% survived to the adult stage, again depending on the specific HP1b allele uses. These data illustrate that, although detrimental to the overall health of the flies, complete loss of HP1B and HP1C is survivable in D. melanogaster, raising the possibility that other HP1 family members may compensate for their loss. For the HP1B/HP1a double-mutants, third instar larvae had a 4.5% survival rate, and none of the animals survived into adulthood. These data show that loss of HP1B and HP1a is not survivable at the adult stage and that animals only rarely live to the third instar larval stage. Ongoing studies focus on HP1a/HP1B double-mutants at earlier stages of development to determine at which stage most animals die and on the HP1a/HP1C double-mutants. Our study highlights possible crosstalk and cooperative functions between HP1 proteins, and future studies will provide insights into the potentially overlapping functions of HP1 homologs.

894C Expression of *HSATII* noncoding RNA in transfected fibroblast cells *Lia D'Alessandro*[1] 1) Swarthmore College, Swarthmore, PA.

Human Satellite 2 (*HSATII*) is a tandemly repeated, ~26bp monomer sequence present in the pericentric heterochromatin on a subset of human chromosomes. Previously, it was demonstrated that *HSATII* RNA is expressed in many cancer types, producing a long non-coding RNA that accumulates in foci in cancer cells, while these transcripts are never observed in normal human cells. Further, this RNA accumulates in cancer cells in *cis* and binds key regulatory proteins, which are recruited to these nuclear foci. In an effort to understand the effect of *HSATII* RNA expression in cells that do not normally express *HSATII*, we created stably transfected primary human cells expressing *HSATII* RNA. In these stably expressing cells, we demonstrate that the *HSATII* expression construct is randomly integrated into the genome and the majority of cells contain a single site of integration. We observe focal accumulations of *HSATII* RNA in a subset of these transfected cells, demonstrating that ectopically-expressed *HSATII* RNA produces focal accumulations in the nucleus, with the capacity to recruit their protein binding partners and elicit changes in nuclear protein distribution.

895A Transcriptomic Response of Housefly to Natural Infection by *Pseudomonas aeruginosa* and *Escherichia coli* *Danial Asgari*[1], Richard Meisel[1], Dana Nayduch[2] 1) University of Houston; 2) USDA-ARS.

Organisms use a variety of defensive strategies against invading pathogens, and many include the production of anti-pathogen molecules. For example, the insect innate immune response includes the production of anti-microbial peptides (AMPs), which can be always present (constitutive) or produced when needed (induced). An induced immune response can be measured by testing for genes that are differentially expressed upon exposure to a pathogen. In general, animals can be infected in two ways: natural infection (ingestion) and septic injury. Natural infection likely provides a more ecologically relevant perspective on interactions between insects and bacteria. Housefly (*Musca domestica*) is as a model system

for studying the immune response to pathogens because it is adapted to septic habitats that include pathogenic bacteria. Previous work demonstrated that immune-related genes are upregulated upon septic injury in house fly, and a shift in expression from metabolism to protein synthesis was observed. Here, we are testing if natural infection in housefly induces an immune response, and if the response to infection is pathogen-specific. We fed houseflies *Pseudomonas aeruginosa* or *Escherichia coli* to determine, separately, the transcriptomic response of gut and the rest of the body. Notably, no genes encoding AMPs were upregulated upon infection. In contrast, 5 AMP genes show high constitutive expression in gut. Constitutive expression was greatest in gut, which suggests that the septic environment houseflies inhabit favored adaptation of a constant defense in the gut against natural infections. This is in contrast to *Drosophila*, where there is induction of AMPs upon natural infection. We hypothesize that differences in life history between house fly and *Drosophila* favor the evolution of different defense strategies. There were no GO categories enriched in house flies infected with *E. coli*. In *P. aeruginosa* infection, genes related to metabolism (including propanoate, galactose metabolism) are upregulated, and genes related to tyrosine metabolism are downregulated. These observations suggest some specificity of responses to different bacteria, but notably not in the AMP response. In conclusion, upon natural infection, houseflies change the expression of metabolic-related genes rather than immune-related ones.

903C The genetic architecture underlying inter-individual variation in the *in vivo* ER stress transcriptional response *Nikki Russell*[1], Katie Owings[1], Clement Chow[1] 1) University of Utah, Salt Lake City, UT.

The phenotypic expression of a disease varies greatly between individuals. Differences in genetic background between patients can be a major contributor to these differences in disease outcomes. Inter-individual genetic variation in important stress responses like the response to endoplasmic reticulum (ER) stress may contribute to the variation observed in disease outcomes. ER stress occurs when misfolded proteins accumulate in the lumen of the ER. ER stress is a primary cause and modifier of many human diseases. Cells respond to ER stress with the Unfolded Protein Response (UPR), which restores ER homeostasis by regulating a large transcriptional response that upregulates genes that encode proteins that function in protein folding, degradation of misfolded proteins, and halt protein translation. We have previously shown that the ER stress response is highly variably across different genetic backgrounds. Our goal is to uncover how the *cis*- and *trans*- regulatory landscape drives this variation in the ER stress response. We are using C57BL/6J (B6), CAST/EiJ (CAST), and F1s between the strains to understand how the regulatory landscape shifts under ER stress. For each response gene, we identify *cis*- and *trans*- regulatory effects by comparing the relative allelic expression in the F1 to the ratio of expression between the parental strains under control and ER stress conditions. In each strain, we induced ER stress, performed RNA-seq on both liver and kidney, and found hundreds of genes that differed in response to ER stress between strains in both tissues. Strikingly, most of the regulatory variation that drives differences in the ER stress response is subject to GxE interactions, with interactions specific to tissue type and stress conditions. In both tissues, most of the regulatory variation was stress-specific. Approximately 75% and 85% of genes with *cis*- or *trans*- effects, respectively, were detectable only under stress or control conditions. For genes that have regulatory variation, there was no enrichment for canonical ER stress genes, demonstrating that regulatory variation in the ER stress response is mostly in peripheral processes. This study demonstrates the importance of considering both genotype- and context- specific effects, such as stress or different tissue types, to understand how genetic variation impacts gene regulation.

914B Characterization of a potential gene interaction between *spr-5, met-2*, and *mep-1* in determining germline versus soma in *C. elegans* *JOVAN BROCKETT*[1], Sindy Chavez[1], Brandon Carpenter[2], Onur Birol[2], Karen Schmeichel[1], David Katz[2] 1) Oglethorpe University; 2) Emory University.

In *C. elegan*s, epigenetic modifier proteins aid in the activation and repression of gene expression that is required to distinguish germline from soma. For example, SPR-5 is a histone demethylase that removes the activating histone modification H3K4me1/2 and MET-2 is a histone methyltrasferase that adds the repressive histone modification H3K9me2. Together, these two epigenetic modifiers, work synergistically to establish a totipotent ground state by shutting down the precocious transcription of germline specific genes inherited from the gametes. Previous studies have shown that unlike *spr-5* or *met-2* single mutants, *spr-5; met-2* double mutants produce progeny that experience a severe developmental delay at the L2 larval stage. Transcriptomic analysis of these animals indicates that the delay is largely due to the inappropriate expression of germline genes in somatic tissues. Here we explore the possibility that the early actions of SPR-5 and MET-2 are reinforced in the embryo by coordinating with other transcriptional regulators. One possible collaborator is MEP-1, a component of the epigenetic deacetylase complex, MEC. Similar to *spr-5;met-2* double mutants, *mep-1* mutants fail to suppress germline genes in somatic tissues, as indicated by the misexpression of PGL-1 and GLH-3 proteins in the soma of L1 larvae. We tested a potential interaction between these two pathways by subjecting *spr-5, met-2,* and *spr-5;met-2* mutants to *mep*-1 RNAi. *mep-1* knockdown when combined with any of these mutant backgrounds, results in an exacerbated developmental delay phenotype. The "triple" mutant is most severe, displaying a full L1 arrest. To fully understand the molecular basis of this synergy, we scaled up *mep-1* RNAi to generate sufficient quantities of L1 isolates to perform an RNA-seq based transcriptomic analysis. Data will be presented at the conference looking at overlapping gene expression patterns between *spr-5, met-2, and mep-1*. If the gene expression changes overlap, it would be consistent with a gene interaction between these pathways. Collectively, these basic findings could help elucidate the functional mechanisms of human orthologues that have been implicated in some neurological diseases.

932B Live cell tracking of yeast IMP Dehydrogenase expression suggests dynamic regulation in response to intracellular purine nucleotide levels *Erica Schwotzer*[1], Kaitlin Sundling[1], Kieran Sweeney[2], Megan McClean[2], David Brow[1] 1) Department of Biomolecular Chemistry, University of Wisconsin- Madison School of Medicine and Public Health, USA; 2) Department of Biomedical Engineering, University of Wisconsin- Madison, USA.

Given the many essential cellular functions of purine nucleotides, selection for maintenance of optimal levels of ATP and GTP is presumably strong. The enzyme IMP dehydrogenase (IMPDH), which catalyzes the first committed step of GTP biosynthesis from the purine nucleotide precursor IMP, helps regulate the balance of ATP and GTP. Mutations in the human IMPDH1 gene cause autosomal dominant retinitis pigmentosa type 10 (adRP10) and Leber congenital amaurosis type 11 (LCA11), progressive and congenital blindness disorders. These mutations are primarily located in the CBS domains of IMPDH, which are thought to regulate IMPDH activity via binding of purine nucleotides or single-stranded nucleic acids. In *S. cerevisiae*, IMPDH is produced from the *IMD2*, *IMD3*, and *IMD4* genes. Imd2 is uniquely resistant to the IMPDH inhibitor mycophenolic acid (MPA) and synthesis of its mRNA is strongly induced by MPA. *IMD2* transcription is regulated by intracellular GTP levels through the use of alternative transcription start sites (TSS) that either elicit or bypass Sen1-dependent transcription termination in the 5'-UTR and early ORF.

Using the CellASIC microfluidic platform and GFP-tagged Imd2, we developed a protocol for live-cell imaging of changes in Imd2 levels over 50 hours of growth in the presence of MPA. Our data show that Imd2 induction by MPA occurs in multiple phases with different rates of induction and is

dependent on cell growth state. Furthermore, Imd2 expression after cells reach maximum density depends on the direction of nutrient flow and is increased or decreased by addition of adenine or guanine, respectively, to the medium. Our data suggest that other regulatory mechanisms, in addition to the transcriptional regulation of Imd2 in response to GTP concentration, control Imd2 protein levels in response to cell growth state. We are further exploring the complex mechanism of regulation of this key enzyme in nucleotide metabolism.

934A Mechanisms of RNA Polymerase II transcription *Craig Kaplan*[1] 1) University of Pittsburgh.

RNA Polymerase II (Pol II) is the engine for gene expression in eukaryotes. Proper expression of genes at the level of transcription involves the coordination of large number of factors that shepherd Pol II through distinct phases of RNA synthesis – initiation, elongation, and termination. We have previously determined that the initiation phase of transcription in *Saccharomyces cerevisiae* is exquisitely sensitive to perturbation of Pol II activity. Mutations altering the Pol II active site and its catalytic activity alter the distribution of transcription start site (TSS) usage at essentially all promoters in yeast. We are employing genetic, genomic, and systems biological approaches to understand why and how Pol II activity controls the process of start site selection, and how Pol II activity integrates with general transcription factors and promoter sequence to accomplish appropriate initiation. Genetic analyses suggest that at least two networks of factors control TSS selection, one network controls initiation efficiency and the other putatively controls the ability of Pol II to survey the promoter for usable start sites – a process known as promoter scanning. We find that scanning appears to operate at almost all yeast promoters and that promoter architecture can shape sensitivity of promoters to specific transcription mutants. We are also quantitavely examining the interaction of the Pol II active site with tens of thousands of designed promoter sequences to determine how Pol II reads promoter sequence to initiate during the promoter scanning process. Our results indicate altered Pol II activity changes usage of essentially all TSSs examined, consistent with the Pol II active site controlling efficiency of initiation at any sequence. However, we also observe alterations to TSS sequence selectivity in distinct Pol II mutants. Our results illustrate the power of basic model systems for genetic and genomic dissections of mechanisms in vivo.

938B Defining the function of SMYD lysine methyltransferases in yeast *Deepika Jaiswal*[1], Rashi Turniansky[1], James Moresco [2], Sabeen Ikram[1], Ganesh Ramaprasad [1], Assefa Akinwole[1], Julie Wolf[1], John R. Yates III[2], Erin Green[1] 1) University Of Maryland Baltimore County; 2) The Scripps Research Institute, La Jolla, CA 92037.

Protein lysine methylation has emerged as a critical regulator of signaling pathways that promote proper growth, development and differentiation in response to environmental signals. The budding yeast enzymes Set5 and Set6 carry the same domain structure as the mammalian SMYD enzymes, which have been identified as regulators of skeletal and cardiac muscle development and linked to cardiac disease. We previously characterized Set5 as a histone H4K5, K8 and K12 methyltransferase that promotes genome stability and gene repression near telomeres and transposable elements in coordination with the H3K4 methyltransferase Set1. Recently we performed mutational analysis of Set5, combined with phosphoproteomics, to identify regulatory mechanisms for its enzymatic activity and subcellular localization. We determined that the interaction of Set5 with chromatin may be associated with DNA-binding activity of its MYND domain, and identified phosphorylation within the C-terminal region as a potential regulator of its catalytic activity. We also determined the role of different domains of Set5 to its regulation of gene expression at subtelomeres. In addition to Set5, the other conserved SMYD protein in yeast is Set6, which has no described biological or biochemical function. Our proteomic data suggest that both Set5 and Set6 methylate multiple, diverse substrates, and new genetic evidence indicates that cells rely on Set5 and Set6 for promoting proper responses to stress. These findings are therefore working to establish yeast as a useful model for understanding SMYD lysine methyltransferase function and their roles in development and disease.

950B Sugar Awakens Cancer Cells: Ras between Survivability & Apoptosis *Mai Rahmoon*[1], Menattallah Elserafy[1], Mohammed Zakaria Gad[2], Sherif El-Khamisy[1] 1) Zewail City for Science and Technology ; 2) German University in Cairo .

Metabolic reprogramming of tumor cells & the conservative activation of Ras is similar to yeast fermentation. Owing to this genetic & metabolic similarities, a functional yeast survival screen was performed exploiting a genetically well-defined yeast system to screen a cDNA library for novel inhibitors of Ras in overactive glycolytic flux to hinder tumorigenesis &/or resolve therapy resistance. We used *tps1Δ* cells since its Ras mimics the oncogenic Ras in cancer cells undergoing Warburg effect. In *tps1Δ* cells, activation of Ras led to cell death, while in mammalian hyper proliferation. High glucose concentrations were selected as the lethal condition & the human kidney cDNA library was used. A proof-of-principle experiments were also performed to evaluate known Ras inhibitors as NF1. The 14 genes identified in 3 screens were categorized into 5 classes encompassing ribosomal proteins, transcription factors, anti-apoptotic proteins, structural proteins & mitochondrial respiration & cell metabolism. 3 genes were known to inhibit Ras, therefore validating the screen system. Of the isolated genes, the human Aldolase B (ALDOB) was chosen. ALDOB expressing plasmid was isolated & its expression was confirmed by blotting against HA tag. Then, ALDOB rescue was confirmed by viability test. The mechanism of this enzyme was then analyzed. ALDOB expression in mutant yeast cells re-corrected the stalled glycolytic flux at GAPDH, so the cell grew on growth media in absence of ethanol. Also, *tps1Δ* cells showed nuclear fragmentation & condensation in the presence of high glucose. But, in the mutant strain expressing ALDOB a repression of apoptosis was observed & the nucleus was round that might indicate that it regained its integrity as assessed by DAPI & Annexin/PI staining. By the DHR123 ROS probe & fluorescence microscopy, ALDOB was also shown to suppress ROS accumulation in mutant yeast cells. Upon, growing ALDOB expressing cells on high concentration of hydrogen peroxide, this strain was able to rescue the cells behaving similar to wild type & different from mutant cells that showed defective growth on the spot test. Our results suggest that ALDOB might be a Ras signaling inhibitor as shown by regulating the defects hyperactive Ras caused in yeast cells. These findings open avenues for understanding the metabolic cues of tumor cells that are activated or inactivated in response to glucose to identify accurate treatment approaches of metabolic disease-associated cancer

952A Investigating the Quantitative Relationship Between Transcription Factor and Target in Yeast *Samuel Linde*[1], Guadalupe Sanchez[1], Joseph Coolon[1] 1) Wesleyan University, Department of Biology, Middletown, CT.

Transcriptional regulation is a critical step in most biological processes. The transcription factor-target relationship is commonly modeled as a boolean relation with genes residing in either an on or off state based solely on the presence or absence of activators and inhibitors. This model does not consider varying levels of transcription factor expression in the system and due to the deterministic nature of the model, information on the network dynamics can be lost. Gene regulatory networks (GRNs) are inherently continuous as genes are expressed at a range of levels throughout a cell's lifetime. We propose a gene regulation function that quantitatively relates transcription factor abundance to target gene expression level. This quantitative relationship is effectively unknown, and we aim to fill this knowledge gap by titrating *Saccharomyces cerevisiae* transcription factors

and quantifying genome-wide gene expression response in their targets using RNA sequencing. We can use this function to uncover the structure of the GRN in yeast by titrating the expression level of the transcription factor RAP1. We performed RNA sequencing (RNA-seq) on yeast grown in media containing a concentration series of doxycycline titrating the expression of RAP1. We found that direct targets of RAP1 expression levels are better correlated with RAP1 expression than non-direct targets as well as that the indegree of a gene does not have an effect on the correlation with RAP1 expression indicating that the identity of the regulators is more important than the quantity. Additionally, the hill function parameters identified by this model are variable suggesting a more complex relationship between transcription factor and target then was previously thought.

953B Genome Wide Effect of Doxycycline, Tetracycline, and 4-epidoxycycline on gene expression in Saccharomyces cerevisiae *Guadalupe Sanchez*[1], Samuel Linde[1], Joseph Coolon [1] 1) Wesleyan University, Department of Biology, Middletown, CT.

Tetracycline (Tet) and derivative chemicals have gained widespread recognition for their antibiotic properties since their introduction in the late 1970s but recent work with these chemicals in the lab has shifted to include multiple techniques in all genetic model systems for the precise control of gene expression. Because Tet works as an antibiotic by inhibiting protein synthesis through binding to the subunits of the bacterial ribosome, it is generally considered to have effects specific to bacteria and should therefore have few off-target effects when used in eukaryotic systems. The most widely used Tet-modulated methodology is the Tet-On/Tet-Off gene expression system. While this system was developed for use with Tet, its derivative Doxycycline (Dox) is now more commonly used because it has increased stability generating more consistent results in a very large number of studies to date. However, not much is known about the effects of Dox on global gene expression. A previous study in the yeast Saccharomyces cerevisiae found that Dox had no effect on genome-wide gene expression as measured by microarray but another study found that the use of Dox in common cell lines and several model organisms led to mitonuclear protein imbalance, suggesting an inhibitory role of Dox in eukaryotic mitochondria. Recently, a new Dox derivative 4-Epidoxycycline (4-ED) was developed that was shown to have less off-target consequences on mitochondrial health. To determine the best tetracycline family chemical to use for gene expression control in S. cerevisiae we performed RNA sequencing (RNA-seq) on yeast grown on standard medium compared to growth on media supplemented with Tet, Dox or 4-ED to identify any genome-wide changes in gene expression they may elicit. While each caused dozens of genes to change expression, each differed in the number and identity of the genes effected. These findings suggest that care should be taken when using Tetracycline family chemicals for S. cerevisiae experiments, with the chemical best-suited to each study dependent on the genes effected by each of these chemicals.

964A Regulation of gene expression by acidic pH in an opportunistic human fungal pathogen *Cryptococcus neoformans*: modulating antifungal susceptibility and iron Uptake Donghyeun Kim[1], *Won Hee Jung*[1] 1) Department of Systems Biotechnology, Chung-Ang University, Anseong, Korea.

Cryptococcus neoformans is an opportunistic human fungal pathogen, but its infection is militated by phagocytosis and phagolysosomes in the host immune system. It has been reported that the pH of phagolysosomes containing *C. neoformans* is approximately 5.3. In general, an acidic pH condition, in comparison with neutral pH, is known to alter several physiological characteristics of fungi, including susceptibility to azole antifungal drugs. Indeed, azole antifungal susceptibility of another well know human fungal pathogen, *Candida albicans*, is modulated by pH, although the underlying mechanism is not clear yet. Therefore, we investigated whether the environmental pH influences the azole antifungal susceptibility of *C. neoformans* and how the fungus responds to acidic pH. We found that the minimal inhibitory concentration (MIC) of *C. neoformans* against fluconazole was increased under an acidic pH condition, and our GC-MS analysis revealed that the ergosterol content in *C. neoformans* that was grown under an acidic condition was greatly increased in comparison to that in cells grown at a neutral pH level. Moreover, a mutant strain lacking *CFO1*, which is the major component in the high-affinity reductive iron uptake system in *C. neoformans*, displayed significantly reduced sensitivity to fluconazole at an acidic pH level. This implies that a different iron uptake pathway governs the transport of iron under such a condition. Considering that a number of the proteins involved in ergosterol biosynthesis require iron as a cofactor, our data implied the involvement of a yet unknown iron uptake pathway, which is independent of the *CFO1* function, in azole antifungal susceptibility of *C. neoformans* under an acidic pH condition. In addition, we investigated the underlying molecular regulatory mechanism to understand how *C. neoformans* responds to an acidic pH condition through transcriptome analysis as well as phenotypic and biochemical analysis of the series mutant strains lacking the genes involved in the major iron uptake pathways.

965B Mapping the early zebrafish gene regulatory landscape Using CUT&RUN *Matthew Hurton*[1], Miler Lee[1] 1) University of Pittsburgh.

After fertilization, thousands of genes influence embryonic development through a maternal contribution of RNAs and proteins in the egg. In zebrafish, several transcription factors rank among the most highly provided mRNA, but it is unclear how they coordinate to regulate gene expression as the embryonic genome is activated. To begin to elucidate this regulatory network, we have embarked on a large-scale effort to characterize early embryonic gene regulatory sequences and their transcription factor binding profiles. To this end, we have adapted Cleavage Under Targets and Release Using Nuclease (CUT&RUN) for zebrafish embryos. CUT&RUN is a more sensitive ChIP-Seq alternative: in our hands, CUT&RUN can be carried out on as few as 10 oblong-stage embryos, i.e., 30-100 fold less material than in a conventional ChIP-Seq experiment. Using CUT&RUN, we have mapped the putative active enhancers and promoters in the early genome, by identifying regions enriched for histone modifications including H3K27ac; and bound by early-acting maternal transcription factors, including Nanog, Pou5f3 and Sox19b. These regions largely correlate with the acquisition of accessible chromatin, as measured by ATAC-seq. Together, these results reveal a rich gene regulatory landscape across the genome as the embryo transitions to pluripotency and beyond.

973A The role of phospholipid metabolism in maize adaptation to highlands Karla Blöcher-Juárez[2], Fausto Rodríguez-Zapata[1, 2], Juan Estevez[2], Rocio Aguilar-Rangel[2], Sergio Pérez-Limón[2], Dan Gates[3], Li Wang[4], Oliver Fiehn[3], Jeffrey Ross-Ibarra[3], Matthew Hufford[4], Ruairidh Sawers[1,5], *Rubén Rellán-Álvarez*[1, 2] 1) National Laboratory of Genomics for Biodiversity; 2) North Carolina State University ; 3) University of California Davis ; 4) Iowa State University; 5) Pennsylvania State University.

After domestication from lowland teosinte in the warm, humid Mexican southwest maize colonized the highlands of Mexico and South America. In the highlands, maize was exposed to a whole range of environmental factors that differ from the site of domestication, including, among others, lower temperatures, soils with lower phosphorus availability and different biological pressures. In my talk, I will present data supporting the hypothesis that glycerolipid metabolism remodeling was important in the process of maize adaptation to highlands.
We will show results from common garden experiments in Mexican lowland and highland common gardens where we grew maize mapping populations and using quantitative biochemical genetics tools we identified major QTLs that explain the conversion of phosphatidylcholines (PCs) to

lyso-phosphatidylcholines (LPCs) leading to a high PC/LPC ratio that is particularly conserved in Mexican highland landraces. We have identified a couple of genes (ZmPla1.2 and ZmLpcat1) that code for enzymes controlling the PCs/LPCs ratio as the most likely causative genes of the PC/LPC conversion QTLs.
We then used GBS data from 3200 maize landraces and whole genome sequences from another 30 landraces across the Americas and identified SNPs within the coding regions of ZmPla1.2 and ZmLpcat1 that show clear signs of selection to highlands. Other genes controlling PCs/LPCs ratio were also found to be under selection in highland maize.

974B Adaptation of the industrial yeast *Saccharomyces cerevisiae* against toxic chemicals for lignocellulose-to-biofuels conversion *Zonglin Liu*[1] 1) BioEnergy Research Unit, National Center for Agricultural Utilization Research, USDA-ARS.

The industrial yeast *Saccharomyces cerevisiae* is a workhorse widely applied in fermentation-based industrial applications. It has a plastic genome and great flexibility in adaptation to varied environmental conditions. A tolerant strain of NRRL Y-50049 was successfully obtained by environmental evolution from a progenitor of industrial type strain NRRL Y-12632. Strain Y-50049 can *in situ* detoxify 2-furaldehyde (furfural) and 5-hydroxymethyl-2-furaldehyde (HMF), major toxic chemicals derived from lignocellulose-to-fuels conversion, while producing ethanol. Based on characterizations of a novel aldehyde reductase gene (*ARI1*) and its protein, a mode of action was established for conversion of furfural and HMF into non-harmful furanmethanol (FM) and furandimethanol (FDM), respectively. By time-course comparative transcriptome and protein expression profiling analyses, glucose-6-phosphate (Zwf1) in Y-50049 was found as the key protein to driving the glucose metabolism in favor of the oxidative branch of the pentose phosphate pathway, facilitating *in situ* detoxification. These results suggested a fine-tuned mechanism of the reprogrammed detoxification pathway in Y-50049. The activated expression of Zwf1 appeared to generate essential cofactor NADPH to enable reduction of furaldehydes through a group of aldehyde reduction enzymes. In return, the active aldehyde reductions released desirable feedbacks of $NADP^+$ stimulating continued oxidative activity of Zwf1. Thus, a well-maintained cofactor regeneration cycle was restored overcoming the furfural-HMF stress. Key transcription factor genes involved in major altered pathways were also identified. Furthermore, numerous pathway-based tolerant phenotypes of Y-50049 were highlighted that distinguished the tolerance components from the innate stress response of its progenitor. Identification of legitimate tolerance phenotypes is critical for continued investigations in dissection of mechanisms of yeast tolerance. Knowledge and insight obtained by this research aid understanding yeast adaptation at the genomic level and development of the next-generation biocatalyst for advanced biofuels production from lignocellulosic materials.

975C Fungal incipient local adaptation through allelic and copy-number variation Anna Bazzicalupo[1], Joske Ruytinx[2], Yi-Hong Ke[3], Laura Coninx[4], Jan Colpaert[4], Nhu Nguyen[5], Rytas Vilgalys[3], *Sara Branco*[1] 1) Montana State University, Bozeman, MT; 2) Vrije Universiteit Brussel, Brussels, Belgium; 3) Duke University, Durham, NC; 4) Hasselt University, Hasselt, Belgium; 5) University of Hawaii, Honolulu, HI.

Human-altered environments can shape the evolution of organisms. Fungi are no exception, though little is known about how they withstand anthropogenic pollution. Here, we document incipient polygenic local adaptation in the mycorrhizal fungus *Suillus luteus* driven by recent soil heavy metal contamination. Genome scans across individuals from recently polluted and nearby unpolluted soils in Belgium revealed no evidence of population structure but detected allelic divergence and gene copy number variation in genes involved in metal exclusion, storage, immobilization, and reactive oxygen species detoxification. Standing genetic variation included multiple alleles of small effects contributing to heavy metal tolerance, suggesting the existence of different strategies to withstand contamination. Variants were shared across the whole population but found to be under selection in isolates exposed to pollution. Together, our results point to *S. luteus* undergoing the initial steps of adaptive divergence and contribute to understanding the processes underlying local adaptation under strong environmental selection.

980B More exquisitely adapted species have lower structural disorder in vertebrate protein domains *Catherine Weibel*[1,4,5], Jennifer James[1], Sara Willis[1,3], Paul Nelson[1], Joanna Masel[1,2] 1) Department of Ecology and Evolutionary Biology,University of Arizona, Tucson, AZ; 2) BIO5 Institute, University of Arizona, Tucson, AZ; 3) High Performance Computing, University of Arizona, Tucson, AZ; 4) Beckman Scholars Program, Irvine, CA; 5) Undergraduate Biology Research Program, University of Arizona, Tucson, AZ.

Past work in the Masel lab suggests that protein domains become more structurally ordered with age. We studied the relationship between the effectiveness of selection and the Intrinsic Structural Disorder (ISD) of Pfam domains across 120 vertebrate species. Using the phylostratigraphy database of James et. al, we compiled the IUPred2 ISD estimates for the Pfams of 120 vertebrate species and estimated ISD Species effects using a mixed linear model with species as fixed effect and Pfam as random effect, thus controlling for Pfam composition across each species' genome. We then calculated each species' codon adaptation index (CAI) from genomic codon frequencies and calculated each species' total GC content (from genic and intergenic regions) using in-house scripts, and linearly modeled CAI vs ISD and Total GC vs ISD.
Initial correlations between ISD and CAI indicate that well adapted species tend to have low ISD (Spearman's R = -0.81, p value < 2e-16). Surprisingly, the initial relationship between Total GC content and ISD is weak and not significant (Spearman's R = 0.27, p value = 0.07). To correct for phylogenetic confounding and pseudo replication, we transformed CAI, Total GC content, and ISD Species effect data using Phylogenetic Independent Contrasts (PIC) and re-plotted. Phylogenetically controlled linear models found a strengthened relationship between Total GC content and ISD (Spearman's R = 0.37, p value = 4.6e-6), indicating that either PIC is artificially amplifying a small Total GC content signal or that mutation bias may have a role in driving structural disorder in protein domains. We plan future work to remove the effect of mutation bias. Phylogenetically controlled linear models confirmed that well adapted species have lower ISD domains than poorly adapted species (Spearman's R = -0.58, p value = 8.5e-8), thus adding evidence to the case for directional protein evolution.

982A Adaptive fitness advantage in ancestors: a major health risk to a present-day Arab population *Eaaswarkhanth Muthukrishnan*[1], Andre Luiz Campelo dos Santos[2,3], Omer Gokcumen[2], Fahd Al-Mulla[1], Thangavel Alphonse Thanaraj[1] 1) Dasman Diabetes Institute, Kuwait; 2) State University of New York at Buffalo, New York, USA; 3) Federal University of Pernambuco, Brazil.

Following the out-of-Africa diaspora, the Arabian Peninsula has experienced several waves of human migrations despite the prevailing extreme and varying environmental conditions. The potential adaptation that shaped the extant human populations of the hot and dry environment of the Arabian Peninsula have been scarcely studied. To explore natural selection in the region, we analyzed 662,750 variants in 583 Kuwaiti individuals applying multiple statistical tests (integrated Haplotype Score, Cross Population Extended Haplotype Homozygosity, Population Branch Statistics) including log-likelihood ratio scores that have become available recently to identify selection.
Through this integrative approach, we identified gene regions that presented strong signals of positive selection in the Kuwait populations. Among

those positively selected gene regions, we highlight a haplotype overlapping *TNKS* gene region that has been previously associated with obesity, hypertension, and asthma. This *TNKS* haplotype exemplifies a general trend in which a more rapid metabolism rate and hypertension have been selected in the Kuwaiti population and potentially conferred some degree of fitness advantage to the Kuwaiti ancestors for surviving in the extremely dry and hot ecological environments while posing a considerable health risk to present-day Kuwait populations.
Our results suggest that past adaptive trends have further predisposed Kuwaiti populations to the health risks at the genetic level. Overall, the mechanisms through which the *TNKS* haplotype conferred a fitness advantage and how the same haplotype predisposes the population to metabolic diseases remain fascinating and could be explored in future research.

984C Using natural genetic variation in *Drosophila* to characterize the underlying mechanisms of hormesis *Katie Owings*[1], Clement Chow[1] 1) University of Utah.

Pre-exposing organisms to a sublethal level of stress can improve their ability to withstand subsequent stress and result in beneficial health outcomes. This phenomenon, termed hormesis, was first reported in 1956 when Maynard Smith noted that a transient exposure to heat stress increases the lifespan of female *Drosophila subobscura*. In the years following, hormesis has been reported in several model organisms exposed to a variety of stresses, such as heat stress, mitochondrial stress, and ER stress. Although this is a widely noted phenomenon, the molecular mechanisms underlying hormesis are still not fully understood. One factor contributing to this lack of understanding is that many stress studies examine a single stress in isolation. In reality, however, stress occurs in a complex milieu of previous and ongoing stresses that likely impact how the cell responds. Studying stress responses in the context of previous stresses will provide insight into how a cell responds to numerous insults in nature and will lead to a stronger understanding of the underlying mechanisms of hormesis. This study begins to investigate the mechanisms of hormesis by characterizing the impact of genetic variation on this phenomenon. Characterizing the impact of genetic diversity on hormesis will allow us to identify modifiers of hormesis that were overlooked when evaluating the phenomenon in a single strain. In order to accomplish this goal, this study utilizes the natural genetic variation of the *Drosophila* Genetic Reference Panel (DGRP), which is a collection of 200 fully-sequenced, inbred lines derived from a natural population. In our model of hormesis, lifespan was assayed in flies from each DGRP strain that were preconditioned with transient heat stress (or controls with no preconditioning) and then exposed to a subsequent ER stress. We found that the strength of the hormetic effect varies greatly with genetic background, ranging from beneficial to detrimental consequences of heat shock preconditioning. Genome-wide association study (GWAS) results from this screen indicate that chromatin organization may play a crucial role in hormesis. This work has important implications for both health and evolution.

993C Functional characterization of *atlas,* a putative *de novo* evolved gene essential for *Drosophila* male fertility *Andrew Ludwig*[1], Emily Rivard[1], Prajal Patel[1], Geoffrey Findlay[1] 1) College of the Holy Cross.

Unlike many genes that arise from duplication, *de novo* evolved genes arise from previously noncoding DNA. Many *de novo* genes are expressed in the male reproductive tract, but their functions remain uncharacterized. We used an RNAi screen to identify several testis-expressed, putative *de novo* genes that are essential for male reproduction. Both the RNAi knockdown and the CRISPR-mediated knockout (KO) of one such gene, *atlas,* resulted in almost complete male sterility. Cytological analyses of spermatogenesis in *atlas* null mutants revealed two defects. First, mutant male testes showed few sperm in the seminal vesicle (SV) and a distended basal end of the testis, suggesting that sperm in *atlas* mutants are not transferred to the SV during spermatogenesis and, thus, to females during mating. Second, phalloidin staining of whole testes revealed that *atlas* KO males inefficiently initiate the process of spermatid individualization. To understand the normal role of *atlas* in spermatogenesis, we used CRISPR to scarlessly insert GFP at the end of the gene's protein-coding sequence, at the endogenous locus. This *atlas*-GFP allele fully rescued the fertility defect of *atlas* null males. We then observed a nuclear-localized GFP signal in the post-meiotic stages of the testes. This signal overlapped partially with fully condensed sperm nuclear bundles marked by protamine-dsRed, but the GFP-positive bundles were not as close as the dsRed-positive bundles to the basal end. These data, along with the biochemical properties of the atlas protein, suggest that *atlas* may encode a transition protein that facilitates the replacement of histones with protamines in late spermiogenesis. These results demonstrate how a putatively *de novo* evolved protein can acquire an essential reproductive function.

1003A Ape-specific *ATF4* retrocopies may act to regulate parental *ATF4* activity and integrated stress response outcomes *Hans Dalton*[1], Katie Owings[1], Nels Elde[1], Clement Chow[1] 1) Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

Multiple stress signals trigger the integrated stress response (ISR) - including endoplasmic reticulum stress and viral infection. Dysregulation of the ISR is implicated in diseases such as cancer and diabetes. The ISR activates kinases that phosphorylate eIF2alpha, causing strong inhibition of protein synthesis machinery while repair occurs. Despite this, certain genes contain specialized upstream Open Reading Frame (uORF) regulatory sequences in their 5'UTR region that allow, or even upregulate, translation during the ISR. One of the most upregulated uORF-containing transcripts is *ATF4*, a highly conserved transcription factor that activates pro-survival or apoptotic genes.

We have found conservation of multiple *ATF4* retrocopies - labeled in humans as pseudogenes *ATF4P1-P4*. *hATF4P1* and *P2* are highly truncated retrocopies that date to the common ancestor between apes and old world monkeys, while *hATF4P3* and *P4* are ape-specific. *hATF4P3* is a full length copy that sits within the gene *RNF157*; it maintains all domains and uORF regulatory regions with 95% amino acid (AA) identity with the parent *ATF4* gene. The 5% AA that are diverged from the parent gene are primarily in the N-terminal side, with no differences in the C-terminal DNA binding domain. Perhaps the most interesting is *hATF4P4* which has a truncation event removing its DNA binding domain, yet maintains 90% AA identity with the parent gene in the truncated half. *hATF4P4* is well conserved in humans, chimps, bonobos, and gorillas (Homininae) and ancestral to their most common ancestor. Strikingly, independent retrocopies with similar truncation events have occurred in orangutan, gibbon, and gorilla genomes (an extra gorilla copy).

We have cloned the full-length (*P3*) and truncated (*P4*) *ATF4* retrogenes into human cell lines and tested for cell viability under ISR activation. Intriguingly, overexpression of *hATF4P4* under ISR activation causes loss of cell viability similar to overexpressing parental *ATF4* (likely due to increased apoptosis). We hypothesize that *hATF4P4*-like retrocopies may regulate the parent *ATF4* gene by interaction with regulatory proteins due to its lack of DNA binding domain. In addition, viruses, like HIV, can hijack important machinery during the ISR, suggesting an alternative hypothesis where hATF4P4 may buffer ATF4 to inhibit viral infection while not affecting *ATF4* transcription. We explore these possibilities through functional analyses.

1007B Inferring adaptive introgression using Hidden Markov Models *Jesper Svedberg*[1], Vladimir Shchur[2], Paloma Medina[1], Rasmus Nielsen[2], Russell Corbett-Detig[1] 1) UC Santa Cruz, Santa Cruz, CA; 2) UC Berkeley, Berkeley, CA.

Adaptive introgression - the flow of adaptive genetic variation between species or populations - has in recent years been attracting more and more scientific interest and it has been implicated in a number of cases of adaptation, from pesticide resistance and immunity, to local adaptation. However, methods for inferring adaptive introgression from genomic data are lacking, due to the fact that the genomic signatures of selection and introgression are similar and difficult to untangle. To fill this gap we have developed Ancestry_HMM-S, a computational tool capable of both identifying adaptively introgressed loci and quantify their selective coefficients. It calculates the expected haplotype length distribution surrounding an adaptively introgressed locus using analytical methods and compares this to sequence data using a Hidden Markov Model. We have validated the method using extensive forward simulations and can show that it works in a parameter space that is expected to be found in nature. We also tested the method on a population genomic dataset of *Drosophila melanogaster* from South Africa, and we identified a number of loci showing strong signals of being adaptively introgressed from European populations. Some of these loci coincide with genes known to increase resistance to insecticides. Ancestry_HMM-S works on whole genome population genomic data of arbitrary ploidy, and can handle low coverage data as well as samples generated through pooled sequencing. It is implemented in C++ and can analyze large samples within a few hours. In conclusion, Ancestry_HMM-S provides population geneticists with a much needed tool for studying how selection shapes interspecies gene flow.

1021A Flexible mixture model approaches that accommodate footprint size variability for robust detection of balancing selection *Xiaoheng Cheng*[1,2], Michael DeGiorgio[2,3] 1) Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA; 2) Department of Biology, Pennsylvania State University, University Park, PA; 3) Department of Computer and Electrical Engineering and Computer Science, Florida Atlantic University, Boca Raton, FL.

Long-term balancing selection typically leaves narrow footprints of increased genetic diversity, and therefore most detection approaches only achieve optimal performances when sufficiently small genomic regions (*i.e*., windows) are examined. Such methods are sensitive to window sizes and suffer substantial losses in power when windows are large. Here, we employ mixture models to construct a set of five composite likelihood ratio test statistics, which we collectively term *B* statistics. These statistics are agnostic to window sizes and can operate on diverse forms of input data. Through simulations, we show that they exhibit comparable power to the best-performing current methods, and retain substantially high power regardless of window sizes. They also display considerable robustness to high mutation rates and uneven recombination landscapes, as well as an array of other common confounding scenarios. Moreover, we applied a specific version of the *B* statistics, termed B_2, to a human population-genomic dataset and recovered many top candidates from prior studies, including the then-uncharacterized *STPG2* and *CCDC169-SOHLH2*, both of which are related to gamete functions. We further applied B_2 on a bonobo population-genomic dataset. In addition to the *MHC-DQ* genes, we uncovered several novel candidate genes, such as *KLRD1*, involved in viral defense, and *SCN9A*, associated with pain perception. Finally, we show that our methods can be extended to account for multi-allelic balancing selection, and integrated the set of statistics into open-source software named BalLeRMix for future applications by the scientific community.

1025B The role of structural variation in human local adaptation *Stephanie Yan*[1], Rachel Sherman[1], Sara Carioscia[1], Michael Schatz[1], Rajiv McCoy[1] 1) Johns Hopkins University, Baltimore, MD.

Humans have developed numerous genetic adaptations to the diverse environments that they inhabit. While previous studies have focused on single-nucleotide polymorphisms (SNPs), due to their ease of discovery from short-read sequencing, the recent discovery of potentially adaptive structural variants (SVs)—large insertions, deletions, or inversions (>50bp)—suggests that SVs may constitute prominent but hidden targets of human positive selection. Recent long-read sequencing studies have expanded the catalog of SVs that segregate in human populations, but have not yet been applied to study selection on SVs on a population-wide scale.

We used the variant graph-based method Paragraph to genotype long read-discovered SVs in samples from the 1000 Genomes Project. Using this method, reads are aligned to a graph representation of the genome that incorporates known variation, including SVs, as alternative haplotype paths, thus facilitating accurate genotyping. Although graph genotyping is limited to SVs previously discovered in long-read sequenced genomes, variants involved in local adaptation are by definition locally common, and are therefore likely to be known. We nevertheless complemented this approach with a k-mer-based scan for allele frequency differentiation, thus circumventing the need to resolve SV sequences and facilitating unbiased discovery of local adaptation targeting complex SVs.

We identified 312 SVs with extreme allele frequency differentiation (population branch statistic [PBS] > 0.5), using neutral coalescent simulations to compare these loci to a null distribution. Only 14% of these putative adaptive SVs were previously discovered in the 1000 Genomes samples using short-read sequencing data, underscoring the power of long-read sequencing and short-read genotyping approaches to uncover novel targets of positive selection. Together, our results highlight the underappreciated role of SVs in human local adaptation.

1027A Uncovering the basis of natural resistance to *S. cerevisiae* killer toxin K28 *Ilya Andreev*[1], Meru Sadhu[1] 1) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Killer yeast are strains of yeast infected with dsRNA killer viruses that do not kill the host, but instead encode a killer toxin that is secreted into the environment to kill susceptible yeast cells. A number of killer toxins have been identified, varying considerably in their mechanism of toxicity. *Saccharomyces cerevisiae* killer virus ScV-M28 encodes killer toxin K28, which enters susceptible cells via receptor-mediated endocytosis, ultimately localizing to the nucleus and causing G1/S cell cycle arrest. Previous studies have uncovered many gene knockout mutations that confer resistance to K28 toxin. However, it is unknown what kind of genetic strategies naturally occurring *S. cerevisiae* employ for developing resistance to K28. We have discovered that there is considerable natural variation in K28 resistance among *S. cerevisiae* strains. We are using quantitative trait locus (QTL) mapping to identify genetic factors responsible for naturally occurring K28 toxin resistance to better understand how killer toxins could have influenced the evolution of *S. cerevisiae*.

1031B Environmental Adaptation in House Mice from the Americas *Megan Phifer-Rixey*[1], Ke Bi[2], Felipe de Mello Martinsde Melo Martin[2], Tiffany Longo[1], Mallory Ballinger[2], Jesse Bragger[1], Michael Nachman[2] 1) Monmouth University; 2) University of California, Berkeley.

House mice (*Mus musculus domesticus*) have expanded their range in association with humans, establishing populations in a variety of novel habitats, including most of the Americas. Their rapid expansion provides a unique opportunity to study the genetic basis of adaptation over short time scales in a mammalian model system. Previous work along a latitudinal transect in North America revealed strong evidence of environmental adaptation. Using phenotypic analysis, RNA-seq, and genome scans, we identified traits and genes underlying environmental adaptation and uncovered a key role for regulatory evolution. Here, we build on those results, identifying candidate loci for environmental adaptation in mice from populations along a latitudinal transect from southern Argentina to equatorial Brazil. Again, we find a key role for regulatory evolution with more overlap than expected by chance between candidate loci from North and South America. Analysis of the transects together uncovered additional candidates, including those relating to metabolism, immunity, and behavior. Finally, we add to previous phenotypic analyses, focusing on morphological plasticity in response to diet and differences in reproductive traits among mice from different locations.

1032C The roles of *kayak* and *center divider* on sperm length in *Drosophila melanogaster* *Phoebe Elizaga*[1], Mollie Manier[1] 1) The George Washington University, Washington, D.C..

Males of *Drosophila* have been found to produce some of the longest known sperm, measuring up to 5.83 cm in *D. bifurca*. Because *Drosophila* females mate with multiple males, sperm must compete within the female reproductive tract in the process of post-copulatory sexual selection. Males with long sperm have been found to experience greater mating success than males with short sperm in females with long sperm storage organs, or seminal receptacles (SRs). Furthermore, there is a significant genetic correlation between sperm length and SR length. These results suggest that the two reproductive traits are coevolving and may be under the influence of pleiotropy. The genes *kayak* and *center divider* (*cdi*) have been previously identified through QTL mapping as candidate genes that may influence sperm length in *Drosophila*. The *kayak* gene encodes a transcription factor that is involved in many processes including embryonic development, wound healing, and cell polarity while *cdi* is present in the sperm proteome and is expressed during spermatogenesis. Previous studies have found that knockdown of each gene results in increased SR length in *Drosophila* females, but have not been able to observe the effect of knockdown on sperm length in males. The goal of this study was to identify the influence of genetic knockdown of *kayak* and *cdi* on sperm phenotype. Because the knockdown of each gene resulted in increased SR length, we expect that knockdown will result in increased sperm length as well. The pleiotropic influence of *kayak* and *cdi* on both SR length and sperm length will identify them as candidate genes for the molecular mechanism of male-female *Drosophila* coevolution.

1039A Experimental and Bioinformatic Analyses of Coevolution of Primate Seminal Proteins and HIV/SIV *Emine Kahveci*[1], Feng Hsiao[2], Ludger Ständker[3], Thomas Mack[3], Jan Münch[3], Nadia Roan[2], Michael Jensen-Seaman[1] 1) Duquesne University, Pittsburgh, PA; 2) University of California, San Francisco, CA; 3) Ulm University Medical Center, Ulm, Germany.

Male reproductive proteins involved in spermatogenesis and fertilization are among the most rapidly evolving proteins in mammals. Selective pressures driving the rapid evolution of these proteins are commonly attributed to sexual selection in the form of sperm competition. However, defense against sexually transmitted pathogens such as HIV/SIV might also be a contributing factor. Previous studies have demonstrated that amyloid fibrils formed from peptides derived from the abundant human seminal proteins semenogelin 1 (SEMG1), semenogelin 2 (SEMG2), and prostatic acid phosphatase (ACPP) dramatically increase the HIV infectivity rate. Presumably, HIV/SIV has evolved to take advantage of the presence of these protein aggregates in semen. Considering the much longer history of SIV prevalence in nonhuman primates compared to humans and the large documented fitness costs associated with SIV infection in chimpanzees, we aim to test whether these seminal proteins in primates have been evolving in response to HIV/SIV. We used maximum likelihood-based sequence analysis of primate homologs to identify codons evolving under positive and purifying selection in primate species grouped by their presence or absence of SIV. However, the homologous regions of these proteins that form fibrils in human semen do not appear to be evolving significantly differently than the non-fibril forming regions. Empirically, we tested synthetic peptides corresponding to the fibril-forming regions of several primate species for their ability to form fibrils in vitro and to enhance HIV infectivity using the TZM-bl reporter cell line. For the four different peptides from three different proteins in nine primate species, the human homologs consistently showed a greater ability to form fibrils and to enhance infection, most notably compared to those from chimpanzee and gorilla. The inclusion of peptides corresponding to hypothetical ancestral sequences allowed us to conclude the direction of change. Furthermore, using phylogenetic approaches to map character states we extended our prediction of ancestral phenotypes more broadly throughout primates. Taken together, our results are consistent with the hypothesis that several seminal proteins have evolved in response to sexually transmitted viruses in primates, with the strongest patterns seen when comparing the SIV-harboring chimpanzees with humans, who have only very recently been exposed to HIV.

1040B Genome scan signals of maize adaptation to soil phosphorus availability. *Fausto Rodriguez Zapata*[1], Rubén Rellán Álvarez[1], Ruairidh Sawers[2] 1) Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, NC; 2) College of Agricultural Sciences, Penn State University, University Park, PA.

Plant phosphorus starving response (PSR) is a coordinated set of biochemical, physiological and developmental reactions to low phosphorus supply. I expect divergent selection in loci involved in PSR if it is the result of local adaptation and consequently an increased frequency of adaptive alleles in populations exposed to low phosphorus availability. Here I use a reverse ecology approach to identify loci that might be involved in PSR and be subject to divergent selection. First I built soilP, an R package for assigning a soil phosphorus availability score to geographic locations. With this phosphorus availability as phenotype I performed environmental genome association on 3238 georeferenced landraces of Zea mays from Latin America and the Caribbean. This resulted in the detection of a a previously reported adaptive introgression from highland teosinte that includes *ZmPho1;2a* an inorganic phosphate transporter. Finally, I propose analysis of phosphorus response in biparental populations polymorphic for *Inv4m* in order to disentangle population structure from local adaptation or other environmental covariates. Variants found in this exploratory study can point to mechanisms of phosphorus use efficiency or acquisition coming from locally adapted maize.

1043B A genome scan for adaptation to high altitude in wild rhesus macaques *Zachary Szpiech*[1], Nicholas Bailey[1], Taylor Novak[1], Laurie Stevison[1] 1) Auburn University, Auburn, AL.

When natural populations split and migrate to different environments, they may experience different selection pressures that can lead to localized adaptation. For aerobic life, the low atmospheric oxygen content of high altitude living presents a special challenge and a strong selection pressure. Searching for evidence of adaptation to high altitude, we analyze the whole genomes of 23 wild rhesus macaques from a population living at high altitude (>4000m above sea level) alongside 22 wild rhesus macaques from a population living at lower altitude (<500m above sea level), which split

approximately 100 kya. Using XP-EHH, a haplotype-based genomic scan for local adaptation, we compare haplotype patterns between the high and low altitude populations. We find that a set of 197 genes related to hypoxia response and oxygen homeostasis is enriched for signals of local adaptation in the high-altitude population. We also find a strong signal at the start of EGLN1 (a classic target for convergent adaptation to high altitude) suggesting a possibly regulatory adaptation. Other signals were found overlapping genes associated with neuronal death during hypoxia, total lung capacity in humans, and a critical enzyme in the citric acid cycle.

1049B The rapidly evolving Drosophila bag-of-marbes gene and its interactions with Wolbachia *Miwa Wenzel*[1], Charles Aquadro[1] 1) Cornell University.

The *Drosophila* protein coding gene *bag of marbles* (*bam*) plays a key role in early male and female gametogenesis by regulating the differentiation of germline stem cells (GSCs). Although the regulation of GSC gene function is essential for reproduction, *bam* shows strong and episodic bursts of protein sequence diversification in several lineages of *Drosophila*. Two hypotheses for the rapid evolution of *bam* include (1) diversifying function of *bam* across the *Drosophila* genus and (2) selective pressure on *bam* from the endosymbiont bacteria *Wolbachia.* Here we are exploring the latter hypothesis based on observations that *Wolbachia* partially rescues the reduced fertility of a *bam* hypomorph mutant in female *D. melanogaster*. The current *bam* hypomorph is defined by a point mutation in a region in where Bam binds to its key partner Bgcn. We are investigating how *Wolbachia* functionally interacts with *bam* by generating new mutants via an alanine scan in the different functional regions of the *bam* gene. Preliminary results suggest that in generating transgenic controls, we have discovered a *bam* variant whose male fertility is rescued by Wolbachia. This suggests a novel genetic interaction between Wolbachia and *bam* in males. Additionally, we are investigating the historical interaction between *Wolbachia* and *Drosophila.* Evidence of a previous infection suggests a historical *Wolbachia* infection that could act as a selective pressure on *bam*. Horizontal gene transfer (HGT) by *Wolbachia* to its host can act as a proxy for us to identify past *Wolbachia* infections. We have developed a pipeline to identify HGTs from whole-genome sequences and are currently going through whole-genome sequences of various *Drosophila* species. Thus far, we have found a few previously unreported HGTs in *D. melanogaster* individuals and are extending this approach to *D. simulans* and other species.

1053C Adaptive mitonuclear interactions are selected for during population stratification in yeast Tuc H.M. Nguyen[1], Sargunvir Sondhi[1], Andrew Ziesel[1], Swati Paliwal[1], *Heather Fiumera*[1] 1) Binghamton University.

Mitonuclear interactions underlie the fundamental roles that mitochondria have in energy production and cellular stress responses. While coevolution between mitochondrial and nuclear genomes must occur, it is not clear how selection operates on various mitochondrial functions. Recent genetic bottlenecks followed by ecological adaptation in *Saccharomyces cerevisiae* yeasts provide the opportunity to determine how selection for mitonuclear interactions occurs. We systematically exchanged mtDNAs between 15 *S. cerevisiae* isolates from a variety of ecological niches, creating 225 strains with unique pairings of nuclear and mitochondrial genomes. We consistently observed that the original coadapted mitonuclear combinations provided fitness advantages over synthetic combinations when yeasts were grown in conditions emulating the isolation habitat. Stress conditions increased phenotypic variances due to mitonuclear interactions although strains with coadapted mitonuclear genomes did not necessarily outperform those with synthetic genome combinations. This reveals that in yeast, selection for mitonuclear interactions is ubiquitous in emerging populations and mainly occurs during growth under basal conditions. Our work shed lights on to how intraspecific incompatibilities arise and how mitonuclear coevolution contributes to early post-zygotic speciation events.

1063A Thermal performance curves: from mitochondrial physiology to population growth rate in *Drosophila* *Omera Matoo*[1], Ibrahim El-Shesheny[1], John DeLong[1], Kristi Montooth[1] 1) University of Nebraska-Lincoln.

Biological processes at the mitochondrial level could play a key role in setting thermal sensitivity of organisms and delineating the thermal range of ectotherms. With the current effects of climate change, many ectotherms will experience greater energetic stress, warranting investigation into the role of mitochondria as an important component of how they will cope with climate change. To determine whether changes in mitochondrial properties could underlie trade-offs and constraints in thermal limits and adaptation, we examined the thermal performance of mitochondrial function in outbred population of *Drosophila melanogaster*. Our approach is to connect thermal performance at different levels of biological organization in order to mechanistically understand how temperature-dependent changes in mitochondrial function scale up to impact population dynamics. Here we present data on thermal performance curves for mitochondrial function and the emergent physiological components and life-history traits including metabolic rate, survivorship, development rate, fecundity and population growth rate in an outbred population of *Drosophila melanogaster*. These data provide the baseline for an experimental evolution experiment to ask how different levels of biological organization together respond adaptively to temperature. We discuss a predictive framework for forecasting the dynamic responses to environmental change from thermal metabolic responses through a series of currently unknown nested functions up to population level responses. This is important for comprehensive and quantitative understanding of the driving forces, tradeoffs and constraints in thermal adaptation.

1065C Evidence for sex-specific genetic architecture of gut length in Lake Malawi cichlid fishes *Aldo Carmona Baez*[1], Emily Moore[1,2], Natalie Roberts[1], Kaitlin Coyle[1], Amanda Cass[1,3], Erin Peterson[1], David Reif[1], Reade Roberts[1] 1) North Carolina State University, Raleigh, NC; 2) University of Montana, Missoula, MT; 3) James Madison University, Harrisonburg, VA.

Trophic specialization is key to the phenotypic and species diversity observed across life. Several characteristics of gut morphology and physiology correlate with trophic levels. The most common example of these correlations is found in vertebrates, where organisms with a plant-based diet generally have longer digestive tracts compared to animals at higher trophic levels. Despite its importance, very few studies have explored the genetic basis of diet adaptation. In this study, we used recently diverged Malawi cichlid species as a model to identify candidate genes involved in gut length variation with a forward genetics approach. We performed QTL mapping of gut length on two F2 mapping populations from hybrid crosses between species of cichlids from different trophic levels. Our results suggest the presence of sex-specific QTL in the genetic architecture of gut length, as well as several inversions between the genomes of the parental species. This analysis represents the first identification of naturally evolved, adaptive genetic variants associated with gut length. In addition, we integrated these mapping results with transcriptomic studies to pinpoint the genes and pathways driving evolution of the gut.

1072A Evolution of cell types in a Drosophila sex organ *Alex Majane*[1], Julie Cridland[1], David Begun[1] 1) Department of Evolution and Ecology, UC Davis, Davis, CA.

Cell type-specific changes in gene expression and function are frequent drivers of adaptation and diversification. While the importance of cell type-specific evolution in metazoans is well-appreciated, little work has addressed how patterns of evolutionary change among closely related species may be influenced by cell type-specific functions and constraints. We are using the Drosophila male accessory gland, the site of seminal fluid synthesis, as a model to study the tempo and mode of evolutionary change among different cell types across a range of time scales. Seminal fluid is essential for successful fertilization in flies, and induces a suite of changes in female post-mating behavior and physiology known as the post-mating response. The gland and its products are an ideal model system for comparing the evolutionary properties of cell types because it is a simple secretory tissue, and seminal fluid evolves rapidly and often adaptively due to sexual conflict between males and females. The gland is comprised of two secretory epithelial cell-types: main cells and secondary cells. Main cells are necessary and sufficient to induce the majority of the post-mating response, while secondary cells have a smaller relative effect. Given these distinct contributions, we expect that each cell type experiences selection at different intensities, and consequently will have distinct evolutionary rates of protein sequence and gene expression divergence across Drosophila lineages. To test this, we used single-nucleus RNA-sequencing of accessory glands on three closely related species to estimate cell type-specificity of gene expression and identify differentially expressed genes across species. We find a greater fraction of differentially expressed genes in secondary cells relative to main cells, and we also observe higher rates of adaptive protein substitution in genes biased towards expression in secondary cells. This system will allow us to compare patterns of divergence within different cell type populations across a broad range of evolutionary time scales, which represents a novel approach towards studying the evolutionary properties of cell types.

1092C Locomotion leads to speciation, in the form of a supergene locus. Yubo Zhang[1], Dequn Teng[2], Wei Lu[2], Min Liu[3], Alan Zhu[3], *Wei Zhang*[4] 1) State Key Laboratory of Protein and Plant Gene Research, Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China; 2) State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences, Peking University, Beijing 100871, China; 3) State Key Laboratory of Membrane Biology, Ministry of Education Key Laboratory of Cell Proliferation and Differentiation, Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, School of Life Sciences, Peking University, Beijing 100871, China; 4) State Key Laboratory of Protein and Plant Gene Research, Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, School of Life Sciences, Peking University, Beijing 100871, China.

Speciation is a comprehensive process, yet the genetic evidence for biomechanical traits and their interplay with other traits involved in this process are still lacking. The *Heliconius* butterflies have undergone adaptive radiation and therefore serve as an excellent system for the study of speciation. Here we identify and characterize a locomotor locus that consistently displays high divergence among multiple groups of subspecies, suggesting it might have been recurrently involved in the *Heliconius* ecological speciation and radiation. We further reveal multiple roles of wing locomotion in speciation and adaptation by illustrating this locus an introgression hotspot, resulting in dynamic combinations of wing locomotion and patterns between sister species and co-mimics. By performing additional functional validation in *Drosophila melanogaster* we show that this locus contains multiple genes related to locomotion and its origin was due to chromosomal rearrangements in Lepidoptera. We therefore suggest it a supergene locus particularly required for speciation accompanying complex wing patterns. In light of these findings, we propose an integrative model of butterfly speciation and adaptation with both morphological and biomechanical traits.

1111A Speciation rate variation due to loss of co-adapted genes *Andrius Jonas Dagilis*[1], Jenn Coughlan[1], Daniel Matute[1] 1) University of North Carolina at Chapel Hill.

When a population experiences selection in a novel environment, mutations experience selection not only on their fitness in this environment, but on their interactions with other newly adaptive mutations. Loss of such co-adapted mutations can cause hybrid fitness to decline. Recent theory has shown that such fitness loss may be a larger contributor to hybrid fitness than novel deleterious interactions (classical Dobzhansky-Muller Incompatibilities) between mutations that have never passed the screen of selection. In an analysis of several data-sets of hybrid fitnesses, we find that speciation occurs more rapidly when one parental species has evolved much more rapidly than the other. Under these circumstances, there are many fewer opportunities for novel deleterious interactions than for loss of co-adapted sets of genes. These results are the first empirical test of several patterns predicted by speciation theory, and inform new theoretical predictions about the probability of introgression.

1114A Identification and characterization of X-linked hybrid male sterilty factors between *Drosophila simulans* and *D. mauritiana* *Rodolfo Villegas*[1], Neal Weldon[1], Gina Mavhezha[1], Colin Meiklejohn[1] 1) University of Nebraska-Lincoln.

Intrinsic postzygotic isolation, resulting in sterile or inviable hybrid offspring, is a powerful mechanism of speciation. In recently diverged species, the negative consequences of hybridization may demonstrate asymmetry between the sexes with the heterogametic sex (males in XY systems, females in ZW systems) generally more severely affected (Haldane's rule). Numerous hypotheses have described the evolutionary forces proposed to underlie this pattern, all with some degree of empirical support. Our research aims to identify and describe X-linked hybrid male sterility factors between *Drosophila simulans* and *D. mauritiana* in an effort to elucidate the evolutionary forces underlying hybrid male sterility.
We have genetically dissected a 4Mb X-linked introgression from *D. mauritiana* into an otherwise *D. simulans* genetic background that causes male sterility. These experiments suggest four distinct regions within this introgression that each cause hybrid male sterility, including a previously identified sterility factor *Odysseus*(*OdsH*). *OdsH* encodes a DNA-binding protein with a homeobox domain and previous work utilizing fluorescently-tagged *OdsH* proteins suggested the *D. mauritiana* allele of *OdsH* (*OdsHmau*) causes sterility through inappropriate binding to the *D. simulans* Y chromosome. We find that introduction of the *D. mauritiana* Y chromosome into our *OdsH*-containing sterile males rescues male fertility, providing genetic evidence for a sterility-causing interaction between *OdsHmau* and the *D. simulans* Y chromosome.
To identify the specific developmental defects underlying male sterility, we examined spermatogenesis in sterile introgression males, using a *ProtamineB* transgene fused to GFP (*ProtB*-GFP) to visualize protamine deposition and sperm nucleus morphology. Testes from males carrying the larger 4Mb introgression show *ProtB*-GFP-positive nuclei scattered throughout the testis, instead of concentrated towards the distal end of the testis as in fertile males, and sperm nuclei fail to complete reshaping. In contrast, in males carrying only the *OdsH* region from *D. mauritiana, ProtB*-GFP-positive sperm nuclei are found at the distal end of the testis, the sperm nuclei reshape and sperm appear to individualize, but they remain in the testis and are not transferred to the seminal vesicle. These results support our conclusion of multiple sterility factors within this 4Mb X-linked region, and suggest different developmental causes of sterility associated with different sterility factors.

1117A *Wolbachia* variants differentially rescue the fertility of a *bag-of-marbles* mutant in *Drosophila melanogaster* *Paula Fernandez-Begne*[1], Jaclyn Bubnell[1], Cynthia K.S. Ulbing[1], Charles F. Aquadro[1] 1) Cornell University.

The vertically-transmitted endosymbiotic bacterium *Wolbachia pipientis* infects a large proportion of arthropod species by manipulating their reproductive biology in order to increase its transmission. In *Drosophila melanogaster*, effects of *Wolbachia pipientis* (wMel) infection on host fitness can range from detrimental to beneficial. Previous work showed that wMel infection rescues fertility defects in a mutant of *bag-of-marbles* (*bam*), a key switch for germline stem cell differentiation that shows patterns of episodic adaptive evolution across the *Drosophila* genus. wMel is therefore of key interest as a potential selective pressure on *bam*. The aim of this project was to further understand the co-evolutionary relationship between wMel and *bam* and determine whether fertility rescue of *bam* mutants by wMel varies due to wMel genotype. We examined female fertility and oocyte production of isogenic *D. melanogaster bam* mutants infected with 11 genetically distinct wMel variants over the course of 17 days. Subsequent analysis led to the result that wMel infection not only rescues fertility of *bam* mutants, but that the degree of rescue depends on the wMel variant. The highest rescue was due to infection by wMelCS-like variants, which typically have higher titer. Future steps include manipulating wMel titer using the wMelPop strain to directly investigate the relationship between titer and degree of *bam* rescue. This and future studies will further elucidate the co-evolutionary relationship between *Drosophila melanogaster* and *Wolbachia pipientis*.

1122C Host-plant specialization and natural history of *Drosophila santomea* *David Peede*[1], Heidi Mavengere[1], Brandon Cooper[2], Daniel Matute [1] 1) Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Division of Biological Sciences, University of Montana, Missoula, MT.

Olfactory genes (genes that regulate chemoreception) play an important role in detecting chemical cues from plant hosts. Notably, these genes also show signatures of positive selection among *Drosophila* sister species pairs, which suggests they have played an important part in specialization to different resources. Here we study the *yakuba* sister species complex (*D. santomea*, *D. yakuba*, and *D. teissieri*) to better understand the dynamics of host-plant specialization and the natural history of *D. santomea*. Using various field assays, and ecological monitoring of *Drosophila* and fruit abundance in the island of São Tomé, we provide evidence that *D. santomea* has specialized not only a preference but also a higher fitness for the endemic fig species (*Ficus chlamydocarpa fernandesiana*). In contrast, its sister species (*D. teissieri* and *D. yakuba*) have not. We also show that there are genomic signatures of positive selection in olfactory genes, which might be related to the specialization of *D. santomea*.

1127B Partitioning reproductive success: experimental evolution of male fertility *Katja Kasimatis*[1,2], Megan Moerdyk-Schauwecker[1], John Willis[1], Patrick Phillips[1] 1) University of Toronto; 2) University of Oregon.

The reproductive phase of the lifecycle is a complex process requiring individuals to find a mate of the opposite sex, share gametes, and fuse gametes. In internally fertilizing organisms, this process occurs in two major stages: pre-insemination mating interactions and post-insemination gametic interactions. The total reproductive fitness of a male, therefore, is determined by the number of successful insemination events which are then translated into successful fertilization events. Studying the relationship between pre- and post-insemination success in determining male reproductive fitness has proved challenged, as post-insemination interactions are difficult to isolate. However, indirect studies indicate that pre-insemination interactions may be more important. We take advantage of the tools and tractability of *Caenorhabditis* nematodes to build a new system for post-insemination mating interactions. First, we design an external, inducible sterility system for *C. elegans*. The sterility induction capitalizes on the auxin inducible degron system to prevent males from producing functional sperm. This tool provides fine-scale temporal control over male fertility, which we use to isolate post-insemination interactions. Specifically, we explicitly test the relative contributions of pre-insemination mating success and post-insemination fertility success to overall male reproductive fitness using an experimental evolution approach. The experimental framework uses a 2x2 factorial design to compare and contrast the effects: i) sexual selection acting on sperm competition, ii) sexual selection acting on male-male competition, iii) gametic selection acting on sperm ability, and iv) lab adaptation. We evolved six biological replicates of each condition with population sizes of ~5,000 worms for 30 generations. We identify a strong response to selection. Total male reproductive success increased across all conditions. Interestingly, the component of total reproductive success attributed to sperm competitive success also increased across all conditions, such that at least 30% of the increase in reproductive success came from increased sperm success. These fitness changes seem attributable to a relatively small number of loci. This work indicates that post-insemination male reproductive success is an important contributor to overall fertility.

1133B Inbreeding slows the spread of selfish *wtf* meiotic drivers in fission yeast populations Jose Fabricio Lopez Hernandez[1], Rachel Helston[1], Jeffrey J. Lange[1], Scott McCroskey[1], Andrew Box[1], SaraH Zanders[1,2] 1) Stowers Institute for Medical Research, MO.; 2) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, KS..

In meiosis, both alleles of a heterozygous organism have equal chances to be transmitted to the offspring. Selfishly, some alleles manipulate meiosis to increase their transmission. This process is called meiotic drive. In the fission yeast *Schizosaccharomyces pombe*, members of the *wtf* gene family are meiotic drivers. The *wtf* drivers are transmitted up to 100% of the viable offspring by eliminating the gametes that do not carry the *wtf* allele. These selfish genes gain transmission by decreasing the species fitness and are thus in conflict with the rest of the genome. Mechanisms that suppress drive in a population are therefore predicted to be favored by natural selection. We found that some strains of *S. pombe* inbreed, which reduces the cost and spread of *wtf*s in a population. We demonstrated that inbreeding is facilitated by mating-type switching, which allows the two sister cells produced by mitosis to mate with each other. Surprisingly, despite low genetic diversity amongst natural *S. pombe* isolates, mating patterns greatly varied between isolates, some mate randomly while others tend to inbreed. We confirmed that a *wtf*+ allele spreads slower in an inbreeding population by monitoring it throughout consecutive sexual cycles. Although the cost of genetic conflict can be alleviated by gene interactions, we show that meiotic conflict is also suppressed by mating behavior in fission yeast.

1140C Dietary impact on starvation resistance in an evolved population of *D. melanogaster* *Zachary Elkins*[1], Jordyn Moaton[1], Elizabeth King[1] 1) University of Missouri.

An organism's access to food fluctuates over the course of its life. In many populations, organisms likely experience prolonged lengths of time with limited or no access to food. In such cases, an organism's ability to resist starvation is essential to its survival. Starvation resistance (SR) can differ among individuals due to both genetic differences as well as environmental differences. We used an experimental evolution framework to assay for SR in three different populations of *D. melanogaster* selected for three different resource allocation patterns. Our goal was to see how three different diets affected SR in each of the three evolved populations.
We placed twelve replicate populations of *D. melanogaster* on three selection treatments: constant high availability (CHA), fluctuating availability

(FA) and deteriorating availability (DA). Flies in the CHA treatment were fed a high sugar diet for their entire lifespan. Flies in the FA treatment are in turn given a standard diet followed by a low yeast diet followed by a standard diet. Flies in the DA treatment start with a standard diet and then are put on a low yeast diet until eggs are collected for the next generation. These three treatments have been ongoing for over 50 generations. For our experiment, flies from all replicates and treatments were placed on one of three diets for 10 days: high sugar, standard and low yeast diets. After 22 days post-oviposition (p.o.), flies were placed on nutrition-less agar. SR was measured as the time it takes for a fly to die starting at the moment it is placed on nutrition-less agar. We determined how these different selection treatments led to the evolution of plasticity in SR in response to diet. We link these phenotypic changes to both genetic mechanisms and environmental conditions.

1143C Methods for inferring haplotype frequencies from pooled genomic samples with applications to evolve and resequence experiments using *Drosophila melanogaster* *Elizabeth King*[1], Zeke Elkins[1], Enoch Ng'oma[1], Paul Petrowski[1] 1) University of Missouri.

Evolution has been directly observed many times in populations in real time. Examples include experimental evolution studies, plant and animal domestication, and wild populations experiencing a known selective pressure. As genome sequencing becomes increasingly commonplace, directly observing how genomes evolve in real time during selection has also become possible. In these studies, our understanding of genome dynamics can be substantially improved when it is possible to infer haplotype frequencies from sets of allele frequencies. We developed two different optimization methods for inferring haplotype frequencies from pooled genomic samples for a population with a known set of ancestors. We used the Drosophila Synthetic Population Resource (DSPR) to simulate data to test the performance of these methods for different window sizes and different sample sizes. In addition, we use multiple empirical datasets to validate the method and demonstrate its use in evolve and resequence studies.

1151B Evolutionary pathways to collateral sensitivity *Kara Schmidlin*[1], Sam Apodaca[1], Kerry Geiler-Samerotte[1] 1) Center for Mechanisms of Evolution, School of Life Sciences, Arizona State University, Tempe, AZ 85287, United States.

Drug resistance represents a vast problem that has yet to successfully addressed. Recent work focuses on collateral sensitivities, where evolution of resistance under drug A results in susceptibility to drug B. At present, studies in both cancer cells and bacteria populations have demonstrated that collateral sensitivity is unpredictable and nonrepeatable. This can be attributed to the wide array of mutations that can occur under the stress of the drug A and the low replicate size used in the experiments. Here, we propose using a barcoded yeast system to track a large population of yeast as they develop resistance to drug A then are subsequently challenged by drug B. This system tracks hundreds of thousands of replicate yeast lineages, thus revealing the full spectrum of adaptive mutants that protect against drug A. Experiments using this system will provide a more quantitative understanding of the likelihood of collateral sensitivity, as well as the evolutionary paths that lead to collateral sensitivities.

1156A Evolution of high mutation rates is generally constrained but permitted during intermediate-level cycles of starvation *Wei-Chin Ho*[1], Megan Behringer[1], Samuel Miller[1], John Meraz[1], Jadon Gonzales[1], Amber Nguyen[1], Gwyneth Boyer[1], Michael Lynch[1] 1) Biodesign Center for Mechanisms of Evolution, Arizona State University.

Mutations are the ultimate source of molecular and phenotypic evolution, and therefore the rate of mutation is a fundamental parameter for determining the level of intraspecific diversity and predicting evolutionary trajectories in a population. Despite of the importance of mutation rates in understanding evolution, how mutation rates evolve still remains largely unknown. Several factors have been proposed to contribute to the evolutionary dynamics of mutation rate, including the biophysical limitation of functional protein complexes, mutational load due to the deleterious effects of mutations, hitchhiking of beneficial mutations, and the genetic-drift barrier. However, how these factors interact is still questionable, partly due to the lack of experimental evidence. Experimental evolution under better-defined conditions can help uncover the constraints of evolutionary predictability and reveal if there are any non-monotonic patterns across the gradients of interest. Here, in order to test what determines the evolutionary outcomes of mutation rates, we performed two sets of experimental evolution of *Escherichia coli* with replicates in Luria-Bertani (LB) broth. Two kinds of gradients were studied: (1) three transfer sizes (TS) related to the ancestral population: 10^{-1}, 10^{-4}, and 10^{-7}, representing different levels of bottleneck effects; and (2) three nutritional transfer periods (TP): every day, every 10 days, and every 100 days, representing different levels of starvation. To further detangle the complex dynamics of gradients on mutation rates, two genetic backgrounds were used: wild-type (WT) and a mismatch-repair defective mutant (MMR-), which results in an 150x increase of mutation rate. After 900 days of evolution, we quantified the mutation rates using fluctuation tests. Across the three TSs, most MMR- populations experienced a reduction of mutation rate by 10-100 fold, while WT populations do not. In contrast, across the three TPs, we surprisingly found both WT and MMR- populations from the intermediate TP evolved higher mutation rates, showing a non-monotonic pattern. We further collected a time series of pooled-sequencing data from these evolved populations and identified the fixed mutations using a clade-aware hidden Markov chain model. This set of fixed mutations contained several genes that are highly relevant to mutation rates, including several structural variations in *mutL* that are found in multiple WT populations evolved under the 10-day period. To sum up, our results show that the mutational load remains an important factor to constrain the evolution of high mutation rate. However, that constraint can be overcome by extra evolutionary opportunities provided under some special conditions, such as a fluctuating environment of resource availability.

1163B Life in evolution's fast lane: extensive loss of cell-cycle and DNA repair genes in ancient budding yeasts *Jacob Steenwyk*[1], Dana Opulente[2], Jacek Kominek[2], Xing-Xing Shen[1], Xiaofan Zhou[3], Abigail Labella[1], Noah Bradley[1], Brandt Eichman[1], Neža Čadež[4], Diego Libkind[5], Jeremy DeVirgilio[6], Amanda Hulfachor[2], Cletus Kurtzman[6], Chris Hittinger[2], Antonis Rokas[1] 1) Vanderbilt University, Nashville, TN; 2) University of Wisconsin–Madison, Madison, WI; 3) South China Agricultural University, Guangzhou, China; 4) University of Ljubljana, Ljubljana, Slovenia; 5) Universidad Nacional del Comahue-CONICET, San Carlos de Bariloche, Rio Negro, Argentina; 6) United States Department of Agriculture, Peoria, IL.

Cell-cycle checkpoints and DNA repair processes protect organisms from potentially lethal mutational damage. Compared to other budding yeasts in the subphylum Saccharomycotina, we noticed that a lineage in the genus *Hanseniaspora* exhibited very high evolutionary rates, low Guanine–Cytosine (GC) content, small genome sizes, and lower gene numbers. To better understand *Hanseniaspora* evolution, we analyzed 25 genomes, including 11 newly sequenced, representing 18/21 known species in the genus. Our phylogenomic analyses identify two Hanseniaspora lineages, a faster-evolving lineage (FEL), which began diversifying approximately 87 million years ago (mya), and a slower-evolving lineage (SEL), which began diversifying approximately 54 mya. Remarkably, both lineages lost genes associated with the cell cycle and genome integrity, but these losses were greater in the FEL. E.g., all species lost the cell-cycle regulator WHIskey 5 (*WHI5*), and the FEL lost components of the spindle checkpoint pathway (e.g., Mitotic Arrest-Deficient 1 [*MAD1*], Mitotic Arrest-Deficient 2 [*MAD2*]) and DNA-damage–checkpoint pathway (e.g., Mitosis Entry Checkpoint 3 [*MEC3*], RADiation sensitive 9 [*RAD9*]). Similarly, both lineages lost genes involved in DNA repair pathways, including the DNA glycosylase gene

3-MethylAdenine DNA Glycosylase 1 (*MAG1*), which is part of the base-excision repair pathway, and the DNA photolyase gene PHotoreactivation Repair deficient 1 (*PHR1*), which is involved in pyrimidine dimer repair. Strikingly, the FEL lost 33 additional genes, including polymerases (i.e., POLymerase 4 [*POL4*] and *POL32*) and telomere-associated genes (e.g., Repressor/ activator site binding protein-Interacting Factor 1 [*RIF1*], Replication Factor A 3 [*RFA3*], Cell Division Cycle 13 [*CDC13*], Pbp1p Binding Protein [*PBP2*]). Echoing these losses, molecular evolutionary analyses reveal that, compared to the SEL, the FEL stem lineage underwent a burst of accelerated evolution, which resulted in greater mutational loads, homopolymer instabilities, and higher fractions of mutations associated with the common endogenously damaged base, 8-oxoguanine. We conclude that *Hanseniaspora* is an ancient lineage that has diversified and thrived, despite lacking many otherwise highly conserved cell-cycle and genome integrity genes and pathways, and may represent a novel, to our knowledge, system for studying cellular life without them.

1165A Alliance of Genome Resources: Towards an extensible, sustainable knowledge commons framework for model organism databases *Carol Bult*[1], Judith Blake[1], Brian Calvi[2], J. Michael Cherry[3], Kevin Howe[4], Chris Mungall[5], Norbert Perrimon[6], Mary Shimoyama[7], Paul Sternberg[8], Paul Thomas[9], Monte Westerfield[10] 1) The Jackson Laboratory, Bar Harbor, ME; 2) Department of BIology, Indiana University, Bloomington, IN; 3) Department of Genetics, Stanford University, Palo Alto, CA; 4) European Bioinformatics Institute, European Molecular Biology Laboratory, Hinxton, UK; 5) Lawrence Berkeley National Laboratory, Berkeley, CA; 6) Department of Genetics, Harvard University, Boston, MA; 7) Medical College of Wisconsin, Madison, WI; 8) Division of BIology and Biological Engineering, California of Technology, Pasadena, CA; 9) Keck School of Medicine, University of Southern California, Los Angeles, CA; 10) Department of BIology, University of Oregeon, Eugene, OR.

Funding models for community genome data resources such as the Model Organism Databases (MODs) and the Gene Ontology (GO) has been a topic of discussion in the biomedical research community for many years. In 2016, NIH's National Human Genome Research Institute, announced their intention to significantly reduce funding for these resources. In response, a campaign to show support for community genome resources was launched by the Genetics Society of America, the Society for Developmental Biology, and the American Society of Cell Biology. Over 10,000 signatures on a petition in support of the MODs and the GOC were collected in a matter of weeks and presented to the Director of NIH at The Allied Genetics Conference in July 2016.

Following the announcement by NHGRI, six MODs (Mouse Genome Database, Saccharomyces Genome Database, WormBase, FlyBase, Zebrafish Information Network, Rat Genome Database) and the Gene Ontology Consortium formed the Alliance of Genome Resources (aka, the Alliance). The goal of the Alliance is to develop a common framework for acquiring, curating, and accessing genome data from different model organisms while preserving the representation of the unique biology of each model organism. The Alliance-developed infrastructure is intended to be extensible so that the integration of data from additional model organisms can be accommodated easily.

The Alliance is comprised of two interdependent functional units: Alliance Central and Alliance Knowledge Centers. Alliance Central coordinates data harmonization efforts across different model organism databases and ensures that there are uniform methods for access to data types that are in common across different model organism resources. Alliance Central has implemented a include a web site (alliancegenome.org) where users can compare biological and functional genome annotations across model organisms and human and maintains common APIs and modular software for displaying annotations. Alliance Knowledge Centers focus on expert curation of organism-specific data and knowledge coming from the biomedical literature as well as high-throughput genomics and phenotyping initiatives. The Alliance represents a unique resource in support of comparative genome biology between diverse model organisms and human.

1182C Mutation rate and spectrum variation in natural isolates of *Saccharomyces cerevisiae* *Pengyao Jiang*[1], Anja Ollodart[1], Alan Herr[1], Maitreya Dunham[1], Kelley Harris[1] 1) University of Washington, Seattle, WA.

Mutation, which provides the raw material for evolutionary change, is usually assumed to have a constant rate over evolutionary time. However, the variation of mutation rate among individuals or over time can be hard to measure precisely. Some extremely high mutation rate, known as mutator phenotypes, have been observed in cancers and in microorganisms with disruptions in DNA repair pathways or high environmental stresses. However, whether and why less extreme mutator alleles might have arisen in the past and been maintained in populations is largely unknown. In order to rigorously investigate patterns and causes of historical mutation process changes using *Saccharomyces cerevisiae*, we compare the spectrum of mutations that have accumulated in the history of natural populations to those that are *de novo* accumulated in the lab.

A mutation spectrum, a measurement of the relative frequencies of different mutation types, is a property of mutation processes that can be correlated with perturbed molecular mechanisms or environments experienced by the organism. We analyzed segregating mutation spectra from recently sequenced *Saccharomyces cerevisiae* populations (the 1011 Genomes Project) and found that the overall mutation spectrum from natural variants differs from the spectrum of *de novo* mutations measured in previous genome-wide mutation accumulation (MA) studies. Patterns of inherited variation suggest that African beer and Taiwanese populations have different mutation spectra from other *S. cerevisiae* populations. To determine whether the same populations have unusual mutation spectra in controlled lab environments, we have developed a cost and time efficient mutation accumulation pipeline, using *CAN1* as a reporter gene followed by pooling and sequencing to measure the rate and the spectrum of *de novo* mutations in different strains. As a proof of principle, we were able to measure the locations and sequence contexts of all 39 mutations from an initial pool of 38 mutant colonies, confirming them by Sanger sequencing with zero false positives. Preliminary results from a subset of non-African, non-Taiwanese strains show a moderate difference in mutation rate, but no significant difference in mutation spectra. Once measurements on the strains with unusual inherited spectra are completed, these results will further the current understanding of mutation rate variations within a species and over time.

1190B Faster mutation rates in New World monkeys *Richard Wang*[1], Muthuswamy Raveendran[2], R. Alan Harris[2], Jeffrey Rogers[2], Matthew Hahn[1] 1) Indiana University; 2) Baylor College of Medicine.

The equivalence of the mutation and substitution rate is a classic result from theoretical population genetics. Under a neutral model of molecular evolution, the constancy of this 'molecular clock' allows species divergence times to be dated using sequence divergence. Studies subsequent to this proposal have, however, revealed a significant degree of substitution rate variation across mammalian lineages. Species with longer generation times appear to have slower rates, as observed from the comparison of humans to Old World monkeys. The increasing accessibility of whole-genome sequencing has enabled a second approach: the estimation of mutation rates by counting the number of *de novo* mutations that appear between generations. These direct estimates of the mutation rate have indicated an even lower mutation rate in humans than expected from studies of sequence divergence. A better understanding of how mutation rates translate into sequence divergence across species, and the underlying

biology driving this variation, requires a broader sampling of mutation rates.

Here, we compare direct estimates of the mutation rate across two groups of primates, the Old World monkeys and the New World monkeys. Our study adds rate estimates from the whole-genome sequencing of 8 trios across two primate species, the olive baboon (*Papio anubis*) and the squirrel monkey (*Saimiri boliviensis*). We combine these with existing direct estimates from primates, including species from these two groups of monkeys as well as from great apes, and contrast them to substitution rates estimated from sequence divergence. Our analyses suggest a much faster rate of mutation in New World monkeys, consistent with studies using sequence divergence. We explain this lower mutation rate in a model of reproductive longevity, highlighting the large influence of life history on evolutionary rates.

1202B Investigating the Evolution of Vocal Communication in *Xenopus* frogs Young Mi Kwon[1], Ursula Kwong-Brown[1], Christa Baker[2], Avelyne Villain[1], Therese Mitros[3], Emilie Perez[1], Mala Murthy[2], Andres Bendesky[1], *Darcy Kelley*[1] 1) Columbia University; 2) Princeton University; 3) UC Berkeley.

For many vertebrate species, vocalizations are essential components of social communication. Animals call in a variety of social contexts including courtship, mating, rivalry, and alarm signaling. In diverging populations, co-evolution of courtship signals and preferential responses to those signals can generate barriers to reproduction and lead to speciation. In the 29 species of the fully aquatic frog genus *Xenopus,*vocal signaling predominates in social interactions and is innate. Males produce an advertisement call in which the combination of spectral (pitch) and temporal (rhythm and pattern) features is a unique species identifier. To uncover genetic differences across species that support divergent advertisement calls, we performed quantitative trait locus (QTL) mapping using F2hybrids between related species with different male advertisement calls: *X. petersii* and *X. laevis.* To phenotype each individual F2 male, we developed a pipeline for automated analysis of spectral and temporal features.We identified ~3.8 million candidate species-specific markers from whole genome sequences (>10x coverage) of 4 *X. laevis* and 2 *X.petersii*including the founders of the F2 intercross, to genotype across the F2 males. We whole genome sequenced the F2s at 0.01-0.03x coverage, and used a HMM-based imputation method and pruned the SNP panel to obtain 533 informative genome-wide markers for QTL analysis. Ourpreliminary results identify QTLs on chromosomes 3, 5, 8, and 9 for vocal spectral features. Determining how variation in spectral features affect female preference will help to elucidate the genetic basis and evolution of courtship-based vocal signaling in *Xenopus*and more broadly, provide further insight to how vocal communication has evolved across vertebrates.

1206C Genome-wide identification of conserved non-coding elements associated with subterranean mammal phenotypes using deep learning *Elysia Saputra*[1], Nathan Clark[1,2], Maria Chikina[1] 1) University of Pittsburgh; 2) University of Utah.

Subterranean environment imposes selection pressures that drive the phenotypic adaptation of fossorial animals, which includes the convergent loss of ocular structures. Previous work in our lab have identified significant correlations between ocular functions and protein-coding elements with accelerated relative evolutionary rates in subterranean mammals. The same study also demonstrated convergent accelerations in evolutionary rates of ocular-specific transcriptional enhancers. In this work, we extended the investigation genome-wide to identify potential regulatory elements that are associated with subterranean adaptation. We used a deep convolutional neural network model developed in the work by Kelley [*biorXiv* 660563 (2019)], which was trained to predict the signal tracks of mouse sequencing assays from the ENCODE and FANTOM databases using DNA sequence as input. The model has been shown to be effective for predicting regulatory activities cross-species. With this mouse-trained model, we predicted CAGE expression of 320,299 conserved non-coding elements in mammals using the annotation for the eyeball tissue of mouse embryo (day 12) from FANTOM, for 3 species: naked mole rat (subterranean), mouse, and guinea pig, the latter being a control aboveground species with the same evolutionary distance from the mouse as the naked mole rat. By evaluating the relative expression with respect to mouse, we identified 16,787 conserved non-coding regions that are significantly repressed in the naked mole rat compared to in the guinea pig. Gene ontology analysis on these regions with the GREAT tool returned hits that were consistent with the subterranean phenotype, including skin thickening, eye degeneration, abnormal kidney functions, and heart enlargement. Next, we evaluated whether the top repressed non-coding elements matched known DNA motifs and found hits consistent with the subterranean traits, such as the repression of ROX1, a repressor of hypoxic genes, and the repression of SRF, which is associated with postnatal mouse retinal hypovascularization that resembles blinding retinopathies in human. We are following up this analysis by predicting the expression of the regions in two other subterranean mammals, the star-nosed mole and the cape golden mole, and control animals selected based on appropriate evolutionary relationships in their clades. This work demonstrated the potential utility of deep learning models for evolutionary comparative genomics studies.

1247B I Against I: Leveraging adversarial training for population genetics *Jeffrey Adrion*[1], Andrew Kern[1] 1) University of Oregon.

There has been a recent explosion in the application of supervised machine learning methods within the fields of population genetics, genomics, and phylogenetics. These tools, however impressive in their performance, come with a unique set of constraints and potential hazards. Perhaps the most obvious of these limitations are the problems of overfitting and out-of-sample prediction, where the training set is a poor match to the test data. Quantifying the robustness of machine learning methods to such misspecification is therefore of great concern to geneticists who rely on these tools to address important and often cryptic biological phenomena. One emerging technique for assessing the robustness of Deep Learning methods is known as Adversarial Perturbation Analysis (APA), where robustness is measured by the minimum perturbation to an input example that results in a maximally erroneous prediction. These perturbed inputs, known as adversarial examples, are indistinguishable from non-adversarial examples to the naked eye. Here we use APA to quantify the robustness of several widely used artificial neural network architectures designed to infer recent population size expansions from genome sequence data. We then leverage adversarial training to produce a robust Deep Learning classifier for inferring the demographic history of a population.

1268B Inbreeding resistance naturally evolved on islands *Longhua Guo*[1], Marta Riutort[2], Joshua Bloom[1], Katarina Ho[1], Zain Kashif[1], Kaiya Kozuma[1], Eyal Ben David[1], Tzitziki Lemus Vergara[1], Alejandro Sánchez Alvarado[3], Leonid Kruglyak[1] 1) Department of Human Genetics, Department of Biological Chemistry, and Howard Hughes Medical Institute, University of California, Los Angeles, CA, USA; 2) Department de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona, and Institut de Recerca de la Biodiversitat (IRBio), Barcelona, Catalonia, Spain; 3) Stowers Institute for Medical Research, and Howard Hughes Institute for Medical Research, Kansas City, MO, USA.

Island species have provided valuable insights into our understanding of genome diversity and evolution since the time of Charles Darwin. Sexual biotypes of the freshwater planarian, *Schmidtea mediterranea*, are distributed largely on the islands of Sardinia, Corsica, and Sicily, and in rare locations in Tunisia. Populations in Sardinia were previously reported to maintain genome heterozygosity during inbreeding. We constructed a genetic

linkage map by single sperm sequencing and found the heterozygosity maintaining regions mapped to chromosome 1. Genotyping 70 animals from all four locations uncovered two population superclusters: Sardinia/Corsica and Sicily/Tunisia. Inbreeding resistance occurs in both Sardinia and Corsica but not Sicily or Tunisia. Sardinia/Corsica populations have higher nucleotide diversity and smaller inbreeding coefficient with significant contributions from chromosome 1. Microscopy analysis revealed a ring structure of chromosome 1 in such heterozygous animals. We hypothesize our data revealed a naturally occurred low-recombining chromosome that is resistant to inbreeding in island populations.

1277B Holocene sea level change drives different fates of two Asian horseshoe crab species *Qian Tang*[1], Frank Rheindt[1], Prashant Shingate[2], Akbar John[3], Yusli Wardianto[4], Boon Hui Tay[2], Laura Yap[5], Jasmin Lim[5], Hor Yee Tong[6], Karenne Tun[6], Byrappa Venkatesh[2] 1) National University of Singapore, Singapore; 2) Institute of Molecular and Cell Biology, A*STAR, Singapore; 3) International Islamic University Malaysia, Malaysia; 4) Bogor Agricultural University, Indonesia; 5) School of Applied Science, Republic Polytechnics, Singapore ; 6) National Parks Board, Singapore.

Holocene sea level change drives dramatic coastal habitat transformation along the Sunda Shelf. Population genomics is an ideal tool to help us understand evolutionary responses of the coastal species to sea level change. In this study, we sampled over 300 individuals of two horseshoe crab species, *Carcinoscorpius rotundicauda* and *Tachypleus gigas*, across the Singapore Strait and used double digest RAD sequencing to collect their genomic information. Our results show that the two species display different population structures even though they are similar in habitat preference and life history. Based on our spatial analyses, we discovered that different dispersal ability, where *C. rotundicauda* has relatively smaller dispersal range and subjected to sea depth, may contribute to the difference in population structure. Reconstructed demographic history also indicates that difference in dispersal ability led different fate of the two species during sea level change, with the less dispersive species continuously declining as the sea level rose and maintaining high level over the recent few thousand years. We speculate that high sea level introduces coastal habitat fragmentation which creates isolated populations for the less dispersive species that are vulnerable to the loss of genomic diversity. Therefore, we suggest that different conservation approaches should be considered for the two horseshoe crab species against sea level rise in the foreseeable future.

1292B *wtf* genes: Killing gametes for more than 110 million years. *Mickael De Carvalho*[1,2], Blake Billmyre[1], Sarah Zanders[1,3] 1) Stowers Institute for Medical Research, Kansas City, USA; 2) Open University, UK; 3) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City.

Killer meiotic drivers are parasitic genes that promote their own transmission into the progeny by actively destroying gametes that do not inherit the driver allele from a heterozygote (driver+/driver-). Meiotic drivers are predicted to play a significant role in shaping genome evolution. The newly discovered *wtf* genes of *Schizosaccharomyces pombe* are a family of meiotic driver genes that encode two proteins: a poison that kills gametes and an antidote that protects only the gametes that inherit the driver. Meiotic drivers are predicted to have a very short lifespan because do not persist for long evolutionary periods. Here we show that the meiotic driver *wtf* is present in *Schizosaccharomyces* lineage for more than 110 million years.
Currently, *wtf* genes are reported to be found only within *S. pombe*. We recently identified 63 *wtf* genes in *S. octosporus* and 5 in *S. cryophilus*. We have shown that *S. octosporus wtf* genes share features with *S. pombe wtf* genes. They share a high number of exons, two predicted translational start sites and are associated to repetitive elements. We show that *S. octosporus wtf* genes can encode both poison and antidote proteins that allows to favorize their own transmission in the population. *S. octosporus* and *S. pombe* have diverge 110 million years ago. The finding of *wtf* genes in *S. octosporus* and *S. cryophilus* mean that *wtf* genes are born more than 110 million years ago. *wtf* family is the oldest active meiotic driver identified.

1298B Skewed sex ratios after partial or complete deletion of the *Obp50a-d* gene cluster in *Drosophila melanogaster* *Ugonna Ihearahu*[1, 2], Joel Johnstun[3], Roberta Lyman[2], Sneha Mokashi[2], Trudy Mackay[2], Robert Anholt[2] 1) University of Surrey; 2) Clemson University; 3) North Carolina state University.

Odorant binding proteins (*Obps*) in Drosophila are generally thought to be associated with olfactory responses, but identification of OBPs in non-chemosensory tissues (*e.g.* accessory glands) suggests that some members of this family may have evolved to acquire different functions. The functions of many *Obps* remain, however, poorly defined. We used CRISPR technology to delete a group of four members of the *Obp50* cluster, *Obp50a-d*, that are in close physical proximity without intervening genes while introducing a PhiC31 integration site. We encountered difficulty in obtaining females from the CRISPR knock-out line when flies were reared at 25°C but slowing their growth at 18°C rescued females. We quantified the sex ratio at 25°C in the CRISPR knockout line versus the co-isogenic Canton-S control. We then reinserted all combinations of intact members of this group in their endogenous location through PhiC31 integration. Deletion of the entire *Obp50a-d* cluster resulted in a female-male sex ratio of 0.33 compared to a ratio of 0.46 after reinsertion of an intact *Obp50a-d* cluster. Reinsertion of constructs with intact *Obp50b*, *Obp50c* and/or *Obp50d* genes failed to restore a normal sex ratio. However, reinsertion of constructs that contained an intact *Obp50a* gene resulted in an average sex ratio of 0.43, close to the control. Thus, the *Obp50* cluster has undergone functional diversification during evolution and *Obp50a* has acquired a function that is essential for enabling balanced development of males and females. The mechanism of sex ratio bias from the *Obp50a-d* cluster remains to be determined. Supported by NIH grant GM128974.

1301B Barley Cytoplasmic Multi Parent Population (CMPP) for Studying Loss of Plasticity Under Domestication *Eyal Fridman*[1], Adi Leker[1], Khalil Kashkush[2], Eyal Bdolach[1,2] 1) Plant Sciences Institute, The Volcani Center, Agricultural Research Organization (ARO); 2) Department of Life Sciences, Ben-Gurion University, Beer-Sheva, Israel..

Although there is evidence that phenotypic plasticity can facilitate the evolution of fixed traits in general, it remains an open question whether it is stability or variability of the different characteristics that have adaptive value and therefore be selected? Besides, recent studies indicate the loss of DNA standing variation underlying interaction with a different environment in modern crops. It remains to explore how much of this left-behind variation is adaptive under current scenarios of climate change, and to what extent neglected cytoplasm variation is involved in providing plasticity? To begin answering these questions, we have generated a new multi-parent population derived from reciprocal crosses between cultivated barley (*Hordeum vulgare* cv Noga) and ten diverse *H. vulgare* ssp. *spontaneum* wild barley ecotypes. Initially, we focus our phenotypic analysis on attributes of the circadian clock plasticity using newly developed high-throughput phenomics platform. Preliminary results indicate trends of lost plasticity of the clock in the transition from wild to the cultivated gene pool. We develop genetic models to allow genome scan for allelic variation driving environmental sensitivity, and follow-up on derived F2 population allows us to estimate and validate the prevalence of specific cytoplasm and nuclear loci epistasis in providing thermal plasticity of the circadian clock. Combining these results with field experiments under thermal gradi-

ents should assist us in questioning the possible pleiotropic effects of clock plasticity and robustness on fitness traits. This study will explore hitherto unknown allelic variation controlling robustness and contribute to our understanding of the mechanisms underlying flexibility vs buffering against a significant ecological constraint.

1304B Metabolic Influences of Bisphenol F Exposure in Population-based Heterogeneous Stock rats *Valerie Wagner*[1,2], Karen Clark[1,2], Katie Holl[1], Derek Simonsen[2], Miriam Velez-Bermudez[2], Kai Wang[2], Leah Solberg Woods[3], Hans-Joachim Lehmler[2], Anne Kwitek[1] 1) Medical College of Wisconsin, Milwaukee, WI; 2) University of Iowa, Iowa City, IA; 3) Wake Forest School of Medicine, Winston-Salem, NC.

Bisphenol F (BPF) is marketed as a 'safe' substitute for bisphenol A (BPA), an endocrine disruptor associated with obesity and heart disease (i.e. cardiometabolic disease), in manufacturing polycarbonates and in common consumer products. BPF's environmental presence is growing rapidly and is detected in 66.5% of U.S. adults. There is evidence of individual variation in bisphenol levels, suggesting that gene x environment (GxE) interactions influence risk of cardiometabolic disease from bisphenol exposure. Few studies of BPF exposure in either humans or animals have been performed, thus the health risks associated with BPF exposure are unknown. Traditional *in vivo* toxicity studies are performed in genetically undefined, outbred rats or genetically homogeneous, inbred mice, leading to conflicting results possibly due to GxE interactions. The N/NIH Heterogeneous Stock (HS) rats are a genetically heterogeneous rat population that is amenable to genetic study.

The overall hypothesis of this project is that BPF-induced cardiometabolic disease has underlying genetic susceptibility, which can be identified using the HS rat model. The goal of this pilot project was to determine if BPF exposure influences growth and adiposity in HS rats. Weanling littermate pairs of male HS rats were randomly exposed to either vehicle (0.1% Ethanol) or 1.125 mg/L BPF in 0.1% Ethanol for five weeks in drinking water. Cardiometabolic measures, tissues, urine, and feces were taken.

Our studies determined that BPF exposure in HS rats significantly increases whole body growth. BPF exposure alters body composition seen in nuclear magnetic resonance measurements (NMR) by increasing % fat and decreasing % lean, mirroring the significant increase in gonadal-white-adipose-tissue (GWAT) mass and perirenal-white-adipose-tissue (PWAT) mass in BPF-exposed males. Heritability estimates of significant phenotypes reveal that BPF exposure may alter trait variation. Our data suggest that BPF exposure increases body growth and adiposity, which are risk factors for cardiometabolic disease. Current studies are assessing adipose tissue homeostasis through gene expression of adipogenesis, fibrosis, and inflammation markers.

1305C The origin of reaction norms *Maximilian Press*[1] 1) Phase Genomics Inc.

Reaction norms are a foundational concept in quantitative genetics. Their perennial relevance to genetic analysis is exemplified in the challenges of present-day studies of the heritable basis of human traits. However, the origin of the term is relatively obscure. *Reaktionsnormen* (the original term) were first described by Richard Woltereck in a 1909 paper as genetically-determined phenotypic responses to environmental variation; until recently the German text of this paper was not available in digital form.

As I was unable to locate a previous translation into English, I provide a draft translation of this paper from German to English (working draft available here: https://github.com/maximilianpress/reaktionsnorm/blob/master/woltereck_reaktionsnorm.pdf). I briefly review the chief findings and their importance in historical context.

I then evaluate some more recent secondary literature published in English citing Woltereck's primary text, and note some incorrect statements about Woltereck's ideas, possibly due to the lack of an available English text. These inconsistencies highlight the importance of the accessibility of primary texts, and specifically the difficulties which can be encountered when important primary texts are not available in an accessible language.

I discuss how our retrospective judgment of Woltereck's paper (contrasted with Wilhelm Johannsen's classic work on pure lines) might be affected by language of publication. I conclude by discussing some of the issues around current publication practices (digital, global, and mostly monoglot) and past publication practices (analog, provincial, and polyglot). I additionally discuss the tradeoffs of language acessibility in the context of language imperialism.

1334B Data-driven identification of environmental variables influencing phenotypic plasticity for grain yield in hybrid maize *Aaron Kusmec*[1], Dan Nettleton[2], Patrick S. Schnable[1,3] 1) Department of Agronomy, Iowa State University, Ames, IA; 2) Department of Statistics, Iowa State University, Ames, IA; 3) Plant Sciences Institute, Iowa State University, Ames, IA.

Phenotypic plasticity describes changes in a genotype's phenotype in response to environmental variation. A key component for the quantification of phenotypic plasticity is the identification of the set of environmental variables that influence alterations in a particular phenotype. These variables are typically selected using domain-specific knowledge or, when the set of variables is suitably small, via exhaustive search. Two factors complicate these strategies. First, environments are shifting and becoming more variable due to global climate change which may introduce novel stresses that are not yet captured by domain-specific knowledge. Second, environments exhibit high dimensionality not only in terms of the variables that can be measured and their temporal resolution but also with respect to the timescales at which organisms perceive different environmental variables throughout development. This size makes exhaustive search impossible without the introduction of potentially erroneous simplifying assumptions, especially when assessing the simultaneous influence of multiple environmental variables on a phenotype. To address these challenges, we propose a heuristic procedure to efficiently identify informative sets of environmental variables for the quantification of phenotypic plasticity. We apply this procedure to a hybrid maize dataset and demonstrate its utility for characterizing phenotypic plasticity and identifying directions for future research into the biology of plastic responses.

1339A Dissecting the Genetic Basis of Variation in Cocaine and Methamphetamine Consumption in *Drosophila melanogaster* *Brandon Baker*[1], Robert Anholt[1], Trudy Mackay[1] 1) Clemson University.

Studies on *Drosophila melanogaster* can identify genetic and transcriptional networks that underlie variation in voluntary consumption of cocaine and methamphetamine to serve as a blueprint for subsequent studies on humans. Exposure to these psychostimulants in flies results in behavioral and physiological effects that resemble those observed in humans. We derived an outbred advanced intercross population (AIP) from 37 of

the sequenced inbred wild-derived lines of the *Drosophila melanogaster* Genetic Reference Panel (DGRP). These lines are maximally genetically divergent, have minimal residual heterozygosity, are not segregating for common inversions, and are not infected with *Wolbachia pipientis*. We assessed voluntary consumption of sucrose, methamphetamine-supplemented sucrose and cocaine-supplemented sucrose and found significant phenotypic variation in the AIP, in both sexes, for consumption of both drugs. We performed whole genome sequencing and extreme QTL mapping on the top 10% of consumers for each replicate, sex and condition, and an equal number of randomly selected flies. We evaluated changes in allele frequencies genome-wide among high consumers and control flies and identified 3,033 variants associated with increased consumption that reside in 1,963 genes, enriched for genes associated with nervous system development. We assessed the effects of ubiquitous RNA interference (RNAi) on consumption for 22 candidate genes, of which 14 showed a significant increase or decrease in consumption. We constructed new AIPs which were homozygous for target alleles in five intergenic SNPs and five genes, tested average consumption for each population and observed extensive sexual dimorphism and differences in genotype- and condition-specific effects. Supported by U01DA041613.

1360A Sprinters versus marathon runners – are there differences in how Drosophila respond to exercise induction? *Nicole Riddle*[1] 1) University of Alabama at Birmingham.

Animal activity is an important trait that influences an organism's fitness. For example, when an organism is active, during the day or the night, is important as this choice greatly impacts its ability to find food or mates and its chances to encounter predators or prey. It is also important how organisms respond to external stimulation, i.e. how much energy to expend on such a stimulation in terms of activity. We have used the DGRP strain collection to investigate variation in activity levels in *Drosophila melanogaster*, both for basal activity levels without stimulation and for activity levels induced by continued rotational stimulation (exercise). We found that within the DGRP strain collection, activity levels varied by approximately 500-fold and that the rotational stimulation is able to increase activity levels in most of the strains. Using a GWAS, we linked the variation in activity levels to over 400 genetic variants present in the DGRP strain collection. Here we use this dataset to investigate how the various Drosophila strains respond to the rotational stimulation, either by a short burst of activity following the start of the rotation (sprinters) or by continued activity throughout the two-hour exercise period (marathoners). We find that the DGRP strains vary greatly in their response pattern, with some strains exhibiting less than 10% of their activity in the first quarter of the exercise period and other strains exhibiting over 85% in this time interval. The amount of activity performed during the first quarter of the exercise period is strongly dependent on the sex of the animals, their genotype, as well as the interaction of sex and genotype. We will present the results of an ongoing GWAS, which suggests that the response type exhibited by Drosophila, either a short burst or sustained activity, might be under genetic control.

1376B Private Genomes and Public Alleles Christian Fischer[1], Pjotr Prins[1], Robert Davies[2], *Richard Mott*[3] 1) Genetics, Genomics & Informatics, University of Tennessee Health Science Center, Memphis TN, USA; 2) Dept Statistics, University of Oxford, IK; 3) Genetics Institute, University College London, UK.

Sharing genetic data makes possible discoveries that would be otherwise inaccessible to analyses of individual under-powered data sets. However, the need for genetic privacy limits data sharing, so we require methods that separate public information about allelic effects from private information about individuals. Here, we describe the use of linear transformations to homomorphically encrypt genotype and phenotype data. Homomorphic encryption is a form of encryption that allows computation an encrypted result which, when decrypted, matches the result of the operations as if they had been performed on the original plaintext. Orthogonal transformations, composed of rotations and reflections, leave unchanged the likelihood of quantitative trait data under a linear model with normally distributed errors. These transformations also preserve linkage disequilibrium between genetic variants and associations between variants and phenotypes, but encrypt the relationships between individuals and between individuals and phenotypes. All standard inferences based on the likelihood are unaffected, including the use of a mixed-model to control for unequal relatedness between individuals, the estimation of heritability, and the inclusion of covariates when testing for association. We will discuss in how far this homomorphic encryption is truly cryptographically secure and discuss several potential attacks, and show that random orthogonal transformations provide at minimum protection from accidental release of data.

1388B MouseGWAS – an R package for easy mouse inbred strains GWAS execution *Asaf Peer*[1], Brian Q. Geuther[1], Vivek M. Philip[1], Vivek Kumar[1] 1) The Jackson Laboratory.

Genome wide association studies (GWAS) provide important insights into genetic architecture of complex traits. This effort has been highly successful in human studies although it requires very large sample sizes. In mice, resources and methodologies for GWAS have been developed. These include, genotyping platforms and generation of reference genotype data for large number of inbred mouse lab strains, such as the Mouse Diversity Array and the Hybrid Mouse Diversity Panel, respectively. Hundreds of inbred and recombinant inbred strains were genotyped using these arrays and their SNP genotyping data are currently available at The Jackson Laboratory's phenome database (https://phenome.jax.org/genotypes). However, conducting a GWAS analysis still requires a lot of preprocessing effort such as extraction of the relevant genotypes, combining of genotypes for F1 mice, filtering of the SNPs and preparing the input files. These hurdles can sometimes be a significant cost to biological researchers. Here, we present an R package that greatly simplifies mouse GWAS studies by creating an automated GWAS analysis pipeline. The package MouseGWAS takes phenotypic measurements of inbred and F1 mice strains along with a yaml file describing the experiment, prepares the input for GEMMA or PyLMM, executes them using a leave-one-chromosome-out approach, runs meta-analysis or multivariate analysis if desired, and generates publication-ready figures of the results. We tested the package with coat color phenotypes and here will present data in which we study the grooming behavior of mice. The package is available on github: https://github.com/TheJacksonLaboratory/mousegwas. The availability of this package will greatly simplify and potentially help community members carry out genetic association studies.

1390A Polymorphic B2 insertions shape tissue-specific gene expression programs in house mice, *Mus domesticus* *Laura Blanco-Berdugo*[1], Beth Dumont [1] 1) The Jackson Laboratory.

Transposable elements (TEs) serve as important sources of evolutionary innovation in gene regulation. As a result of recent transposition events, TEs are also highly polymorphic across individuals within populations. Despite these well-established observations, the extent to which polymorphic TE insertions (polyTEs) influence global gene expression programs in diverse tissues has not been thoroughly explored. To address this gap, we leveraged a publicly available dataset of wild house mice (*Mus musculus domesticus*) that features whole-genome and RNA-seq data for 10 distinct tissues collected on an identical set of 24 animals from three different populations (France, Germany and Iran). We systematically identified 11,507 SINE B2 polyTEs across these wild mouse genomes and assessed their distribution across populations. The majority of B2 polyTEs segregate among

populations and we observed very few population private polyTEs (France = 29, Germany = 18, and Iran = 36). To determine the impact of these polyTEs on gene expression, we performed tissue-specific eQTL analyses to map approximately 260 polyTE insertions with significant effects on gene expression. Several key findings emerge from this analysis. First, many eQTL are directly related to conserved housekeeping functions such as cell division and cellular metabolism. Second, we identify numerous tissue-specific eQTL. For example, a B2 PolyTE located on chromosome 1(181 Mbp), affects the expression of multiple genes related neural plate elongation in the brain, but does not influence expression programs in other surveyed tissues. Third, multiple polyTEs exert regulatory effects on clusters of genes located in both *cis* and *trans*. In several cases, this coordinately regulated *cis* and *trans* eQTL are implicated in common biological networks. For example, we identified a PolyTE near the transcription factor *Stat3* that drives significant changes in liver gene expression of 13 genes in the same co-expression network. Overall, our findings underscore polyTEs, an underappreciated form of genetic variation, as an important source of regulatory variation across diverse mammalian tissues.

1397B Ras-RalGEF-Ral-dependent developmental events in *C. elegans* development and metabolism *You Wu*[1], Jacob Mardick[1], Hanna Shin[1], Neal Rasmussen[1], David J. Reiner[1] 1) Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX.

Ras is the most mutated oncoprotein, with ~30% of tumors driven by activated Ras. Among three Ras effectors of roughly equivalent oncogenicity, Ras-Raf and Ras-PI3K are well studied, but the downstream components and mechanisms of Ras-RalGEF-Ral signaling remain poorly understood. Ral (Ras-like) is a small GTPase related to Ras. The fundamental gap in our understanding of Ras-RalGEF-Ral signaling is because Ral is both a component of the heterooctomeric exocyst complex and uses the exocyst as a signaling intermediary, which precludes conventional biochemical bootstrapping to identify signal transduction components downstream of the exocyst. Consequently, we are using genetic analysis of Ral signaling in *C. elegans* to identify roles of Ral in development and metabolism, and investigate activities downstream of Ral. In addition to our previous publication of a Ral-dependent 2˚-promoting cascade in in vulval cell fate patterning (Shin *et al*, *Cell Reports*, 2018)), we found that Ral may function in six different contexts. 1) Constitutively activated endogenous Ral (RAL-1(gf)) causes a defect in lipid synthesis and/or storage in the intestine. 2) RAL-1(gf) in combination with deletion of GCK-2 confers phenotypes consistent with defective development of the utse cell and the ventral uterus. 3) Constitutively activated Ras may signal through RalGEF-Ral as a parallel input into DAF-2/InsR-mediated dauer formation. 4) Ral effector Rlbp1 (a GAP for Cdc42/Rac) and mir-61 may repress an anti-2˚ signal in the in vulval cell fate patterning. 5) Ras-RalGEF-Ral contributes to CAN cell migration and other events during sculpting of the nervous system. 6) Redundant Ral and Rap1 (Ras Proximal) are required for progression through embryonic morphogenesis, possibly through regulation of the exocyst.. Taken together, our results highlight diverse roles of Ral signaling in development, and positions us for mutant screens to identify downstream components of Ral signaling in multiple contexts.

1426A RhoGAP RGA-8 supports morphogenesis in *C. elegans* by polarizing epithelia through CDC-42 Shashikala Sasidharan[1], Luigy Cordova Burgos[1], Hamidah Raduwan[1], Andre Wallace[2], *Martha Soto*[1] 1) Rutgers - RWJMS, Piscataway, NJ; 2) Fairleigh Dickinson University, Teaneck, NJ.

CDC-42 regulation of non-muscle myosin/ NMY-2 is required for polarity maintenance in the one-cell embryo of *C. elegans*. CDC-42 and NMY-2 regulate polarity throughout embryogenesis, but their contribution to later events of morphogenesis are less understood. We have shown that epidermal enclosure requires the GTPase CED-10/Rac1 and WAVE/Scar complex, its effector, to promote protrusions that drive enclosure through the branch actin regulator Arp2/3. Our analysis here of RGA-8, a homolog of SH3BP1/Rich1/ARHGAP17/Nadrin, with BAR and RhoGAP motifs, suggests it regulates CDC-42, so that NMY-2 promotes two events of epidermal morphogenesis: ventral enclosure and elongation. Genetic and molecular data suggest RGA-8 regulates CDC-42, and the CDC-42 effectors WSP-1 and MRCK-1, in parallel to F-BAR proteins TOCA-1 and TOCA-2. The RGA-8-CDC-42-WSP-1 pathway enriches myosin in migrating epidermal cells during ventral enclosure. We propose TOCA proteins and RGA-8 use BAR domains to localize and regenerate CDC-42 activity, thus regulating F-actin levels, through the branched actin regulator WSP-1, and myosin polarity through the myosin kinase MRCK-1. Regulated CDC-42 thus polarizes epithelia, to control cell migrations and cell shape changes of embryonic morphogenesis.

1429A A role for COP9 signalosome component CSN-5 in stabilizing stem cell regulators FBF-1 and FBF-2 *Emily Osterli*[1], Mary Ellenbecker[1], Xiaobo Wang[1], Ekaterina Voronina[1] 1) University of Montana.

The highly conserved COP9 (constitutive photomorphogenesis 9) signalosome complex can affect protein stability through a range of mechanisms including deneddylation, deubiquitination, and phosphorylation (Wolf et al., 2003). The most extensively studied mechanism of COP9 is its deneddylating activity on the cullin family of ubiquitin-ligases. Furthermore, the catalytic subunit of COP9, CSN-5, can interact with other cellular proteins beyond COP9 components (Shackleford & Claret, 2010). Our lab identified an interaction between CSN-5 and the RNA-binding proteins FBF-1 and FBF-2, both of which are required for stem cell maintenance in *Caenorhabditis elegans*. We also discovered that CSN-5 promotes the accumulation of FBF-1 and FBF-2 proteins in *C. elegans* stem and progenitor cells, therefore contributing to stem cell maintenance in the organism. Additionally, genetic analysis suggests that the CSN-5/FBF interaction functions in the context of COP9 holoenzyme because FBF-1 protein levels are also decreased in *csn-2* and *csn-6* mutant worms. Both COP9 subunits CSN-5 and CSN-6 have an MPN metalloprotease domain (Mpr1/Pad1 N-terminal), which is essential for integration into the COP9 complex, but only CSN-5 is catalytically competent (Cope et al., 2002; Lingaraju et al., 2014; Birol et al., 2014). Our preliminary results support the hypothesis that the metalloprotease domain of CSN-5 binds to the conserved RNA-binding domains of FBF-1 and FBF-2, thus identifying a protein complex that is evolutionarily conserved. Interactions between CSN-5 and other cellular proteins outside of the COP9 signalosome complex have not been extensively studied, but since the FBF stabilization depends on multiple subunits of the COP9 complex we expect the FBF/CSN-5 interaction to also occur when CSN-5 is incorporated into the COP9 complex. Analysis of the interaction between CSN-5 and the FBF proteins will elucidate how assembly of this protein complex is mediated and provide tools to further test our hypothesis that FBF/CSN-5 interaction stabilizes FBFs in stem and progenitor cells.

1436B *C. elegans* proteasomal subunit RPN-12 is essential for germline sex determination and oocyte quality but not viability or proteolytic activity *Lourds Fernando*[1], Anna Allen[1] 1) Howard University.

The 26S proteasome is a major proteolytic machinery that is conserved across species. Proper function of the proteasome is crucial for the survival of organisms. The proteasome is composed of nearly 33 different subunits arranged into two 19S regulatory particles (RP) capping a cylindrical 20S core particle (CP). Recent evidence suggests that specific 19S RP subunits of the proteasome perform non-proteolytic roles in various cellular processes such as transcription, mRNA export and chromatin structure. However, there is still a large knowledge gap in our understanding of specific canonical and non-canonical roles of each proteasome subunit. In *Caenorhabditis elegans,* depletion of individual proteasome subunits typically results in embryonic lethality and thus elucidating explicit roles of individual subunits is challenging. We recently found that the 19S RP subunit

RPN-12 is not essential for the general proteolytic function of the proteasome but for hermaphrodite gametogenesis in *C. elegans*. A null mutant of RPN-12, *rpn-12(av93),* is homozygous viable, however the hermaphrodites lack sperm. The oocytes of the *rpn-12(av93)* animals can be cross fertilized by mating with males but the hermaphrodites do not reach wild-type reproductive capacity. Therefore, we hypothesized that RPN-12 plays a role in *C. elegans* germline sex determination pathway and oocyte quality respectively. Loss of RPN-12 causes nuclear accumulation of the oocyte meiotic kinase WEE-1.3 which is essential for oocyte quality and *rpn-12(av93)* hermaphrodites can partially suppress the *wee-1.3(RNAi)* infertility phenotype. As chemical inhibition of the proteasome using bortezomib neither causes WEE-1.3 nuclear accumulation in oocytes nor suppresses the *wee-1.3(RNAi)* infertility phenotype, this suggests a potential non-proteolytic role for RPN-12 in WEE-1.3 regulation. Current investigations are underway to identify the mechanism by which RPN-12 regulates the germline sex determination pathway and its interaction with WEE-1.3 in maintaining oocyte quality. To further characterize RPN-12 we generated an endogenous GFP::RPN-12 tagged strain which shows ubiquitous expression in the germline and somatic cells. The *gfp::rpn-12* strain will be used to perform *in vitro* and *in vivo* studies to identify interactors of RPN-12 and further determines its non-proteolytic roles in the *C. elegans* germline.

1438A The role of RPN-12, a subunit of the proteasome's 19S regulatory particle, in *C. elegans* male fertility *Jeandele Elliot*[1], Lourds Fernando[1], Anna Allen[1] 1) Howard University.

The 26S proteasome is a complex which degrades proteins and maintains protein homeostasis in animal cells. The proteasome consists of a 19S regulatory particle (RP) lid and base component capping a cylindrical 20S core particle (CP). The proteasome is conserved in all eukaryotes and our lab uses *Caenorhabditis elegans* to investigate possible distinct roles of individual proteasomal subunits. In *C. elegans*, the proteasome consists of nearly 33 different subunits of interacting proteins. Our research investigates roles for individual 19S RP proteasomal subunits in *C. elegans* fertility. This research focuses on characterizing the role of the 19S RP subunit RPN-12 in *C. elegans'* male fertility. Our lab has shown that in *C. elegans,* an endogenous *rpn-12* deletion mutant [*rpn-12(av93)*] causes feminization of the hermaphrodite germ line, hermaphrodite fertility issues and a subtle decrease in proteolytic function. Here we will show that male *rpn-12(av93)* animals show no apparent defects in growth, survival, or fertility. To investigate the role of RPN-12 in the fertility of *C. elegans* males, we quantified the *rpn-12(av93)* male spermatids and performed fertility assays to assess the ability of the males to successfully fertilize oocytes. Quantification and characterization of the *rpn-12(av93)* male spermatids allowed us to determine that although there is a consistent decrease in the spermatid count compared to wild-type animals, this difference is not statistically significant. There is also no statistically significant difference in the average brood resulting from *rpn-12(av93)* males mated to feminized females compared to wild-type males. This indicates that loss of RPN-12 has no significant effect on sperm production in males. Interestingly, we see a high percentage of *rpn-12(av93)* males run off the agar and dry on the walls of the plate. Therefore, we hypothesize that while RPN-12 may not be necessary for sperm production in *C. elegans* males, it may be essential for sensing the presence of the female and the act of copulation. Future experiments will be performed to assess the mating behavior of *rpn-12(av93)* mutant males. Collectively our data provides further support that specific proteasome subunits perform different, non-proteolytic functions in specific tissues.

1439B Functional analysis of RPN-13, a C. elegans proteasomal subunit, using a strain that indicates proteolytic function in the germline *Caroline Ugoaru*[1], Lourds Fernando[1], Anna Allen[1] 1) Howard University, Washington, DC .

The 26S proteasome degrades proteins tagged with ubiquitin. It is composed of a cylindrical 20S core particle (CP) capped with two 19S regulatory particles (RP). The 19S RP is composed of many subunits, and in many cases the specific proteolytic and non-proteolytic roles of individual 19S RP subunits are not fully understood. Our lab identified that certain 19S RP subunits appear to play non-proteolytic roles in the C. elegans germline. To learn more about the function of particular 19S RP subunits, we are using a recently published strain, IT1877, to monitor proteolytic activity of the 26S proteasome under various conditions, such as RNAi depletion of different 19S RP subunits. The IT1877 strain contains a mutated ubiquitin fused to GFP under control of the germline promoter [pie-1p::Ub(G76V)::GFP::H2B::drp-1 3'UTR; unc-119(+)] and results in continuous degradation of GFP during optimal 26S proteasome activity. If a certain condition causes proteasome dysfunction, the degree of dysfunction can be quantified by measuring the intensity of the GFP signal in the IT1877 strain. A strong GFP signal indicates 26S proteasome dysfunction, and a dim or no signal indicates the proteasome is functioning properly. As our lab is interested in utilizing the IT1877 strain to monitor proteasome function upon depletion of genes that potentially affect the fertility of the animals, we wanted to determine if the IT1877 strain itself had any fertility defects. A fertility assay was conducted to compare the average 72 hour brood in IT1877 and wild type hermaphrodites. Our data shows that IT1877 hermaphrodites have a significantly lower average brood compared to wild-type animals. Although the IT1877 strain will not be used for future fertility screens in the lab, it will continue to be a useful tool to measure proteolytic activity of the proteasome in the C. elegans germline. We are currently using the IT1877 strain to investigate the proteolytic function of the RPN-13 subunit. RPN-13 is an uncharacterized 19S RP subunit in C. elegans. Since little is known about this subunit, we will explore its role in C. elegans fertility and identify any potential phenotypes observed upon RNAi depletion. Functional analysis of 19S RP subunits, such as RPN-13, will increase our understanding of how the 26S proteasome influences C. elegans reproduction.

1455C Characterizing the role of SCF-mediated protein degradation in meiosis *Joshua Blundon*[1], Jocelyn Haversat[1], Yumi Kim[1] 1) Johns Hopkins University.

Skp1-Cullin-F-Box (SCF) complex is the E3 ubiquitin ligase, which plays important roles in cell cycle progression by polyubiquitinating its targets, thereby promoting their destruction via the proteasome. The SCF complex has previously been shown to control aspects of meiotic prophase in *C. elegans*. However, it has remained elusive what are the key targets that need to be degraded or how proteolysis controls chromosome segregation during meiosis. Here we report that SCF components are robustly expressed throughout the germline, consistent with their functions during meiotic prophase. Cytological analyses of mutants lacking two paralogous Skp1-related genes, *skr-1* and *skr-2,* have revealed that SKR-1 and SKR-2 play distinct functions in meiotic progression. While SKR-1 is dispensable, SKR-2 is required for timely onset of meiosis, proper synapsis, and pachytene exit. Interestingly, SKR-2 associates with protein complexes containing synaptonemal complex (SC) components, and is enriched along the SC. We are currently investigating to identify an F-box protein and key substrates required for proper synapsis and will present our findings.

1457B Genetic suppressor screen of separase mutants identifies cohesin subunits Michael Melesse[2], Dillon E. Sloan[1], Amy Fabritius[3], Harold Smith[3], Andy Golden[3], Györgyi Csankovszki[1], Joshua N. Bembenek[1] 1) MCDB, University of Michigan, Ann Arbor, MI; 2) BCMB, University of Tennessee, Knoxville, TN; 3) NIDDK, NIH, Bethesda, MD.

Separase is a well-conserved protease best known for its function in promoting anaphase onset by cleaving cohesin. However, other roles for separase have been implicated in cytokinesis and vesicular trafficking in different model organisms. In *C. elegans*, it was demonstrated that separase has

a role in the formation of the eggshell during cortical granule exocytosis just after anaphase onset of meiosis I, a role independent of chromosome segregation which requires its proteolytic activity. To elucidate the mechanism of separase activity during cortical granule exocytosis, we conducted an ENU mutagenesis screen for suppressors of a temperature sensitive, partial separation-of-function allele of separase that covered nearly a million haploid *C. elegans* genomes. At the restrictive temperature, this allele has minimal issues in chromosome segregation but fails to localize to or exocytose cortical granules. In our screen, we identified 68 suppressor mutations of this allele in 7 different genes, including 14 intragenic suppressors, 47 mutations in *pph-5*, and 7 mutations in previously unidentified genes including *hsp-90* (which regulates *pph-5*), and 3 cohesin genes not directly cleaved by separase. Interestingly, while the mutations in these cohesin genes suppress the lethality associated with this allele, RNAi depletion at varying levels does not. This may indicate that the mechanism of suppression is not simply due to a loss of cohesin function. Currently, our work is aimed at verifyingthese suppressors by CRISPR and investigating the mechanism of suppression by observing the cellular phenotypes of these cohesin mutants.

1461C Elucidating the Role of Securin in Regulating Separase during Cortical Granule Exocytosis *Christopher Turpin*[1], Marian LaForest[1], Joshua Bembenek[2] 1) University of Tennessee; 2) University of Michigan.

Meiosis is a tightly regulated series of events leading to the production of haploid gametes. A key player in this process is the cysteine protease separase (SEP-1). Known for its role in chromosome segregation, recent studies suggest that SEP-1 has an additional function in vesicular trafficking during cell division. We hypothesize that cell cycle machinery known to control SEP-1 protease activity for chromosome segregation also controls its localization to the cortex and subsequent exocytic activity. Following spindle attachment and chromosome alignment during the meiotic M phase, the anaphase promoting complex/cyclosome (APC/C) is activated, resulting in the degradation of SEP-1 inhibitory chaperone securin (IFY-1) and entry into anaphase I. In recent studies, we have observed that SEP-1 localizes to specialized vesicles called cortical granules and regulates their exocytosis during anaphase I. Before it appears on cortical granules, SEP-1 localizes to cytosolic filaments near the plasma membrane. We have shown that SEP-1 colocalizes with its inhibitor, IFY-1, on filaments during prometaphase, and both disassociate from these structures after anaphase I onset. Inhibition of APC/C activity prevents SEP-1 and IFY-1 from leaving the filaments, and depletion of IFY-1 via RNAi causes premature localization of SEP-1 to cortical granules and blocks their exocytosis. These data suggest the hypothesis that degradation of IFY-1 may allow SEP-1 to localize to vesicles and act on substrates to promote exocytosis. To test this further, we generated a non-degradable IFY-1 (IFY-1^{DM}::GFP). Consistent with enhanced IFY-1 stability, IFY-1^{DM}::GFP is not degraded following anaphase I onset and causes embryonic lethality. IFY-1^{DM}::GFP persists on chromosomes in anaphase and inhibits chromosome segregation. Interestingly, IFY-1^{DM}::GFP causes polar body extrusion failure, which could be related to defects in cortical granule exocytosis. IFY-1^{DM}::GFP, in contrast to wild-type IFY-1, localizes to vesicles with SEP-1 during anaphase I. In the future we will investigate how IFY-1^{DM}::GFP affects SEP-1 localization to cortical granules. This will provide insight into how key regulatory components of the cell cycle control SEP-1 localization to promote timely cortical granule exocytosis during anaphase I.

1466B Establishing *C. elegans* as a Model for Studying the Biological Effects of Therapeutic Ultrasound *Louise Steele*[1], Troy Kotsch[1], Catherine Legge[1], Delores Smith[1] 1) Kent State University, Salem, OH.

Ultrasound is frequently used in medicine for diagnostic imaging and therapeutic procedures. Other than a weak association between ultrasound exposure and non-right handedness in males, there is no evidence that diagnostic ultrasound produces deleterious effects in humans. Research in flies, mice, and chicks has, however, suggested that various doses of ultrasound can affect mortality, birth weight, and learning in those species. In amoebae and cultured cells, ultrasound exposure led to cavitation, release of reactive oxygen species (ROS), and apoptosis. Additional research in animals is needed because the output and availability of ultrasound machines have increased, the mechanisms of some therapeutic approaches remain poorly understood, and ultrasound is becoming an important tool in biological research. We developed methods for exposing *C. elegans* to therapeutic ultrasound and found that worms exhibited dose-dependent reductions in movement, fecundity, and survival. The effects were prevented by polyvinyl alcohol, which is known to minimize cavitation (formation and collapse of gas-filled spaces) in tissues. Using a genetic approach, we also looked for evidence of other mechanisms of ultrasound-induced damage. Exposed *sod-2;sod-3*, *clk-1*, and *ced-3* mutants exhibited the same changes in mobility and fecundity that exposed N2 worms did. Thus, at the intensities tested, cavitation may have masked ROS release, and apoptosis may have been insufficient to mitigate the tissue damage. This work provides a foundation for characterizing the effects of lower-intensity ultrasound at the cellular and molecular levels. A thorough understanding of bioeffects is needed to realize the full potential of ultrasound's usefulness in science and medicine, and to ensure continued patient safety.

1470C Suppression of nekl-associated molting defects by induction of L2d *Shaonil Binti*[1], Sam Miller[1], Rosa Melinda[1], David Fay[1] 1) University of Wyoming.

Molting is required for *C. elegans* to develop through larval stages into the adult. In addition, the timing of molting must be tightly coordinated with developmental progression. We have shown that the NIMA-related kinases, NEKL-2 and NEKL-3 (the NEKLs), promote molting through their involvement in the trafficking of epidermal cargos. This includes LRP-1/megalin, which is involved in the internalization of cholesterols, which can serve as precursors in steroid hormone synthesis. To better understanding the molting process and the functions of NEKLs, we screened for mutations that suppress molting defects in *nekl* mutants. Through this screen we identified *catp-1*, which encodes a predicted ATP-dependent Na^+/K^+ pump. Previous studies indicate that CATP-1 functions in the epidermis and may be involved in L2 developmental timing, as its loss of function results in a lengthened L2 stage. Notably, L1 larvae that are starved or exposed to dauer pheromone enter a lengthened L2 stage termed L2d. L2d comprises a predauer stage from which worms can later molt into dauers (L3d) or proceed with normal development (L3), depending on environmental conditions. Our findings indicate that loss of *catp-1* induces an L2d-like state and that its pump activity is critical for CATP-1 function. Consistent with this, mutations that inhibit entry into L2d, such as *daf-5*/SKI loss of function, strongly reduce suppression by *catp-1*. In addition, we find that starvation, exposure to dauer pheromone, growth on heat-killed bacteria, and daf-C RNAi, conditions which induce L2d, can also suppress *nekl* molting defects. Notably, suppression by L2d occurs only with weak or partial loss-of-function (LOF) *nekl* alleles, which typically arrest at the L2/L3 molt, but not stronger LOF alleles, which typically arrest at ~L1/L2.
Notably, previous studies by Bessereau and colleagues demonstrated that loss of *catp-1* suppressed a lethal L2/L3 heterochronic phenotype induced by dimethylphenylpiperazinium, which causes cell divisions to become slowed and uncoupled from the molting timer. More recently, Ilbay and Ambros showed that induction of L2d or diapause suppressed abnormal cell divisions in heterochronic backgrounds that reiterate the L2 stage. We are currently testing the model that that *nekl* mutants may suffer from heterochronic defects that stem from an inability to endocytose precursors required for the synthesis of steroid hormones that control developmental timing.

1481B Apico-basal distribution of the GPCR Smoothened and its impact on Hedgehog transduction Marina Gonçalves Antunes[1], Matthieu Sanial[1], Anne Plessis[1], Isabelle Bécam[1] 1) Institut Jacques Monod CNRS, Université de Paris.

During development, morphogen form gradients that pattern organs by controlling the fate of the receiving cells in a dose dependent fashion. In epithelial cells, which are polarized, apical and basal regions are functionally separated and are in contact with different extracellular environments, which can differ in the nature and dose of signaling molecules. The control of the accumulation and localization of the receptors for these signals, in specific sub-domains along the apico-basal axis is thus critical for correct signal transduction.
This project aims to study the impact of apico-basal localization on signaling, using as a model the Hedgehog (HH) morphogen in the *Drosophila* wing primordium (wing disc). In this epithelial tissue, HH molecules emanating from posterior cells form two gradients: an apical gradient, required for long-range responses, and a basal one, for short- distance responses (High HH). However, how these two gradients are interpreted remains unknown. HH transduction requires the conserved G-protein coupled receptor smoothened (SMO) which doesn't bind HH but is regulated by the HH receptor Patched (PTC). SMO is activated by relocalization from internal vesicles to the plasma membrane which depends on the extensive phosphorylation of its intracellular C-terminal tail by multiple kinases. However, no studies have addressed the impact of SMO apico-basal localization on HH transduction.
We have undertaken a careful analysis of SMO apico-basal distribution in the wing disc epithelium. We have identified a basal sub-population of SMO that is present specifically in cells responding to high levels of HH. Moreover, this basal enrichment depends on SMO activation and hyper-phosphorylation. Our goals are to tackle how this specific localization is regulated by HH and to understand its role in SMO's ability to transduce the high level HH signal. For that purpose we have developed a method to specifically label and follow -by fluorescent microscopy- different populations of SMO in the developing disc: intracellular versus at the surface and apical versus basal. By combining these methods with genetic approaches to modulate SMO activity and the expression of its regulators, we address the three following questions:
1. What are the effects of HH on the apico-basal distribution of SMO?
2. How does SMO phosphorylation regulate these effects?
3. What are the consequences of SMO basal localization on its activity?

1502B Motor Neuron Connections and Innervation of Muscles in *Drosophila melanogaster* *Sharon Tang*[1], Krista Dobi[1,2] 1) Baruch College, CUNY, New York, NY; 2) PhD Program in Biology, The Graduate Center of the City University of New York (CUNY), New York, NY.

Drosophila melanogaster have two sets of muscle systems: larval muscles and adult muscles. The larval somatic muscle system is established during embryogenesis and is necessary for hatching, feeding, and crawling of the larvae. Like humans, *Drosophila* muscles have individual characteristics, like unique sizes, shapes, orientations, attachment sites, and innervation patterns by motor neurons. These properties are encoded by a group of transcriptional regulators that are expressed in specific muscle subsets. Currently, there are over twenty known transcription factors required for the development of the 30 distinct larval somatic muscles in each abdominal hemisegment and specification of those muscle properties. Previous work has shown that the transcriptional regulator Krüppel functions in directing axons to their respective muscle groups; however, the role of additional transcription factors in muscle-specific innervation has not been tested. We are examining how muscle-specific neuron defasciculation and innervation occurs using genetics, immunohistochemistry, and fluorescent microscopy. We are testing how both loss- and gain- of function mutations in lateral transverse muscle-specific transcription factors affect innervation of the lateral transverse muscles, by the segmental nerve. This work will identify muscle-specific factors that regulate the process of neuron innervation. We are using markers like horse radish peroxidase (HRP), connectin, and Fasciclin III to examine the architecture of the segmental nerve in these genetic backgrounds.

1505B Cleavage of Dally-like protein by Matrix Metalloprotease 2 inhibits Wg/Wnt signaling by sequestration of Wnts *Indrayani Waghmare*[1], Xiaoxi Wang[1], Andrea Page-McCaw[1] 1) Vanderbilt University School of Medicine.

Extracellular distribution of secreted Wg/Wnts from source cells to recipient cells is crucial for proper tissue development and patterning. The recipient cells respond to secreted Wg/Wnts in a dose-dependent manner, and thus the concentration of Wg/Wnts available for signaling in the extracellular space must be tightly regulated. Dally-like protein (Dlp) is a cell-surface glypican that promotes long-range extracellular distribution of Wg from the source cells, modulating the concentration of extracellular Wg available for signaling. Genetic studies suggest that the cleavage of Dlp by Matrix Metalloprotease 2 (Mmp2) attenuates long-range distribution of Wg from source cells to recipient cells in the *Drosophila* germarium, and Mmp2 cleaves Dlp within the N' terminal subunit in S2R+ cell-culture experiments. However, how the cleavage of Dlp by Mmp2 attenuates Wg/Wnt signaling is poorly defined. Interestingly, the cleavage of Dlp by Mmp2 results in increased co-immunoprecipication of Wg/Wnt2/Wnt4/Wnt6 ligands with cleaved Dlp compared to full-length Dlp in cell culture. These findings suggest that the cleavage of Dlp by Mmp2 increases the binding affinity of Wnts to Dlp either by creating new binding sites on Dlp or by recruitment of scaffolding proteins to cleaved Dlp, which provide additional docking sites for Wnt binding. We are currently testing the hypothesis that the Wnts sequestered by cleaved Dlp are endocytosed and degraded to attenuate Wnt signaling. Thus, our study sheds light on how metalloproteases and glypicans regulate signaling ranges of extracellular Wnts.

1507A Alary muscles and TARMS, a novel type of striated muscles maintaining internal organs positions Laetitia Bataillé[1], Nathalie Colombié[1], Gaelle Lebreton[1], Aurore Pelletier[1], Jean-Louis Frendo[1], Achim Paululat Paululat[2], *Alain Vincent*[1] 1) CBI, CNRS/University Toulouse 3, France; 2) Zoology and Developmental Biology, University of Osnabrück, Germany.

Alary muscles (AMs) have been described as one component of the cardiac system in various arthropods. A novel type, lineage-related thoracic muscles (TARMs), have recently been discovered in *Drosophila* embryos, which connect the exoskeleton to specific midgut regions (Boukhatmi et al., 2014, doi: 10.1242/dev.111005). Asymmetrical attachments of AMs and TARMs to the exoskeleton on one side, and to internal organs on the other, suggested an architectural function in moving larvae. We analysed AMs and TARMs striated structure, and live-imaged their atypical deformability in crawling larvae. We then selectively eliminated either AMs and TARMs, or TARMs only, by targeted apoptosis. Elimination of AMs revealed that AMs are required for suspending the heart in proper intra-hemocelic position and opening of the heart lumen. They also constrain the curvature of the trachea, the respiratory system, during crawling. TARMs are required for proper positioning of the visceral mass and dorsal gastric caeca, and efficient food transit. While the *org1*/*Tbx1-tup*/*islet1* genetic hierarchy controls AM/TARM development, cardiac versus visceral attachment depends on Hox control, with visceral attachment representing the ground state. To the best of our knowledge, TARMs and AMs are the first example of multinucleate striated muscles connecting the skeleton to the cardiac and visceral systems in bilaterians, with multiple physiological functions. This instates novel anatomical and evolutionary perspectives and questions.

1515C Mib2, A key regulator of cytoskeleton and border cell migration in Drosophila egg chambers *Sunny Trivedi*[1] 1) UMBC, Baltimore, MD.

Collective cell migration is an essential part of many developmental processes and disease pathologies, where a group of cells coordinates its signaling and movements to translocate as a cohort. Border cell migration in the Drosophila egg chamber stands as an excellent model to study collective cell migration. We identified mind bomb2 (mib2) as a regulator of follicle cell cytoskeleton and migration. Other researchers have shown that Mib2 is an E3 ubiquitin ligase involved in Drosophila embryo muscle maintenance via Myosin regulation and a negative regulator of Signal Transducer and Activator of Transcription (STAT) activity in Drosophila cell culture. We found that mib2 loss of function in border cells results in their delayed migration. Expression analysis by antibody supports Mib2 having a role in the border cell cluster. Mib2 domain deletion experiments uncovered the Mib2 domains that are important for cell migration. Furthermore, using clonal analysis we show that Mib2 regulates E-cad, Arm and cortical Actin. We suspect that Mib2 could be a cytoskeletal regulator which is required for border cell migration. We also propose that Mib2 is a JAK (Janus Kinase)/STAT signaling regulator in border cells and hypothesize it may function more broadly in collective cell migration regulation.

1519A Lztr1 is a conserved regulator of Ras/MAPK activity *Giovanna Collu*[1], Jeremy Baidoo[2], Marek Mlodzik[1] 1) Icahn School of Medicine at Mount Sinai, New York, USA; 2) Center for Excellence in Youth Education at Icahn School of Medicine at Mount Sinai, New York, USA.

LZTR1 was recently identified in a screen of human chronic myeloid leukaemia cells aimed at discovering the genetic basis of drug resistance mechanisms. Specifically, LZTR1 was shown to regulate RAS ubiquitination and RAS/MAPK pathway activation in cell culture models. Here we demonstrate a conserved function for the fly orthologue Lztr1/CG3711 in regulating Ras activity *in vivo*. Knockdown of *CG3711* leads to Ras gain-of-function phenotypes in the wing, which can be rescued by loss of one copy of *Ras*. Further, through epistasis experiments we show that CG3711 acts in the Egrf/Ras/MAPK cascade to control wing vein patterning. We are currently investigating the role of *Lztr1* in the developing eye.

1543A A *Drosophila* larval screen of postnatal growth retardation related genes reveals lozenge as a regulator of growth in response to hypoxia *Danting Zeng*[1], Julian Martinez-Agosto[1] 1) University of California, Los Angeles, CA.

The biological process of postnatal growth is of great importance for all organisms, and the essential gene interactions and pathways required for its regulation are highly conserved. Understanding basic growth signaling pathways helps provide insight into diseases related to postnatal growth retardation in humans. One pathway involved in the regulation of growth during low oxygen availability is the HIF-mediated hypoxia pathway. Low oxygen level causes developmental suppression and growth restriction in many animals, including both mammals and *Drosophila melanogaster*. Gene expression in a low oxygen environment is controlled by hypoxia-inducible factor HIF for both mammals and *Drosophila*. We utilize larval growth and its adaptation to hypoxia as a model for human diseases associated with prenatal and postnatal undergrowth. We have previously proposed a role for specific signaling pathways in the larval fat body, including insulin/Warts, as potential mediators of this adaptive response to hypoxia, with missing links yet to be identified. To identify additional novel mediators of the larval response to hypoxia our study pursued a screen of 134 *Drosophila* lines that are homologs of human genes associated with genetic disorders related to postnatal growth retardation. Overexpression or downregulation of these genes in the *Drosophila* fat body using an R4-gal4 driver identified four genes that cause significant larval size enlargement or size reduction under normoxia, phenocopying the larval response to hypoxia. Each of these genes represents a novel regulator of larval growth and potential mediator of the hypoxia pathway. Overexpression of one of those genes, *lozenge*, the homologue of human *RUNX* genes, in the larval fat body under normoxic conditions causes a decrease in larval size, increases larval translucency, and promotes lipid droplet aggregation in the fat body, similar to starvation and hypoxic conditions. Rearing lz^{r15} mutants under hypoxic conditions rescues larval size but is associated with significant lethality. Similarly, Lozenge downregulation specifically in the fat body also rescues the growth restriction under hypoxic conditions but also increases larval survival. Previously established mouse models have suggested an interaction between RUNX and HIF-α homolog in vertebrates, further supporting the conservation of this adaptive mechanism to hypoxia. These findings further expand our understanding of the pathways activated in response to hypoxia during larval growth and will aid in better understanding postnatal growth retardation in both humans and *Drosophila*.

1554C Mechanism of cell survival of executioner caspase activation during recovery from apoptotic stress *Gongping Sun*[1, 2], Xun Ding[2], Yewubdar Argaw[2], Denise Montell[2] 1) School of Basic Medical Sciences, Shandong University, Jinan, Shandong, China; 2) University of California Santa Barbara, Santa Barbara, CA, US.

Apoptosis is an ancient and evolutionarily conserved cell suicide program. During apoptosis, executioner caspase enzyme activation has been considered a point of no return. However, emerging evidence suggests that some cells can survive caspase activation following exposure to apoptosis-inducing stresses, raising questions as to the physiological significance and underlying molecular mechanisms of this unexpected phenomenon. Previously, we developed a sensor, CasExpress, which converts transient executioner caspase activation to permanent GFP expression. Here, using this sensor, we show that following severe tissue injury, wing disc cells that survive executioner caspase activation contribute to tissue regeneration. Through RNAi screening, we have identified several genes that are essential for survival of executioner caspase activation. We also show that cells expressing an activated oncogene experience apoptotic caspase activation, and the capacity to survive such caspase activation is required for oncogenic overgrowth. Thus, survival following executioner caspase activation is a normal tissue repair mechanism usurped to promote tumor growth.

1557C The translational repressor Brat constrains regenerative growth to ensure proper patterning after tissue damage Syeda Nayab Abidi[1], *Rachel Smith-Bolton*[1] 1) University of Illinois Urbana-Champaign.

A key question in regeneration biology is how regenerating tissue undergoes repatterning and ensures replacement of the correct cell types. By using genetic tools to damage and induce regeneration in third-instar wing imaginal discs, we have identified mutants that have aberrant patterning in the regenerated structure. Through this screen we have shown that the translational repressor *brain tumor (brat)* is a regulator of both growth and patterning during regeneration. While *brat/+* wing discs regenerated better than controls, the resulting adult wings had disrupted wing margins. The enhanced regeneration in *brat/+* mutants was due to elevated expression of Wingless and Myc, which promote regenerative growth, as well as elevated expression of Dilp8, which delays pupariation. However, it was unclear why regenerating tissue would constrain expression of these pro-regeneration factors. Interestingly, overexpressing Myc after tissue damage to replicate the enhanced-regeneration phenotype also caused a disrupted margin phenotype. We determined that this aberrant patterning was not caused by enhanced growth itself, but rather by elevated expression of Myc targets such as the transcription factor Chinmo, which negatively regulates the margin cell fate gene *cut*. Thus, Brat constrains expression of the pro-regeneration factor Myc, and this constraint prevents aberrant patterning of the regenerated structure.

1558A The non-receptor tyrosine kinase Btk29A plays a role in early wing imaginal disc regeneration *Matthew Contreras*[1], Benjamin Wang[1], Mabel Seto[1], Amanda Brock[1], Rachel Smith-Bolton[1] 1) University of Illinois at Urbana-Champaign.

After sustaining an injury, some organisms rely on the complex biological process of regeneration to repair damaged tissue. *Drosophila melanogaster* serves as a good model organism to study the tissue regeneration and repair process due to its regenerative capabilities during the larval stage. We study the regeneration of the imaginal wing disc following tissue ablation. The ablation procedure uses the GAL4/UAS/GAL80^{TS} transcriptional regulatory system to conditionally induce apoptosis via *reaper* in the wing pouch. This system can be used to screen for mutations that affect regenerative capacity by measuring adult wing size and patterning after damage and regeneration of the disc.

The actin cytoskeleton is important for wound closure and regeneration; however, little is known about the proteins responsible for its regulation during a regenerative response. In a pilot genetic screen of known actin regulators, we found that animals heterozygous for *Btk29A* mutations exhibited poor regeneration. Btk29A is a non-receptor tyrosine kinase, which plays a part in many events in *Drosophila* development that involve actin reorganization, and can regulate cell-cell adhesion by phosphorylating β-catenin. The regenerating *Btk29A/+* wing primordia are smaller than wild-type regenerating wing primordia at 24 hours after damage. In addition, we have found no significant difference in Jun N-terminal Kinase signaling or cellular proliferation at one day after damage between mutant and wild-type regenerating wing discs. These results suggest that Btk29A is required for an early regeneration event, such as wound closure or the regulation of epithelial junctions and tension in the regenerating disc. We are currently examining wound closure, debris clearance, epithelial integrity, tension, and β-catenin phosphorylation in *Btk29A/+* damaged discs to clarify the role for this kinase in regeneration.

1570A Stem cell mitotic drive ensures asymmetric epigenetic inheritance and distinct cell fates *Rajesh Ranjan*[1], Jonathan Snedeker[1], Xin Chen[1] 1) Johns Hopkins University.

Through the process of symmetric cell division, one mother cell gives rise to two identical daughter cells. Many stem cells utilize asymmetric cell division (ACD) to produce a self-renewed stem cell and a differentiating daughter cell. Since both daughter cells inherit the identical genetic information during ACD, a crucial question concerns how non-genic information could be inherited differentially to establish distinct cell fates. It has been hypothesized that epigenetic differences at sister centromeres could contribute to biased sister chromatid attachment and segregation. We found that a stem cell-specific 'mitotic drive' ensures biased sister chromatid attachment and segregation. First, during stem cell ACD, sister centromeres are asymmetrically enriched with proteins involved in centromere specification and kinetochore function. Second, temporally asymmetric microtubule activities and polarized nuclear envelope breakdown likely allow for the preferential recognition and attachment of microtubules to asymmetric sister kinetochores and sister centromeres. Furthermore, this communication occurs in a spatiotemporally regulated manner. Abolishment of either the asymmetric sister centromeres or the asymmetric microtubule activities results in randomized sister chromatid segregation. Our results demonstrate that the asymmetry at sister centromeres tightly coordinates with the asymmetry from the mitotic machinery to allow for differential attachment and segregation of genetically identical yet epigenetically distinct sister chromatids. Together, these results provide the cellular basis for partitioning epigenetically distinct sister chromatids in asymmetrically dividing stem cells, which opens a new direction to study how this mechanism could be used by other stem cells to achieve distinct cell fates through ACD.

1573A Yorkie regulates nutrient-independent proliferation of mushroom body neuroblasts (MB NBs) in *Drosophila* *Md Ausrafuggaman Nahid*[1], Conor W. Sipe[2], Sarah Siegrist[1] 1) University of Virginia; 2) Shepherd University.

Drosophila neural stem cells (NSC), known as neuroblasts (NB), undergo asymmetric cell division throughout development producing neurons and glia present in adult brains. Dietary nutrients provide essential building blocks necessary for cell division thus acting as a major determinant of stem cell growth and proliferation. We have found that a subset of NBs, known as mushroom body neuroblasts (MB NBs), continue proliferation regardless of extrinsic dietary nutrient availability. Our research aims to understand the molecular mechanism regulating nutrient-independent NB proliferation. We have previously shown that Eyeless (Ey) is both necessary and sufficient to drive NB proliferation in the absence of dietary nutrients (Sipe CW et. al, 2017). In response to dietary nutrient withdrawal, MB NBs stop proliferating when Ey is knocked down and when Ey is overexpressed non-MB NBs proliferate ectopically. Here we report that Yorkie (Yki) also regulates NB proliferation in response to dietary nutrient availability. Yki is the downstream transcriptional coactivator that functions in the evolutionarily conserved Hippo signaling pathway. When Yki is knocked down, MB NBs also stop proliferation in response to dietary nutrient withdrawal and resumed proliferating upon refeeding. Upon expression of a constitutively active form of Yki, non-MB NBs continue cell division independent of dietary nutrient availability. This suggest that Yki like Ey regulates NB proliferation decision in response to nutrient availability. Yki requires a DNA binding partner and Scalloped (Sd) is the most well characterized in this regard. However, it is still not clear if Sd is the binding partner of Yki in NBs. Surprisingly, Sd knock down didn't affect the proliferation of the NBs. This suggests that Sd is not the DNA binding partner of Yki in the NBs. We conclude that Yki is required for the nutrient-independent proliferation of MB NBs and our future work will determine the DNA binding partner of Yki in the NBs.

1590C *Long non-coding RNA regulation of spermatogenesis via endo/lysosome activity and cytoskeletal elements in Drosophila* *Mark Bouska*[1], Hua Bai[1] 1) Iowa State University.

LncRNAs have high testis expression and their interactions with testis specific spectrin protein complexes are expected to provide novel insights into disorders such as male idiopathic sterility as well as provide unique targets for contraceptives. We used CRISPR to knock out the lncRNA CR45362 and revealed an impact on endo/lysosomal activity, but also a male sterile phenotype in which the spermatid nuclei are dispersed throughout the cyst. To determine the molecular partners of CR45362 we performed chromatin isolation by RNA precipitation which demonstrated that the lncRNA binds to spectrin and spectrin-related proteins that are conserved in human spermatogenesis. RNAi confirmed α-spectrin knockdown flies manifest a spermatid nuclear dispersion phenotype similar to that of CR45362 knockout flies, while immunostaining revealed α-spectrin was dispersed throughout CR45362 knockout cyst cells in the same cell type and region of the testis where CR45362 is located in wild type flies. Furthermore, we demonstrate that CR45362 can localize to endosomes which is particularly relevant to fertility as endocytic cycling has been shown to affect junctional proteins that normally hold sperm cells in place during their maturation process. We propose that the lncRNA CR45362 regulates spectrin cytoskeletal complexes to mediate endosomal recycling of junctional proteins that maintain cyst cell-spermatid junctions during spermatogenesis. Our results provide evidence that lncRNAs are viable targets for contraceptive development and are expected to play a genetic role in cases of human infertility.

1595B The behavior of the fourth chromosome in Drosophila melanogaster Spermatogenesis Camila Avelino[1], Carolina Mendonça[1], Mara Pinheiro[1], *Maria Vibranovski*[1] 1) University of Sao Paulo.

Meiotic Sex Chromosome Inactivation (MSCI) is a process of transcriptional silencing of the sex chromosomes in the early stages of meiosis in spermatogenesis. Scientists have investigated the existence and impact of MSCI on *D. melanogaster* and some aspects related to spermatogenesis raised a great debate about the existence of this mechanism. Recently, the IV chromosome of *D. melanogaster* (dot chromosome) was described as an ancient X chromosome. This finding suggests that possibly the IV chromosome has characteristic sequences used in regulations related to the sex chromosome, including regulations during meiosis. The relationship between the IV chromosome and MSCI in *Drosophila* male germline is not known. In order to verify the behavior and the activity of IV chromosome during meiosis I, cytogenetic experiments (double immunofluorescence-FISH) were performed in meiotic cells of the adult testis. We found that evidence of depletion of active RNA polymerase II (Pol-IISer2p) on IV chromosomes in the late stages of meiotic prophase I, compared to the autosomes. Surprisingly, the antibody for total RNA polymerase II (both active and non-active) was not observed to be depleted on the IV chromosome. These results suggest that occur a binding of RNA polymerase II in IV chromosome but generally fails to initiate or elongate the mRNA in late spermatocytes.

1597A *fs(1)K741* and *fs(1)A1304* are female sterile mutations in the genes *Sxl* and *sov* Jillian G. Gomez[1], Myles Hammond[1], Stephen Kucera[1], Brian Oliver[2], *Leif Benner*[2,3] 1) Department of Biology, University of Tampa, Tampa, FL; 2) Section of Developmental Genomics, Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; 3) .Department of Biology, Johns Hopkins University, Baltimore, MD.

X-linked female sterile screens in *Drosophila* have led to a tremendous increase in our understanding of the genetic control of oogenesis. However, many of the loci in these screens have not been mapped to a single gene and are therefore a rich resource in further elucidating the genetic control of this process. Two such alleles, *fs(1)K741* and *fs(1)A1304*, have been shown to be germline dependent and result in female specific sterility. We were interested in determining the mutation that leads to female sterility in *fs(1)K741* and *fs(1)A1304* females. Meiotic mapping placed both alleles within the *crossveinless* and *singed* region of the X chromosome. We began complementation and rescue tests with known deficiencies and duplications tiling the *crossveinless* and *singed* region. *fs(1)A1304* mapped to a roughly 180 kb region while *fs(1)K741* mapped specifically to the genomic location of the gene *Sxl*. Whole genome DNA sequencing was performed and we found a 324 nucleotide deletion in the promoter for the gene *sov* in *fs(1)A1304* females. We complementation tested *fs(1)A1304* with known alleles of *sov* and found that *fs(1)A1304* failed to complement female sterility of the amorphic alleles sov^{EA42} and sov^{ML150}, while it did complement the hypomorphic allele sov^{2}. This indicates that the deletion in *fs(1)A1304* disrupts the activity of *sov* and thus is an allele of *sov*. Since *sov* is an essential gene and has two annotated promoters, it would be interesting to determine if the deletion abolishes *sov* expression specifically in the germline and not other tissues or if transcriptional levels of *sov* are reduced leading to female sterility. For *fs(1)K741*, we found a single point mutation in the male-specific exon of *Sxl*. We checked splicing of *Sxl* in *fs(1)K741* ovaries and found the presence of both male- and female-specific splicing products. This suggests that the point mutation in *fs(1)K741* disrupts *Sxl* in the female germline and leads to sterility. In the future, we are interested in determining if this point mutation specifically disrupts male-specific splicing silencing or if transcriptional regulation is altered leading to insufficient levels of *Sxl* in the female germline.

1602C Calcium independent phospholipase A2 -VIA affects female but not male fertility in *Drosophila melanogaster*, with altered mitochondrial distribution in the developing female germ cells Surya Banerjee[1], Matthew Lubin[1], Liam Eliach[1], Adina Schonbrun[1], Sogol Eizadshenass[1], *Josefa Steinhauer*[1] 1) Yeshiva University, New York, NY, USA.

iPLA2-VIA, the *Drosophila melanogaster* homolog of human *PLA2G6/PARK14*, encodes a calcium independent phospholipase A2 that hydrolyzes glycerophospholipids into free fatty acids and lysophospholipids, thereby regulating membrane phospholipid composition and signaling pathways that can affect neuronal function, fertility, inflammation, metabolism, and apoptosis. Human *PLA2G6* mutations are associated with neurodegenerative and locomotor disorders, including inherited dystonia-parkinsonism, in which neuroaxonal dystrophy is correlated with mitochondrial degeneration. However, the underlying mechanisms behind these conditions are not clear. We generated a null mutation in *iPLA2-VIA* and found that homozygous mutant flies show age-dependent locomotor defects and shortened lifespan, hallmarks of neurodegeneration, as reported in previous studies. Additionally, we observed high expression of *iPLA2-VIA* transcripts in both the wild-type male and female germlines, and reduced fertility was observed in mutant females. Mutant females laid significantly fewer eggs than controls, and no maternal lethality was seen, suggesting that the reduced fertility results from defects during oogenesis. On account of iPLA2-VIA's biochemical function in releasing free fatty acids, which are precursors of prostaglandin lipid signals that regulate the actin cytoskeleton, we carefully examined the actin cytoskeleton in mutant germ cells but did not see any defects. Furthermore, although many reports have implicated iPLA2-VIA in apoptosis, we detected little difference in apoptosis markers in mutant ovaries relative to the controls. Still, wild-type tagged iPLA2-VIA colocalized with a mitochondrial marker in both male and female germlines, and an abnormal distribution of mitochondria in the female germline was observed in the mutant flies. We developed a quantitative method to score this distinct phenotype in mid-oogenesis stage egg chambers. Surprisingly, male fertility as well as many aspects of spermatogenesis were normal in *iPLA2-VIA* mutants, despite the requirement of the mammalian homolog for male fertility in mice. In summary, reduced female fertility in *iPLA2-VIA* mutant flies is correlated with mitochondrial defects in the developing female germ cells.

1614C Yorkie facilitates cell survival during larval eye development in *Drosophila melanogaster* *Brooke Allen*[1], Tiffany Cook[2], Jacob Kagey[1] 1) University of Detroit Mercy, Detroit, Michigan; 2) Wayne State University, Detroit, Michigan.

We are using the *Drosophila* eye as a model system because it is a well characterized developmental model that grows from a single layer of epithelial tissue. YAP1, the human homolog of Yki, is a transcription factor that has been found to be highly expressed and localized in the nucleus of several human cancers, suggesting that having a better understanding of the time and spatial role of Yki in eye development may help to better understand the molecular consequences of YAP1 up-regulation in human cancer. During *Drosophila* development, the eye utilizes the morphogenetic furrow to facilitate a progressive pattern of differentiation. To understand how key signaling pathways result in not only furrow progression but also cell survival we are primarily focusing on the time dependent knockdown of the transcription regulatory protein Yorkie (Yki) by utilizing both RNAi and the Flp/FRT systems to disrupt Yorkie expression. Previous studies have shown Yki to be involved with cell survival, cell growth and cell proliferation, though many of those studies have been in genetic systems in which Yki is over active. Here we show that Yki is essential for survival and a knockdown of *Yki* results in increased cell death in larval discs, disrupted ommatidia in pupal discs, and drastically smaller adult eye phenotypes. This increase in cell apoptosis coincides with a decrease in DIAP1 (a known Yki transcriptional target). To investigate additional potential roles

of Yki in eye development we used the FLP/FRT system to create mosaic eye discs where clones have both a loss of Yki expression and a block in the canonical apoptotic pathway. Cell death was inhibited through a loss of function *Ark* allele. With the addition of the *Ark* allele, the *Yki/ark* mosaic eyes show a rescue of clone size in both larval and adult eyes. Contrary to the understood role of Yki involved with cell growth and cell cycle regulation we see no disruption in patterns of mitosis, differentiation, or other developmental signaling pathways. Our studies suggest that while Yorkie may facilitate overgrowth and over-proliferation in certain mutant genetic contexts, that its role in early eye development is primarily cell survival.

1616B Sufficiency of active Rac to drive whole tissue phagocytosis in vivo *Abhinava Mishra*[1], Denise Montell[1] 1) University of California Santa Barbara.

The Rho family of small GTPases, Rac, Rho, and Cdc42 represent central nodes in the cytoskeletal and signaling networks that drive cell migration and engulfment. How the cells utilize the same RhoGTPase networks to promote different processes in different contexts is not thoroughly understood. We address this question in the *Drosophila* ovary, which contains ~850 somatic follicle cells and 16 much larger germline cells. Previously we reported that local and transient activation of Rac using a photoactivatable Rac (PA-Rac) induced protrusions and motility in border cells. However, persistent expression of a constitutively active form of Rac (Rac-CA) in just a small subset of follicle cells, using a cell type specific enhancer (*slbo-Gal4*), results in destruction of the entire egg chamber, a phenotype that is significantly suppressed by mutation of a single engulfment receptor, Draper. Therefore, expression of Rac-CA in a few cells destroys the whole tissue likely by stimulating engulfment.
To understand the mechanisms underlying Rac-CA mediated tissue destruction, we performed fixed tissue imaging with cell death markers. We observed that expression of Rac-CA caused caspase activation in follicle cells non-autonomously and increased acidification of germline nurse cells evident by lysotracker staining. To further assess the effects of Rac-CA mediated engulfment, we generated FlpoutGal4 driven clones to express Rac-CA in a subset of follicle cells. These clones displayed a characteristic cell-in-cell phenotype resulting from clonal cell engulfing the non-clonal cell. Interestingly, the cell-in-cell phenotype was also observed when constitutively active form of Rho was expressed in border follicle cells. These results indicate that focal and transient activation of Rac promotes protrusion and motility, whereas high and sustained Rac activation promotes phagocytosis, initially of living cells, ultimately resulting in destruction of the whole tissue. This mechanism may underlie some of the abundant cell-in-cell structures found in human tumor samples as well as emerging forms of immune-therapy such as CAR-P.

1625B A tissue communication network coordinating innate immune response during muscle stress *Samantha Goetting*[1], Nicole Green[2], Justin Walker[1], Alexandria Bontrager[1], Molly Zych[3], Mikelle Read[1], Erika Geisbrecht[1] 1) Kansas State University; 2) University of Iowa Medical School; 3) University of Washington.

Tissue communication is required for maintaining organismal homeostasis during development. The coordination of metabolism, immune activation, and circadian rhythms typify the complex tissue networks necessary for organismal health. Our lab is using the *Drosophila* muscle attachment site (MAS) as a model to understand the connection between innate immune activation and muscle maintenance. Previously, a pupal lethal screen revealed an unknown role for the extracellular matrix (ECM) protein Fondue (Fon) in muscle development. Loss of *fon* caused a reduction in larval locomotion due to the detachment of body wall muscles, and a sensitized background screen revealed that *fon* is secreted from the fat body and incorporated into MASs for stabilization. Loss of *fon* also activates innate immune processes such as melanin accumulation along the MAS, pathogen-independent translocation of Dorsal (Dl) in the fat body, constitutive expression of the antimicrobial peptide (AMP) *drosomycin*, and recruitment of hemocytes to damaged muscle. Furthermore, the Toll pathway, an evolutionarily conserved innate immune pathway, is activated upon loss of *fon*. This project seeks to elucidate the mechanisms by which *fon* mutants activate this immune process.

We previously confirmed that Toll is activated in *fon* mutants via translocation of Dl into the nucleus in a pathogen-independent manner. Here we seek to understand the role of hemocytes in this Toll activation. We ablated hemocytes by expression of the cell death gene *reaper (rpr)* in recombinant *fon* mutants and assayed for the nuclear localization of Dl as a proxy for Toll activation. Our preliminary results show that this mechanical stress-induced Toll activation may be hemocyte-independent. Upon analyzing Dl activation in the fat body, *fon* mutants with hemocyte ablation showed significant levels of Toll activation compared to controls. The Toll pathway is still activated upon loss of hemocytes and *fon*, suggesting that hemocytes are not necessary for Toll activation upon muscle detachment. Current efforts to further confirm this result will be presented.

1628B *Drosophila* blood cell numbers are determined by a novel subpopulation of blood cells that express PDGF/VEGF receptor ligands. *Daniel Bakopoulos*[1], James Whisstock[2], Coral Warr[3], Travis Johnson[1] 1) School of Biological Sciences, Monash University, Clayton, Victoria, Australia.; 2) Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia.; 3) School of Medicine, University of Tasmania, Hobart, Tasmania, Australia..

In *Drosophila*, macrophage-like phagocytes known as plasmatocytes play critical roles in immunity and tissue remodelling. Several studies have suggested that plasmatocyte numbers are influenced by the sole *Drosophila* homologue of the mammalian Platelet-Derived Growth Factor (PDGF) and Vascular Endothelial Growth Factor (VEGF) receptors, Pvr. However, it remains unknown how Pvr regulates this process and which of its ligands, Pvf1-3, activate Pvr in this role. Here, we analysed *Pvf* mutants and found both *Pvf2* and *Pvf3*, but not *Pvf1*, to be necessary for normal larval plasmatocyte numbers. Using an endogenous *Pvf2-GAL4* line, we also reveal that *Pvf2* is expressed in a subpopulation of blood cells that are present in the embryo and larva. RNAi experiments indicate that these blood cells are a major source of Pvf2 in this role, and ectopic expression of *Pvf2* in all blood cells induces their gross overproliferation. Together, these data suggest that *Pvf2* expression in a novel population of blood cells is necessary for determining correct overall larval plasmatocytes numbers. These findings define a new mechanism for controlling blood cell numbers and may provide critical insights into the regulation of macrophage self-renewal in other organisms such as mammals.

1646B Escort-like somatic cells mediate early mouse fetal ovarian development but surface-derived LGR5+ cells support primordial follicles *Wanbao Niu*[1], Allan Spradling[1] 1) Department of Embryology, Carnegie Institution, Baltimore, MD.

Ovarian murine somatic cells are essential to form first wave medullar follicles and second wave primordial follicles. Using single cell RNA sequencing we characterized the transcriptomes of both somatic and germline ovarian cells during fetal and early neonatal development. WNT4-expressing somatic cells we term "escort-like cells(ELCs)" interact with incoming germ cells and early developing cysts of both sexes. In the medullar region, ELCs differentiate into the granulosa cells of fast-growing first wave follicles. In contrast, after E12.5, LGR5+ pre-granulosa cells ingress from the ovarian surface epithelium and replace cortical escort-like cells. These surface-derived cells become the main population of granulosa cells supporting primordial follicles, and differ in transcription from ELC derivatives. Reflecting their different cellular origins, ablation of LGR5+ cells at E16.5 using *Lgr5*$^{tm2(DTR/EGFP)Fjs}$ (Lgr5-DTR-EGFP) eliminates second wave follicles, but first wave follicles continue to develop normally and support fertility.

Our findings provide striking evidence that somatic cell behavior supporting germline cyst development in mice and Drosophila has been evolutionarily conserved.

1661B A comparative developmental genetic study of branchiopods: measuring hedgehog (*hh*) gene expression across embryonic development of *Daphnia* and *Artemia* *Todd Fairbanks*[1], Laura Fricke[1], Brittany Stokes[1], Olivia Fitch[1,2], Matt Beckman[1] 1) Augsburg University, Minneapolis, Minnesota; 2) Michigan State University, East Lansing, Michigan.

Daphnia magna (branchiopoda), a freshwater microcrustacean, possesses a unique morphological feature: a cyclopic eye. Within class Branchiopoda, we observe one-eyed and two-eyed species with varying degrees of eye fusion. Importantly, we find a correlation between the degree of eye fusion and branch distance from a basal member (*Artemia franciscana*) in the branchiopod phylogenetic tree. The molecular genetic program underlying the development of cyclopia has not been elucidated, but models of cyclopia have been created using teratogens and genetic manipulations. These models, along with the analysis of the hedgehog (*hh*) gene sequence in humans with holoprosencephaly have implicated the Hedgehog signaling pathway in cyclopia and other midline defects. The Hedgehog signaling pathway is highly conserved across animal taxa playing an important function in directing midline and other features of embryonic development for both invertebrates and vertebrates. We sought to determine whether the *D. magna* and *A. franciscana* hedgehog gene sequences and expression profiles play a role in cyclopic development through a comparative genetic study of one-eyed and two-eyed organisms. Our sequence analysis shows that *Daphnia* spp. *hh* genes share unique amino acid substitutions that correlate to known mutations in human families exhibiting midline defects, while the *A. franciscana* sequence and all two eye organism sequences do not. We performed RT-qPCR on four stages of eye development in *D. magna* and *A. franciscana* and found hh expression decreases over developmental time in *Daphnia* but increases during *Artemia* development. In order to further determine the spatial and temporal expression of hh we have created a series of plasmids and made digoxigenin-labeled probes for in situ hybridization in *Daphnia* and *Artemia*. Preliminary data from *Daphnia* demonstrate strong expression in the midline head region in early stages of embryonic development. Continued in situ hybridization experiments will establish the expression profile for stages that match those studied by RT-qPCR in both *Daphnia* and *Artemia*.

1671C *C. briggsae* TRA-2 interacts with TRA-1 to prevent spermatogenesis *Yongquan Shen*[1], Ronald E Ellis[1] 1) Rowan University SOM.

We are studying *C. briggsae* TRA-1, to learn how this Gli transcription factor regulates sexual identity in worms. To begin, we generated *cbr-tra-1(v455),* which has an OLLAS tag inserted near the N-terminus, and *cbr-tra-1(v424),* which has one near the C-terminus. Both alleles develop normally, so neither tag affects function. On western blots we see that *Cbr*-TRA-1 is cleaved to form a product that is slightly larger than its *C. elegans* counterpart. This product is predicted to be a repressor that blocks the transcription of male genes. Finally, sequence alignments reveal a small conserved domain near the putative cleavage site. To analyze TRA-1 function, we used gene editing to make the following mutations: (1) *cbr-tra-1(v197)* is a frameshift *upstream* of the cleavage site. When its mRNA is stabilized, the truncated product makes a functional TRA-1 repressor, since *XX* animals develop normal hermaphrodite bodies. However, they make *only* oocytes and no sperm. (2) *cbr-tra-1(v405)* and *cbr-tra-1(v406)* are frameshifts located *downstream* of the cleavage site. They develop normal hermaphrodite bodies, so TRA-1 repressor is functioning. However, these mutations also cause animals to make oocytes instead of sperm, which implies that full-length TRA-1 normally promotes spermatogenesis. (3) *cbr-tra-1(v197v383)* alters 30 residues in the domain that binds TRA-2, and disrupts interactions between *C. briggsae* TRA-2 and TRA-1 in yeast two-hybrid assays. These *XX* mutants make significantly more sperm than the wild type. Furthermore, this mutation restores spermatogenesis to many *she-1(v35) XX* animals. We conclude that TRA-2 normally binds TRA-1 to block spermatogenesis. Analyses of *tra-2(null); fem-3(null); she-1(null)* animals support this model. This result is surprising, since *C. elegans tra-2* mutations that block the interaction cause oogenesis. (4) We are now making mutants that alter the putative cleavage domain without affecting upstream or downstream regions, to confirm that full-length *Cbr*-TRA-1 activates some genes, as does full-length *Ci* in flies. Finally, we and others in our lab found that mutations in several chromatin regulators alter the sperm/oocyte decision. Thus, we propose that full-length TRA-1 promotes spermatogenesis by working with chromatin regulatory factors, whereas the cleaved form of TRA-1 represses spermatogenesis. In *C. briggsae,*TRA-2 blocks the function of full-length TRA-1.

1675A Zic5 is required for rod photoreceptor layer differentiation through stabilizing Gli3 during Xenopus eye development *Jian Sun*[1], Jaeho Yoon[1], Moonsup Lee[1], Yoo-Seok Hwang[1], Ira Daar[1] 1) National Cancer Institute, NIH, Frederick, MD.

Members of the Zic family of zinc finger transcription factors play critical roles in a variety of developmental processes. They are involved in the development of neural tissues and the neural crest, left-right axis patterning, somite development, and formation of the cerebellum. Among all five Zic genes, zic5 is strongly expressed in the developing eye in Xenopus embryos. However, the role of Zic genes in eye development is still poorly understood. By loss of zic5 function, we found zic5 was required for rod photoreceptor layer differentiation. RT-qPCR of embryonic eyes revealed that knockdown of zic5 decreased Hedgehog (Hh) pathway target genes Gli1 and Patch1. Hybridization chain reaction (HCR) indicates the expression pattern of zic5 is overlapped with Gli2/3 but bot Gli1 in early eye development. Morpholino knockdown or CRISPR knockout of Gli3 didn't affect Rhodopsin (rod photoreceptor marker) expression on-site of the photoreceptor layer but induced ectopic Rhodopsin expression in the inner nuclear layer(INL). Interestingly, we found knockdown of Gli3 could rescue Rhodopsin expression impaired by zic5 knockdown. In HEK293 cells, we also found zic5 could bind and stabilize Gli3 through reducing its ubiquitination. Knockdown of zic5 in the eyes or in 293 cells could significantly increase Gli3 repressor level, indicating inhibition of Hh pathway. Our findings show the role of zic5 in the eyes development in Xenopus and reveal a mechanism by which Gli3 is stabilized by zic5, another zinc finger transcription factor.

1685B Expression Pattern in the Developing Zebrafish of the Two Paralogs of *Activating Transcription Factor 5* (*atf5a* and *atf5b*) *Roberto Rodriguez Morales*[1], Aranza Torrado Tapias[1], Viveca Velez[1], Maria Colon[1], Gaurav Varshney[2], Martine Behra[1] 1) University of Puerto Rico, Medical Sciences Campus, San Juan, PR; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK.

The Activating Transcription Factor 5 (ATF5) belongs to the basic leucine-zipper (bZIP) family of transcription factors (TFs) which binds DNA as homo or heterodimers with other family members. Numerous *in vitro* and *in vivo* cell system studies established the anti-apoptotic and proliferative role of ATF5 which is in line with strongly upregulated expression in glioma and other cancer cells and a postulated oncogenic role. A role in cellular-stress response was also demonstrated which appears to depend on hetero-dimerization with ATF4. Importantly, ATF5 action seems cell line/tissue specific suggesting the importance of the multiple binding partners which have been described for this promiscuous TF. Conflicting functional data showed the importance of ATF5 in promoting proliferation of neuroprogenitors while seemingly triggering differentiation of bone and liver

progenitor cells. Surprisingly, little is known in the physiological context of a whole animal. The available expression data is mostly from rodents showing expression in adult liver and heart. During development, expression was detected in neuroprogenitors in several brain regions, as well as in the olfactory pits where it was limited to differentiated olfactory sensory neurons (OSNs). To resolve discrepancies in the role of ATF5, additional expression data is needed. ATF5 is well conserved with homologs even found in invertebrates. In Zebrafish, two paralogs were described: *atf5a* and *atf5b.* Here, we present the developmental expression pattern of both starting in 6-hour post fertilization (hpf) embryo and up to 5day post-fertilization (dpf) larvae. We performed whole-mount *in-situ* hybridization (WISH) and semi-quantitative retro-transcription PCR (RT-PCR) and found an early ubiquitous expression of both genes that was gradually restricted in 24hour post-fertilization (hpf) embryos to the olfactory placodes for *atf5a,* or to the sensory patches of the lateral line (LL) and inner ear for *atf5b*. In addition, we found both genes to be expressed in discreet brain regions, namely in the midbrain-hindbrain (MHB) boundary, and in older animals in the tectum and hindbrain. Our work lays the ground for future functional analyses addressing the respective role of each paralog in expressing tissues/organs during development and regeneration.

1706B The role of collagen 11a2 in zebrafish vertebral development *Denise Rebello*[1], Brian Ciruna[1] 1) The Hospital for Sick Children.

Abnormal curvature in the spine, known as scoliosis, presents in several different forms. One form is congenital scoliosis, which is caused by vertebral malformations. Vertebral defects in humans can arise from somite patterning errors during embryonic development. Somites are patterned by oscillations in Wnt, Notch, and FGF signalling pathways, and mutations in these pathways result in congenital scoliosis in animal models. However, few human patients bear mutations in these pathways, which suggests that mutations in other genes may be causative to congenital scoliosis. To find other genetic causes of this disease, whole-exome sequencing on congenital scoliosis patients was performed by a collaborator, revealing 2 patients with mutations in *COL11A2*, which encodes a cartilaginous collagen. This gene has been previously implicated in several diseases characterized by abnormal bone and connective tissue development. This project aims to examine the role of *COL11A2* in development with a focus on the spine, using *Danio rerio*, the zebrafish. I have created a zebrafish loss-of-function mutant for *col11a2*, and characterized a mild vertebral fusion phenotype in these animals. I have also created a loss-of-function allele of this gene which avoids a genetic compensation response by deleting the *col11a2* open reading frame. These mutant fish exhibit a much more severe vertebral fusion phenotype. In this project, I have visualized these fusion defects across development using *in vivo* staining techniques to show when the fusions first arise and how they progress over time. I have ongoing work to rescue these mutants using a wildtype *col11a2* transgene. I am also working on expressing the specific patient mutations from our collaborator endogenously and exogenously in the fish. This project utilizes the zebrafish to explore the developmental consequences of mutations in *col11a2*, and also shows the relevance of robustness mechanisms, such as genetic compensation, in fully investigating the function of a specific gene.

1709B Short-range Sonic hedgehog mediates branching morphogenesis for fin appendage patterning *Amy Robbins*[1], Joshua Braunstein[1], Scott Stewart[1], Kryn Stankunas[1] 1) Institute of Molecular Biology, University of Oregon, Eugene, OR.

Zebrafish fins robustly develop and regenerate with elaborately branched bony ray skeletons. We recently showed Sonic hedgehog (Shh) signaling specifically promotes ray branching during adult caudal fin regeneration by basal epidermal-initiated signaling that directs adjacent progenitor osteoblasts (pObs) into split pools. We investigated if and how Shh signaling similarly functions during developmental ray branching. *shha* is uniquely expressed by small basal epidermis domains overlying pOb pools at the distal aspect of outgrowing fins. Kaede photoconversion of *TgBAC(ptch2:-Kaede)* fish revealed Hedgehog/Smoothened (Hh/Smo) signaling is restricted to *shha*+ basal epidermis and neighboring pObs. Basal epidermal cells continuously migrate distally, upregulating *shha* only when passing over pObs. Lateral splitting of each *shha*+-epidermal domain followed by Hh-responsive pObs precedes ray branching. Hh/Smo-inhibition using the small molecule BMS-833923 (BMS) prevents branching in all fins with minimal effects on fin outgrowth or skeletal differentiation. Phased Hh/Smo inhibition indicates Hh/Smo signaling acts throughout the branching process, suggesting a sustained mechanism. As with regenerating fins, *shha*+ basal epidermal cells and pObs form extensive surface contacts in distal developing rays. We used live time-lapse imaging to find Hh/Smo signaling restrains basal epidermal cell distal migration by apparent tethering to pObs. We propose instructive basal epidermal movements and Shh/Smo-promoted heterotypic cell adhesion directs zone of polarizing activity (ZPA)-independent branching morphogenesis to pattern fin skeletons during both development and regeneration.

1711A Artery/Vein plasticity after vessel injury *Leah Greenspan*[1], Daniel Castranova[1], Brant Weinstein[1] 1) National Institutes of Health.

Blood vessels are vital for the supply of oxygen and nutrients to all organs and tissues. To ensure proper circulation, balanced arterial and venous blood flow must be specified during development. However, vascular injury due to disease or physical damage can disrupt this balanced flow and lead to severe consequences such as ischemia or hemorrhage. Blood vessel repair is key to reestablishing continuous blood flow and full perfusion after injury, but little is still known about how vascular endothelial cells react to injury and flow conditions *in vivo*. Using transgenic zebrafish with fluorescently labeled vasculature and/or blood cells we are visualizing trunk intersegmental vessels (ISVs) and assessing their responses to vasvcular injury and/or blockage. We find that most vessels reattach after injury or blockage, although they can form aberrant connections to neighboring vessels in order to maintain flow. Newly reconnected vessels can change vessel identity converting from an artery before ablation to a vein after repair or vice a versa. Interestingly, unmanipulated ISVs neighboring blocked or severed ISVs were also observed to switch flow directions, suggesting that the arterial-venous identity of ISVs is plastic and that these vessels adapt to changing hemodynamics to ensure continuous blood flow. Future work using mutant and experimentally manipulated animals will allow us to probe the molecular mechanisms regulating arterial-venous vascular plasticity and potentially help identify potential targets for vascular disease and regenerative therapies.

1718B Distinct dynamics of the extracellular matrix during heart regeneration in different *Astyanax mexicanus* populations *Zhilian Hu*[1], Madeleine Lemieux[2], Mathilda Mommersteeg[1] 1) The University of Oxford, Oxford, OX1 3PT, United Kingdom; 2) Bioinfo, Plantagenet, Ontario, K0B 1L0,Canada .

Heart failure is a costly and incurable disease affecting about 40 million people worldwide and continues to be the leading cause of global death. Heart failure often develops after a heart attack when insufficient blood flow to the heart leads to oxygen deficiency in the cardiac muscle and irreversible cell loss. The impaired heart tissue is replaced by a collagen-rich permanent scar. In zebrafish, however, the scar formed after injury can be dissolved and the injured part of heart can be regenerated. In a novel fish model *Astyanax mexicanus,*scar deposition is completely different even at the early phase after injury between surface fish and cavefish populations. The consequence in response to heart injury is also different: surface fish can regenerate the lost heart muscle (similar to zebrafish), but cavefish cannot (like the situation in human patients). This is particularly interesting because it allows us to compare whether the molecular mechanisms underlying difference in regeneration capacity are distinct between these pop-

ulations. Through histochemistry, RNAscope, quantitativePCR and cardiac transcriptome analysis, we have found that some ECM candidates (such as collagen) and regulators (including *mmp9*and *mmp13*) are differentially regulated between surface fish and cavefish Pachón. The hypothesis is whether we could reverse a permanent scar to a regenerative scar by regulating ECM deposition and degradation. To address this issue, we have performed Quantitative Trait Loci (QTL) analysis. This strategy allows us to identify genomic regions that regulate fibrosis versus regeneration. Our findings could contribute to plug the gap of current knowledge of a degradable versus permanent scar in response to injury and to facilitate further investigation in mammals. It could also contribute to the improvement of therapeutics strategies targeting the ECM in human patients.

1735A An unexpected role for a conserved ADAM-family matrix metalloprotease, ADM-2, in C. elegans molting control Sarina Bernazzani[1], Braveen Joseph[1], *David Fay*[1] 1) University of Wyoming, Laramie, WY.

Mutations in the C. elegans NIMA-related kinase family members, nekl-2 and nekl-3, lead to defects in the ability of larvae to shed their old cuticles during molting cycles. In a screen for suppressors of nekl-2; nekl-3 molting defects, we identified two independent mutations affecting ADM-2, the ortholog of human ADAM9/12/19 and the sole meltrin-class matrix metalloprotease in C. elegans. Notably, it is loss of function in adm-2, including CRISPR-generated null alleles, that facilitate separation of the old and new cuticles in nekl-2; nekl-3 mutants. Although loss of function in several proteases has been reported to cause molting defects, no proteases have been previously reported to suppress a molting defect. This suggests that ADM-2 may promote molting through a mechanism other than cuticle degradation.
By carrying out a structure function analysis of adm-2 using CRISPR mutagenesis, we identified several critical domains required for ADM-2 function. We find that disruption of the extracellular Zn-binding protease active site is sufficient to yield high levels of suppression, demonstrating the importance of this catalytic activity. Moreover, we find a critical role for the cytoplasmic C-terminus of ADM-2, including one of the three SH3-binding domains. This domain in humans has previously been shown to interact with sorting nexin-9 (SNX9), providing a potential link between ADM-2 and the NEKLs, which function in clathrin mediated endocytosis.
Most surprisingly, we find a major function for a putative nuclear-localization signal in the C-terminus of ADM-2, as well as a predicted furin cleavage site located just inside the transmembrane domain. These latter findings suggest that the cytoplasmic domain of ADM-2 may be cleaved and enter the nucleus. Consistent with this possibility, a ADM-2::GFP reporter is observed on plasma membranes and in the cytoplasm and nuclei of ectodermal cell types, including hyp7, which is the cellular focus of NEKL-2 and NEKL-3 activity. Notably, several recent studies have suggested functions for the C-terminal domains of ADAM-family proteases, however, little is known about their potential roles in signaling.

1743C Tension-sensitive recruitment of the RhoGEF RHGF-1 promotes actomyosin contractility in the *C. elegans* spermatheca Shiri Kela Avivi[1], Kriti Sethi[2], *Ronen Zaidel-Bar*[1] 1) School of Medicine, Tel Aviv University, Israel; 2) Mechanobiology Institute, National University of Singapore, Singapore.

The *C. elegans* spermatheca, a flexible pouch like structure and a site for sperm storage, is an elegant model to study contractility at the tissue level. Each ovulation event involves the entry of a mature oocyte into the spermatheca, where it resides while it is fertilized and is then propelled forward into the uterus for further development. Oocyte entry dramatically stretches the myoepithelial cells that make up the spermatheca, and pushing out of the oocyte is the result of a wave of contractile forces generated by a circumferential actomyosin network within the spermatheca. Spermathecal contractility is regulated by calcium signaling and by the small GTPase protein, RHO-1.
Previously, we identified a RhoGAP protein, SPV-1, which functions to inhibit premature RHO-1 activity and thus spermathecal contractility (Curr Biol. 2015 Jan 19;25(2):141-151.). However, the identity of the GEF/s that activate RHO-1 in the spermatheca was still unknown. Here, we performed an RNAi screen for all the known *C. elegans* RhoGEFs in an *spv-1* loss-of-function mutant, to identify RhoGEFs that upon being depleted could correct for the overcontractile defect of the *spv-1* mutant. We identified RHGF-1 as a RhoGEF that contributes to the contractility of the spermatheca. Loss of function of RHGF-1 results in an increase in oocyte retention time, suggesting a reduction in contractile activity of the spermatheca. Using a RHO-1 biosensor, we found that upon depletion of RHGF-1, active RHO-1 levels were reduced in the spermatheca as compared to wild-type worms. Using CRISPR-Cas9, we tagged RHGF-1 with tagRFP and live cell imaging showed recruitment of RHGF-1::tagRFP to the basal side of spermathecal cells upon oocyte entry. High resolution imaging revealed punctate structures of RHGF-1 which assembled onto circumferential F-actin bundles of the spermatheca. RHGF-1 affects the organization of the actin network, as depletion of RHGF-1 increased tortuosity of the actin network, and it is recruited to F-actin in a tension-dependent manner. In conclusion, RHGF-1 appears to function as a mechanosensor, detecting the stretch of the spermatheca upon oocyte entry and responding by relocating to F-actin and activating RHO-1 to induce spermatheca contraction.

1752C Control of clathrin-mediated endocytosis by NIMA family kinases *Braveen Joseph*[1], Yu Wang[2,3], Phil Edeen[1], Vladimir Lažetić[1], Barth Grant[2], David Fay[1] 1) University of Wyoming, Laramie, WY; 2) Rutgers University, Piscataway, NJ ; 3) Huazhong University of Science and Technology, Wuhan, Hubei, China.

NEKL kinases are highly conserved members of the NIMA-kinase family, which have been predominantly implicated in cell division and ciliogenesis. We previously reported, that NEKL-2/NEK8/9 and NEKL-3/NEK6/7 are required within the *C. elegans* epidermis, and that their knockdown causes molting defects that results in larval lethality. In addition, we discovered a conserved set of ankyrin repeat proteins, MLT-2/ANKS6, MLT-3/ANKS3, and MLT-4/INVS, that partner with the NEKLs to regulate their intracellular localization.

To better understand the mechanistic functions of NEKL-MLTs we obtained genetic suppressors of molting defects in *nekl-2*; *nekl-3* double mutants. Strikingly, our screen identified several mutations that directly affect the activity of AP2, a conserved heterotetrametric adaptor complex required for clathrin mediated endocytosis. Knockdown of individual AP2 subunits, or an allosteric activator of AP2, FCHO-1, strongly suppress molting defects in *nekl* mutants. Conversely, mutations that increase the levels of active AP2 strongly enhance *nekl* molting defects. In addition, inhibition of *nekls* suppress morphological defects associated with reduced AP2 function, attesting to the tight functional connection between the NEKLs and AP2.

To determine the functions of NEKLs in endocytosis, while avoiding potential confounding effects caused by *nekl*-mutant larval arrest, we used auxin inducible degradation methods to deplete NEKLs specifically at the adult stage. Loss of NEKLs led to greatly elevated levels of clathrin at the epidermal apical membrane and to a profound reduction in apical clathrin mobility. Adult NEKL depletion also adversely affected uptake of LRP-1, an epidermal cargo critical for molting. These results are consistent with depletion of NEKLs leading to a defect in the ability of vesicles to release clathrin after scission and suggest that NEKLs promote clathrin uncoating. Notably, both defects were largely alleviated by a reduction in AP2 or FCHO-1

activities. These findings suggest that reduced AP2 activity may facilitate clathrin uncoating and indicate that reduced AP2 activity suppresses *nekl* molting defects through the restoration of normal trafficking.

Lastly, we show that human NEK6 and NEK7, orthologs of NEKL-3, can rescue both molting and trafficking defects in NEKL-3 depleted strains, suggesting that the control of intracellular trafficking is an evolutionarily conserved function of NEK family kinases.

1756A Using single molecule resolution to understand how phase-separated condensates organize the siRNA pathway *Celja Uebel*[1], Fabien Pinaud[1], Carolyn Phillips[1] 1) University of Southern California, Los Angeles, CA.

Phase separation has emerged as a crucial cellular strategy for many biomolecular functions. Phase separation occurs when proteins, typically containing low complexity domains or intrinsically disordered regions, coalesce to create a dense and highly dynamic condensate that is sensitive to changes within the cellular environment. These condensates maintain distinct structures within the surrounding bulk phase, similar to the immiscibility of oil droplets in water. This strategy of condensation is seemingly ubiquitous in the biological world, occurring in cells ranging from bacterial to human. Consequently, phase-separated condensates underlie many cellular processes, and investigating the interactions between distinct biological phases is imperative to understanding cellular organization.

Here we utilize prominent phase-separated condensates in the *C. elegans* small interfering RNA (siRNA) pathway to probe the interactions between condensates and the potential for pathway organization and coordination. *Mutator* foci, phase-separated siRNA amplification centers, are exclusively found adjacent to P granules, phase-separated germline-specific mRNA surveillance centers. We aim to understand how the compartmentalization of crucial pathway components into condensates elicits a robust and heritable siRNA silencing signal.

We utilize ectopic protein expression, aliphatic alcohol, and heat stress to probe the interactions and characteristics of P granules and *Mutator* foci, and have discovered that these granules remain separate, yet adjacent, in ectopic environments, respond differentially to perturbation of hydrophobic interactions, and, after disruption, can re-establish adjacency in a dynamic manner. This may indicate the separate and adjacent nature of these condensates is essential for proper siRNA routing and silencing.

To understand the single molecule composition of a condensate and to model the interface between P granules and *Mutator* foci at single molecule resolution, we are using 3D Stochastic Optical Reconstruction Microscopy (3D-STORM). Currently we have revealed the true nanomolecular scale of *Mutator* foci and captured protein density and distribution within the condensate. Understanding interactions between these condensates not only allows us to build a more accurate model of mRNA surveillance, but also provides basic biological insight to how phase-separated condensates work together to coordinate cellular processes.

1760B Ring canal formation in the Drosophila male germline occurs via a midbody-like intermediate *Kari Price*[1], Lynn Cooley[1] 1) Yale School of Medicine.

Cytokinesis, the final step in cell division that results in the physical separation of nascent daughter cells, normally proceeds to completion at the end of mitosis. However, during male and female gametogenesis in various invertebrate and vertebrate species, cytokinesis is incomplete resulting in the formation of cells connected by a shared cytoplasmic intercellular bridge. The best understood example of incomplete cytokinesis is found in the Drosophila germline with the formation of intercellular bridges called ring canals (RCs). RCs are thought to form when cleavage furrows arrest and transform into stable bridges, circumventing the normal process of cellular abscission, to connect the cytoplasm of cells in syncytial groups. RCs are membrane-attached cytoskeletal structures that stabilize the cytoplasmic opening between cells and are comprised of several contractile ring (CR) components suggesting that RCs are derived from the CR; however, the mechanism that transforms a CR into a RC is not known.
Time lapse imaging of GFP-tagged RC components during male germline RC formation has revealed novel insights into the mechanism of RC biogenesis. In contrast to proposed models of contractile ring arrest, we find that RCs are formed from an unexpected midbody-like intermediate. Live imaging of RCs labeled with Pavarotti-GFP (Pav/MKLP1/Kif23), a subunit of the centralspindlin complex and major component of RCs, shows that Pav-labeled cleavage furrows constrict to a dense midbody-like focus that resolves into an open ring over the course of one hour. We find that known midbody ring and RC components localize in a ring around the Pav-labeled focus, similar to the localizations of midbody ring proteins during conventional cytokinesis. Despite the appearance of a midbody and midbody ring, we find that RC formation occurs in the absence of microtubule severing or depolymerization, events associated with canonical midbody formation and function. Furthermore, while ESCRT-III components are not recruited to the midbody-like intermediate as would normally occur during cytokinesis, we observe enrichment of the upstream ESCRT-associated protein Alix-GFP at the intercellular bridge but not mature RC. Taken together, these data suggest a model wherein additional components or protein modifications at the “midbody” inhibit membrane abscission and facilitate the formation of RCs. We are employing a combination of proteomics and electron microscopy approaches to identify potential targets for future investigation.

1765A Is the deubiquitinase *Usp5* required for cell cycle exit? *Jennifer Bandura*[1], Collin Wesley[1], Blake Neal[1] 1) Lock Haven University.

The coordination of cell proliferation and differentiation is crucial for proper development. Through a genetic screen, we previously identified *Usp5* as a gene that is potentially required for cell cycle exit after terminal differentiation. Our results indicated that cells lacking *Usp5* experience ectopic E2F activity, based on the expression of an E2F-responsive reporter gene in *Drosophila* eye cells. In addition, cells undergo ectopic cell divisions in the absence of *Usp5*. *Usp5* encodes a deubiquitinase (DUB) in the ubiquitin-specific protease (USP) subfamily. This is particularly interesting, as proteolysis and reversible ubiquitination are known to play important roles in cell cycle regulation. Our efforts to further characterize the cell cycle defect in cells lacking *Usp5* will be discussed.

1790B Septins are required for collective cell migration in the *Drosophila* ovary *Allison Gabbert*[1], Jim Mondo[1], Joseph Campanale[1], Denise Montell[1] 1) UC Santa Barbara.

Collective cell migration is essential for embryo development, wound healing, and cancer metastasis. Border cells in the *Drosophila* ovary have served as a pioneering model for collective cell migration that is amendable to genetic analysis and live imaging. Yet the molecular mechanisms driving border cell migration remain incompletely understood. Septins are filament-forming proteins sometimes referred to as the fourth cytoskeletal element and have diverse functions that include curving membranes and bending and bundling actin. Microarray analysis shows that septin mR-

NAs are enriched in border cells and/or centripetal cells. I found that multiple septins are required for border cell migration. Knockdown of Septin 1, 2 or 5 reduced the expression of Pnut, which is the *Drosophila* ortholog of human Septin 7. More detailed localization and functional studies of Septins in border cell migration will be presented.

1800C Uncovering functional roles in development for differentially expressed ribosomal protein eRpL22-like using a conditional gene knockout strategy *Caroline Pritchard*[1], Brett Gershman[1], Vassie Ware[1] 1) Lehigh University, Bethlehem, PA.

The *Drosophila melanogaster* eRpL22 ribosomal protein family contains two structurally divergent and developmentally essential paralogues: eRpL22 and eRpL22-like - the latter exhibits tissue-specific expression across development; the former is ubiquitously expressed. Multi-tissue co-localization comparison of eRpL22-like and core ribosomal components indicates eRpL22-like may have functional roles both within the ribosome itself and apart from ribosomal processes.

To elucidate roles for eRpL22-like, we characterized morphological defects in an eRpL22-like heat-shock conditional knockout homozygote (CKO). Preliminary ovary-specific phenotypes include: sudden mid-oogenesis arrest, ectopic rounded polar-like follicular epithelium cells, and elongated-double-anteriorized poles in surviving eggs. Eye-specific defects include: subtle bristle mis-orientation, shortness-stubbiness, and missing bristles, as well as ectopic cone cells and reduced expression of epidermal growth factor receptor (EGFR).

Sequencing of RNAs enriched on eRpL22 and eRpL22-like polysomes in adult testes revealed differential enrichment of mRNAs (also expressed within the ovary and eye) involved in cell polarity, suggesting that paralogue-specific “specialized ribosomes” translate specific mRNAs. Therefore, we expect eRpL22-like CKO mutants to have reduced expression of the following genes with homologues associated with human disease: (I) Dally-like protein - omodysplasia-1, (II) Belle - x-linked mental retardation, and (III) Par-1 - visual impairment and progressive phthisis bulbi. Interestingly, eRpL22 ribosomes show differential enrichment of kekkon-1 (Kek-1), a negative regulator of EGFR. We have previously shown that eRpL22-like knock-down results in a compensatory increase in eRpL22 expression, suggesting that Kek-1 may be overexpressed in eRpL22-like CKO mutants. Many human diseases result from EGFR signaling disruption. Disease-states presently associated with eRpL22 include cancer susceptibility and T-cell abnormalities – no conditions are as yet associated with eRpL22-like.

Immunohistochemical characterization of our eRpL22-like CKO mutant allows us to tease-apart individual ovarian phenotypes, elucidate putative extra-ribosomal functions of eRpL22 paralogues, and potentially reveal a novel model useful for studying specific human conditions arising from a spectrum of epithelial polarity defects.

These preliminary data broaden the context for investigation of the role of eRpL22-like as an essential player across multiple developmental processes.

1802B Sequence Requirement for Protein Trafficking to B-bodies, a Novel Type of Nuclear Domains *Miranda Adams*[1], Anton Bryantsev[1] 1) Kennesaw State University.

The eukaryotic nucleus is a busy place with a high concentration of proteins performing diverse functions. Despite the crowded environment and absence of internal membranes, nuclear organization remains structured via distinct areas that selectively recruit and release proteins – nuclear domains. How nuclear domains are formed and maintained is currently not well understood.

B-bodies are a newly discovered type of nuclear domains that can be found in the indirect flight muscles of Drosophila; their principle resident is the RNA-binding protein Bruno. This protein has 3 RNA-recognizing motifs (RRM1,2,3) as well as two intrinsically disordered regions. B-bodies are highly dynamic domains which size and appearance changes during myogenesis. We used ectopic expression of GFP-tagged Bruno mutants to identify critical regions in the primary sequence that are required for B-body targeting.

Our results indicate that a functional RRM2 domain is required for Bruno trafficking to B-bodies. In contrast, the functionality of RRM3 domain is dispensable in this regard. We speculate that RRM2 domain may be necessary to interact with yet unknown structural RNA molecule(s) in order to retain Bruno in B-bodies.

1819A Addressing the physiological role of endosomal Microautophagy *Satya Surabhi*[1], James Glenn[1], Ana Mesquita[1], Andreas Jenny[1] 1) Albert Einstein College of Medicine,Bronx, New York.

Autophagy is a catabolic process which degrades damaged organelles and aggregate prone proteins and thus is essential for development and cellular homeostasis. It is induced in response to different stressors including starvation, oxidative stress and accumulation of misfolded proteins. As such, it counteracts various human diseases, and its reduction leads to aging like phenotypes. Of the three major forms of autophagy, Macroautophagy can degrade organelles or aggregated proteins, and Chaperone-mediated autophagy is specific for proteins containing KFERQ-related targeting motifs. Endosomal Microautophagy (eMI), a form of autophagy during which substrates are taken up into multivesicular bodies for degradation in a KFERQ-specific manner or in bulk . Among the three form of autophagy the physiological role of eMI is poorly understood.
Using a KFERQ-tagged fluorescent biosensor, we are characterizing the physiological role of eMI with a focus on what types of cellular stress activate eMI. Our data suggest that oxidative stress and DNA damage, but not ER stress can elicit an eMI response in an ESCRT machinery dependent manner, implying a stress-selectivity of the process. Further, we are trying to understand the mechanism of stress induced eMI by identifying novel regulators of the eMI pathway. We identified a candidate regulator, that, when upregulated, results in premature induction of starvation induced eMI. Since targeting autophagic pathways has been proposed as treatment strategy particularly in non-dividing neurons, we are also testing eMI candidate regulators as a possible modifiers of fly models of human neurodegenerative diseases. We anticipate that the physiological role of eMI in stress regulation and neurodegeneration are conserved in humans.

1826B Microtubules regulate intracellular trafficking to mediate apical constriction during tissue invagination *Thao Le*[1], SeYeon Chung[1] 1) Louisiana State University.

The Drosophila embryonic salivary gland (SG) invaginates by budding to form a three-dimensional tube. Coordinated apical constriction during SG

invagination is critical for proper tube shape. We previously showed that Folded gastrulation (Fog)-dependent Rho-associated kinase (Rok) accumulation in the apicomedial region of the SG cells is required for apicomedial myosin formation and clustered apical constriction near the invagination pit. Here, we show that microtubule (MT)-dependent intracellular trafficking has a role in regulating apical constriction during SG invagination. Key components involved in protein trafficking, including dynein heavy chain, Rab11 and Nuclear fallout (Nuf), are apically enriched near the invagination pit in a MT-dependent manner during SG invagination. This enrichment is crucial for apical constriction as disruption of the MT networks or intracellular trafficking impairs formation of apicomedial myosin, which leads to apical constriction defects. We show that apical transport of several proteins along MTs, either in a Rab11-dependent or independent manner, mediates clustered apical constriction during SG invagination. Key proteins that are transported include the Fog ligand, the apical determinant protein Crb, the key adherens junction protein E-Cad and the scaffolding protein Bazooka/Par3, and knockdown of these genes in the SG results in apical constriction defects. These results define a role of MT-dependent intracellular trafficking in regulating the actomyosin networks ands cell junctions to coordinate cell behaviors during tubular organ formation.

1833C The effects of fertilization and sex peptide on the survival of mated female *Drosophila melanogaster* exposed to high fat diet *Dustin Rousselle*[1], Matthew Talbert[1] 1) University of Louisiana at Monroe, Monroe, LA.

We use *Drosophila melanogaster* to study the adverse effects that obesogenic diets have on physiological systems and many prior experiments explore the effects to high fat diets (HFDs). We see a significant decrease in lifespan when mated females are exposed to HFD, which is manifested in mass death within the first couple of days of exposure. These adverse effects are not seen in virgin female flies or on other obesogenic diets, including diets that are disproportionately high in sucrose relative to yeast. The HFD used by us utilizes coconut oil as a source of saturated fat. The cause of mass death isn't known, but we believe that it may be rooted in the behavioral changes due to mating, such as greater contact with the media to lay fertilized eggs. It may also be due to physiological changes resulting from the transfer of seminal proteins to female flies. An initial test involved male flies with reduced Sex Peptide (*SP*) production due to RNAi produced by the GAL4/UAS system[U1] . Males of BDSC strain *1947* have the insertion *P{GAL4-prd.F}RG1,* which causes expression of GAL4 in the accessory glands. These males were crossed with females of BDSC strain *25998* that have the insertion *P{TRiP.JF02022}attP2* that produces dsRNA for RNAi of *SP*. Males with RNAi against *SP,* along with isogenic controls, were then mated with virgin w^{1118} females. The resulting mated females were then put on a HFD (20% w/v coconut oil). Reduction in *SP* in the males involved in the mating showed very little impact on the abruptly shortened lifespan in mated females on a HFD. Further experiments that we are conducting will examine the effect of mating between females and sterile males using the tud^{1} allele of the tudor (*tud*) gene. Sons of homozygous tud^{1} females don't form a germline and thus are sterile, but still make all accessory proteins, and exhibit normal mating patterns. In addition, examining egg-laying on a high fat diet after mating will allow us to gain additional insight on how the observed mass death occurs.

1835B The impacts of obesity-promoting diets on feeding quantity over time in mated female w^{1118} *Drosophila melanogaster* *Elizabeth Long*[1], Dustin Rousselle[1], Matthew Talbert[1] 1) University of Louisiana at Monroe, Monroe, LA.

High-sugar diets (HSDs) and high-fat diets (HFDs) cause pathogenic outcomes such as insulin resistance, obesity, shortened life span, cardiomyopathy, and hyperglycemia in *Drosophila melanogaster*. The HFD we utilize contains a 20% w/v coconut oil content compared to a normal 0% w/v coconut oil content, while the HSD contains 20% w/v sucrose as opposed to a normal 3% w/v sucrose. Previously, flies were found to reduce their feeding quantity on HSD relative to NM (normal medium) after 7 days of exposure. This behavior raised questions as to what extent these diets induce aversion or changes in feeding behavior, particularly in mated w^{1118} females. We found that when placed on a HSD for 7 days and then exposed to normal and HSD for 4, 8, 16, 24, and 48 hours, flies generally had increased feeding quantity on NM compared to HSD. However, flies with previous maturation on NM exhibited a semi-cyclic pattern of feeding, where there was a significant downregulation in response to HSD at 4 hours that does not exist at 8 hours, only to reemerge at 24 hours, and disappear again at 36 hours. Flies with previous maturation on HSD exhibited no such pattern, showing general reduction in feeding on HSD, but there were no statistically significant differences at each time point. When flies were placed on a HFD for 7 days and then exposed to HFD and NM for 4, 8, 16, 24, and 48 hours, they again showed an overall decrease in feeding on the HFD compared to normal diet. When flies previously exposed to NM were exposed to NM or HFD, they exhibited a cycle pattern of feeding with two distinct feeding peaks over a 48 hour period. In contrast, when flies previously exposed to HFD were exposed to NM or HFD, they exhibit only one distinct feeding peak over 48 hours. HFD pre-exposed flies did not experience the same downregulation in response to HFD feeding at around 36 hours compared to NM pre-exposed flies. However, feeding did decrease at around 48 hours in HFD pre-exposed flies. These observations pose questions regarding appetitive homeostasis, circadian rhythm, and potential disruption with HSD and HFD exposure.

1841B Early-life hypoxia alters adult physiology and reduces stress resistance and lifespan in *Drosophila* *Danielle Polan*[1,2,3,4], Mohammed Alansari[1,2,3,4], Byoungchun Lee[1,2,3,4], Savraj Grewal[1,2,3,4] 1) Cumming School of Medicine University of Calgary, Calgary AB; 2) Clark H. Smith Brain Tumour Centre, Calgary AB; 3) Arnie Charbonneau Cancer Institute, Calgary AB; 4) Alberta Children's Hospital Research Institute, Calgary AB.

In many animals, short-term fluctuations in environmental conditions in early life often exert long-term effects on adult physiology. In Drosophila, one relevant environmental variable is oxygen. In their natural ecology, larvae grow by burrowing into rotting, fermenting food that is rich in microorganisms. This environment is likely low in oxygen and, as a result, Drosophila have evolved mechanisms to tolerate hypoxia. While the acute effects of hypoxia in larvae have been well studied, whether early-life hypoxia affects adults is less clear. We have begun exploring this issue. Here we show that Drosophila exposed to hypoxia during their larval period subsequently show reduced starvation stress resistance and shorter lifespan as adults, with these effects being stronger in males. We find that these effects are associated with reduced expression of brain-derived Drosophila insulin-like peptides and a decrease in whole-body insulin signaling. In contrast, we see elevated TOR kinase activity, a manipulation known to reduce lifespan. We also identify a sexually dimorphic effect of larval hypoxia on adult nutrient storage and mobilization. We find that males, but not females, adults exposed to hypoxia as larvae had elevated levels of lipids and glycogen. Moreover, we see that both males and females exposed to hypoxia as larvae show defective lipid mobilization upon starvation stress as adults, which may explain why they are starvation sensitive. Together, these data show how early-life hypoxia can exert persistent long-term effects on Drosophila adult physiology and lifespan.

1860C Regulation and functional differentiation of two actins in *Chlamydomonas* *Masayuki Onishi*[1,2], Friedrich Fauser[3], Josep Vilarrasa-Blasi[3], Robert Jinkerson[3], Michal Breker[4], Martin Jonikas[3], Frederick Cross[4] 1) Duke University, Department of Biology, Durham, NC; 2) Stanford University School of Medicine, Department of Genetics, Stanford, CA; 3) Carnegie Institution for Science, Department of Plant Biology, Stanford, CA; 4) The Rockefeller University, New York, NY.

Actin has a deep root in eukaryotic evolution and plays important roles in a wide range of biological processes, including the determination of cell

shape and polarization, vesicle transport and endocytosis, cell motility, and cytokinesis. Many model and non-model organisms possess multiple and often divergent actins, but their regulation and functional differentiation are not understood in detail. For example, the green alga *Chlamydomonas* has both a conventional actin (IDA5) and a highly divergent actin (NAP1); only IDA5 is expressed in normal proliferating cells. We showed previously that the latrunculin toxins (LatA and LatB) cause loss of filamentous (F-) IDA5 and strong upregulation of NAP1, which then provides essential actin function(s) by forming LatA/B-resistant F-NAP1. Thus, *Chlamydomonas* provides a unique opportunity to study the regulation and functional differences of multiple actin isoforms. In addition, it provides an avenue to identify factors required for normal function of conventional actin, loss of which would normally be lethal but can be rescued by NAP1 in this organism.
By combining RNA-seq analyses, high-throughput quantitative genetic screens, and live-cell imaging of actin dynamics, we have uncovered the general principle of the regulation of two actins and the components involved (the LAT genes), as well as several other genes involved in actin functions:
(1) Perturbation in F-IDA5 structure and function by LatB triggers the "LAT pathway (LAT1, LAT2, LAT3)," which induces expression of NAP1 and other genes in the SCF/ubiquitin-proteasome system.
(2) The SCF (LAT5, LAT6) targets the non-polymerizable LatB-IDA5 complexes for ubiquitination and degradation. Inhibition of this process by mutations cause reduced cellular fitness, presumably by interference with NAP1 polymerization.
(3) Homologs of some of the *LAT* genes in *Arabidopsis* showed phenotypes indicative of actin defects, suggesting a basal evolutionary history of this mechanism.
(4) LatB treatment also induces cofilin and profilin. Cofilin may further the clearance of IDA5 by severing F-IDA5, whereas profilin appears to function in protecting monomeric IDA5 from degradation.
(5) A genetic screen for synthetic lethality with *nap1* identified a mutant of cyclase-associated protein (CAP1; SRV2 in budding yeast), which, together with cofilin and profilin, is involved in actin recycling in other organisms. In the mutant, F-actin forms multiple small rings in the cytoplasm, and the cell fails to divide properly.
(6) Complete removal of F-actin by combining *nap1* and LatB caused only a partial inhibition of cleavage-furrow ingression, suggesting that an actin-independent mechanism drives furrowing in this *Chlamydomonas,* which has no type-II myosin.

1863C Integration of macromolecular complex data into the *Saccharomyces* Genome Database Edith Wong[1], Marek Skrzypek[1], Shuai Weng[1], Gail Binkley[1], Birgit Meldal[2], Livia Perfetto[2], Sandra Orchard[2], *Micheal Alexander*[1], Stacia Engel[1], J Michael Cherry[1] 1) Stanford University, Palo Alto, CA, USA; 2) Wellcome Genome Campus, Hinxton, Cambridge, UK.

Proteins seldom function individually. Instead, they interact with other proteins or nucleic acids to form stable macromolecular complexes that play key roles in important cellular processes and pathways. One of the goals of *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) is to provide a complete picture of budding yeast biological processes. To this end, we have collaborated with the Molecular Interactions team that provides the Complex Portal database at EMBL-EBI to manually curate the complete yeast complexome. These data, from a total of 589 complexes, were previously available only in SGD's YeastMine data warehouse (yeastmine.yeastgenome.org) and the Complex Portal (www.ebi.ac.uk/complex-portal). We have now incorporated these macromolecular complex data into the SGD core database and designed complex-specific reports to make these data easily available to researchers. These web pages contain referenced summaries focused on the composition and function of individual complexes. In addition, detailed information about how subunits interact within the complex, their stoichiometry and the physical structure are displayed when such information is available. Finally, we generate network diagrams displaying subunits and Gene Ontology annotations that are shared between complexes. Information on macromolecular complexes will continue to be updated in collaboration with the Complex Portal team and curated as more data become available.

1869C Developmentally regulated GTP-binding protein 1 modulates ciliogenesis via an interaction with Dishevelled *moonsup lee*[1], yoo-seok hwang[1], jaeho yoon[1], jian sun[1], adam harned[2], kunio nagashima[2], ira daar[1] 1) National Cancer Institute, Frederick, MD; 2) Electron Microscope Laboratory, Frederick National Laboratory for Cancer Research,Frederick, MD.

Cilia are critical for proper embryonic development and maintaining homeostasis. Although extensively studied, there are still significant gaps regarding the proteins involved in regulating ciliogenesis. Using the *Xenopus laevis* embryo, we show that Dishevelled (Dvl), a key Wnt signaling scaffold that is critical to proper ciliogenesis, interacts with Drg1 (developmentally regulated GTP-binding protein 1). The loss of Drg1 or disruption of the interaction with Dvl reduces the length and number of cilia and displays defects in basal body migration and docking to the apical surface of multiciliated cells (MCCs). Moreover, Drg1 morphants display abnormal rotational polarity of basal bodies and a decrease in apical actin and RhoA activity that can be attributed to disruption of the protein complex between Dvl and Daam1, as well as between Daam1 and RhoA. These results support the concept that the Drg1–Dvl interaction regulates apical actin polymerization and stability in MCCs. Thus, Drg1 is a newly identified partner of Dvl in regulating ciliogenesis.

1875C Facultative sex regulated by a prion-like switch associated with Sli15p in budding yeast *Raymond Futia*[1], Daniel Jarosz[2,3] 1) Department of Biology, Stanford University, Stanford, CA; 2) Department of Chemical and Systems Biology, Stanford University, Stanford, CA; 3) Department of Developmental Biology, Stanford University, Stanford, CA.

Prions are proteins that can adopt multiple conformations, at least one of which is self-templating. Ensuing changes in protein activity are heritable over long biological timescales, in a manner that does not follow Mendelian laws of inheritance. Aggregation of PrP, the first prion protein discovered, is associated with devastating neurodegenerative disease. Yet other prions can promote adaptive traits in organisms ranging from yeast to mice. A screen of all *Saccharomyces cerevisiae* open reading-frames identified proteins for which transient overexpression induces prion-like states associated with fitness benefits in various conditions. The induction of these yeast prions influences cellular decisions to optimize growth, often in a manner sensitive to the environment through shifts in proteostasis.
In budding yeast, mating is an important, well-characterized decision. Mating is driven by a central MAPK signaling pathway, and its outcome determines ploidy and outcrossing. Protein-based systems rooted in mechanisms of self-templating aggregation, in the case of Whi3p, and cytosolic titration, in the case of Far1p, have been shown to inform this decision with memories of pheromone exposure. However, memories associated with Whi3p aggregation are not transmitted trans-generationally, and the Far1p titration system does not benefit from the meso-scale stability afforded by self-templating. A prion-based system could inform mating with a stable, yet adaptive, memory, sensitive to various environmental stimuli. This motivated us to explore the degree to which yeast prions regulate mating. Overexpression of *SLI15*, a component of the yeast chromosome passenger complex (CPC), results in a prion-like state characterized by a distinct re-localization of Sli15p, starvation tolerance, an insensitivity to

mating pheromone, and a consequent inability to mate. We therefore refer to this state as *Ste*$^{+}$. Acquisition of *Ste*$^{+}$ modulates histone patterning and demonstrates global transcriptomic repression, downregulating the mating pathway and metabolic components. The CPC is a master regulator of mitosis, most notably promoting mitotic chromosome condensation, monitoring chromosome segregation, and regulating the spindle assembly checkpoint – activities that are not obviously relevant to mating behavior. This novel sterile behavior associated with a CPC component represents a molecular relationship between the decisions to reproduce sexually and asexually, delineated by a prion-like memory.

1879A Functions for Cdc42p BEM Adaptors in Regulating a Differentiation-Type MAP Kinase Pathway Sukanya Basu[1], Beatriz Gonzalez[1], Boyang Li[1], Garrett Kimble[1], Keith Kozminski[2], *Paul Cullen*[1] 1) University at Buffalo; 2) University of Virginia.

Rho GTPases regulate cell polarity and signal transduction pathways to control morphogenetic responses in different settings. In yeast, the Rho GTPase Cdc42p regulates cell polarity, and through the p21-activated kinase Ste20p, Cdc42p also regulates mitogen-activated protein kinase (MAPK) pathways (mating, filamentous growth or fMAPK, and HOG). Although much is known about how Cdc42p regulates cell polarity and the mating pathway, how Cdc42p regulates the fMAPK pathway is not clear. To address this question, Cdc42p-dependent MAPK pathways were compared in the filamentous (∑1278b) strain background. Each MAPK pathway showed a unique activation profile, with the fMAPK pathway exhibiting slow activation kinetics compared to the mating and HOG pathways. A previously characterized version of Cdc42p, Cdc42p^{E100A}, that is specifically defective for fMAPK pathway signaling, was defective for interaction with Bem4p, the pathway-specific adaptor for the fMAPK pathway. Corresponding residues in Bem4p were identified that were required for interaction with Cdc42p and fMAPK pathway signaling. The polarity adaptor Bem1p also regulated the fMAPK pathway. Versions of Bem1p defective for recruitment of Ste20p to the plasma membrane, intramolecular interactions, and interaction with the GEF, Cdc24p, were defective for fMAPK pathway signaling. Bem1p also regulated effector pathways in different ways. In some pathways, multiple domains of the protein were required for its function, whereas in other pathways, a single domain or function was needed. Genetic suppression tests showed that Bem4p and Bem1p regulate the fMAPK pathway in an ordered sequence. Collectively, the study demonstrates unique and sequential functions for Rho GTPase adaptors in regulating MAPK pathways.

1881C A MAPK inhibitor that is targeted for destruction by the anaphase promoting complex links spore differentiation to the completion of meiosis *Abhimannyu Rimal*[1], Zeal P. Kamdar[1], Chong Wai Tio[1], Edward Winter[1] 1) Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA 19107 .

Smk1 is an atypically activated MAPK in yeast that is controlled by the transcriptional program of meiosis. It is phosphorylated on its activation-loop threonine during MI by the CDK activating kinase, Cak1, and it autophosphorylates its activation-loop tyrosine upon completion of MII in response to the MAPK-binding protein, Ssp2. The activation of Smk1 by Ssp2 is stimulated by Ama1, a meiosis-specific activator of the Anaphase Promoting Complex (APC) E3 ubiquitin ligase. In this study, we identified Isc10 as an inhibitor that links activation of Smk1 to the APC^{Ama1}. *SMK1*, *ISC10*, *AMA1*, and *SSP2* are transcriptionally induced as cells enter MI. At this time, Isc10 and Smk1 form an inhibited complex. Ssp2, whose mRNA is translationally repressed until MII, forms a ternary complex with Isc10 and Smk1 that is poised for activation. Upon completion of MII, Ama1 promotes the ubiquitylation of Isc10, thereby allowing Ssp2 to activate Smk1. Mutations that cause Ssp2 to be translated precociously or *isc10Δ* modestly reduced tetrad formation while the double mutant almost completely eliminated tetrad formation. These findings suggest a mechanism for coupling differentiation (spore formation) to the G1/G0 phase of the cell-cycle.

1906A Human optic atrophy associated *OPA1* gene induces mitochondrial dysfunction in *Saccharomyces cerevisiae* *Annabel Vivian Almazan*[1], Shuzhen Chen[1], Shulin Ju[1], Quan Zhong[1] 1) Wright State University, Dayton, OH.

Mitochondria are the primary source of energy production and a key cellular compartment that controls cell death and survival. An intricate balance between mitochondrial fusion and fission, which is the dynamic joining and dividing of this tubular-structured organelle, is essential to mitochondrial function. Mutations in genes regulating mitochondrial dynamics have been found to be the causal factors for several inherited diseases. Among the genes that regulate this pathway of mitochondrial dynamics, *OPA1* has been genetically linked to a dominantly inherited optic neuropathy, optic atrophy type 1. Both *OPA1* and its yeast homolog, *MGM1,* encode a dynamin-like GTPase that is required for the fusion of the mitochondrial inner membrane and the integrity of the cristae structure. Both proteins are cleaved by mitochondrial proteases into long and short isoforms, with the long form anchored in the mitochondrial inner membrane and the short form residing in the intermembrane space. A balanced ratio between the long and short forms of OPA1/Mgm1 appears to be critical for the maintenance of mitochondrial structure and function. Interestingly, human OPA1 can be proteolytically processed by yeast mitochondrial proteases into primarily short isoforms. Using yeast as a model system, we found that high-level expression of human OPA1 leads to distorted mitochondrial morphology, collapse of membrane potential, and defective growth, resembling the phenotypes induced by the deletion of *MGM1*. We found evidence supporting that mitochondrial dysfunction induced by OPA1 expression is linked to specific proteolytically processed short forms of OPA1. Surprisingly, the toxic forms of OPA1 appear to be present in the mitochondrial matrix. Furthermore, deletion of *MGM1*, but not its overexpression, alleviates the cellular toxicity induced by OPA1. Such a synthetic rescue effect does not occur by depleting the short form of Mgm1. Overall, our data suggest that human OPA1 is processed into biochemically active proteins in yeast. The toxicity of OPA1, at least in part, is mediated through its interaction with Mgm1. We, therefore, propose to use such a yeast model to characterize the effect of distinct short forms of OPA1 on mitochondrial structure and function. Cross-species genetic approaches may provide novel insights into OPA1 protein function and regulation.

1913B Regulation of the Cardiac Potassium Channel Kir2.1 by α-Arrestins *Natalie Hager*[1], Ceara McAtee[1], Marcel Bruchez[2], Adam Kwiatkowski[3], Jeffrey Brodsky [1], Allyson O'Donnell[1] 1) Dept. of Biological Sciences, University of Pittsburgh ; 2) Dept. of Biological Sciences, Carnegie Mellon University ; 3) Dept. of Cell Biology, University of Pittsburgh .

Ion channels are dynamically relocalized to or from the plasma membrane in response to physiological changes, allowing organisms to maintain osmotic and salt homeostasis. Critical to cardiac function is the cell surface localization of Kir2.1, an inward rectifying potassium channel that controls the influx of potassium into cardiomyocytes after hyperpolarization. Thus Kir2.1 restores the cell to its initial resting potential and sets the stage for the next action potential. Mutations in Kir2.1 lead to heart disease, with 21 disease-causing mutations identified. Loss-of-function mutations in Kir2.1 lengthen the QT interval in EKGs, and result in Andersen-Tawil Syndrome (ATS). Mutations that disrupt Kir2.1 function are often linked with its defective delivery to the plasma membrane (PM), and thereby altered Kir2.1 function.
To identify regulators of Kir2.1 trafficking we used a yeast model system where the endogenous potassium channels were deleted and Kir2.1 was expressed; In this system, Kir2.1 was promotes yeast growth on low potassium medium. Using this approach, we discovered that specific α-arrestins,

an emerging class of protein trafficking adaptors, regulate Kir2.1 trafficking to the cell surface. We are now defining the trafficking machinery needed for the α-arrestin-dependent trafficking of Kir2.1. We find that α-arrestin Aly1 requires AP-1, a clathrin adaptor complex thought to recruit clathrin to vesicles shuttling between the endosomes and the Golgi, to promote Kir2.1-mediated growth on low potassium. Curiously, α-arrestins Aly2 and Ldb19 did not require AP-1 for their role in promoting Kir2.1-mediated growth, but require a distinct array of factors suggesting that these α-arrestins operate in distinct pathways.
Based on our work in yeast, we now hypothesize that mammalian α-arrestins regulate trafficking of Kir2.1. We expressed mammalian α-arrestins in our yeast model and find that two mammalian α-arrestins show robust rescue on low potassium. Transitioning into a mammalian cells, we expressed each mammalian α-arrestin in HEK293T cells and mouse cardiomyocytes and found that α-arrestins increased Kir2.1 abundance and co-localized with Kir2.1 at the cell surface and in intracellular puncta. We are currently assessing the α-arrestin pathways defined in our yeast system to those that regulate Kir2.1 trafficking in mammalian cells.

1920C Lipid accumulation adapts hepatocytes to ER stress *Anjana Ramdas Nair*[1], Leonore Wunsche[1], Catherine Palmer[1], Priyanka Lakhiani[1], Jeremy Teo[1], Kirsten Sadler Edepli[1] 1) New York University Abu Dhabi.

The Unfolded Protein Response (UPR) serves to counteract the accumulation of proteins in the Endoplasmic Reticulum (ER) during stress. The initial phases of the UPR are adaptive, but chronic activation can lead to cell dysfunction and pathology. We used transcriptomic analysis of zebrafish exposed to known stressors including arsenic and ethanol and uncovered distinct UPR subclasses to be activated in response to each stressor. We integrated this genomic subclassification with ER morphological analysis by *in vivo* live imaging in the liver of 5 days post fertilization (dpf) transgenic zebrafish expressing an ER-marker specifically in hepatocytes (*Tg(fabp10a:ER-tdTomato))*. Unstressed hepatocytes displayed a highly reticular ER whereas hepatocytes in larvae with ER stress showed extensive loss of ER reticularity and, like the unique gene expression patterns observed with each stressor, the changes to ER structure were also distinct in each model. Only Tunicamycin and arsenic stimulated the most dramatic response, inducing motile punctae which retained the fluorescent ER label in approximately 80% of the livers and gene expression analysis showed that such livers also had reduced UPR activation. We further assessed fatty liver using *in vivo* imaging using Nile Red. We asked whether the heterogeneity observed in ER stress markers was reflected in lipid accumulation, and found that some larvae did not develop fatty liver. Surprisingly, we uncovered an inverse correlation between high lipid accumulation and UPR gene expression, suggesting that fatty liver associated with an adapted response to ER stress. To test this, we used a transgenic zebrafish line overexpressing the cytoplasmic lipid droplet protein Perilipin-2, which is predicted to protect against ER stress (PLIN2, *Tg(fabp10a:EGFP-hPLIN2*)). PLIN2 overexpression had a dramatic effect in larvae exposed to ER stressors: it increased the number of fish with fatty liver and increased the amount of lipid in hepatocytes, prevented ER morphology changes and blunted UPR gene expression. This suggests that lipid accumulation is associated with improved cellular function in response to ER stress.

1925B RNA binding proteins coordinately control lifespan in *C. elegans* *Rebekah Napier-Jameson*[1], Adam Norris[1], Victoria Schatzman[1] 1) Southern Methodist University, Dallas, TX.

Regulation of gene expression affects lifespan in *Caenorhabditis elegans*. While transcription factors have been extensively studied for their role in aging, less is known about how RNA binding proteins may contribute to the aging process. We recently performed a CRISPR/Cas-9 based Synthetic Genetic Interaction (CRISPR-SGI) screen in *C. elegans* focused on conserved neuronally-expressed RNA binding proteins, and identified many double mutants with fitness defects. In one notable interaction between the MBNL1/2 ortholog *mbl-1* and the ELAVL ortholog *exc-7*, double mutants displayed a severely shortened lifespan (~70%). Both genes are required for regulating hundreds of transcripts and isoforms, and both play a critical role in lifespan extension through insulin signaling. Additional interactions between *mbl-1* and *fox-1*, and *exc-7* and *fox-1* showed ~10% and ~20% lifespan shortening respectively, while the constituent single mutants had no effect on lifespan. We have therefore identified a trio of RNA binding proteins combinatorically required for proper lifespan in *C. elegans*. The *mbl-1;exc-7* double mutant appears to develop into healthy young adults after which their health rapidly declines. We have used RNA seq data to investigate which RNAs may be uniquely dysregulated in the *mbl-1;exc-7* double mutant. Our preliminary data has led us to hypothesize that immune gene dysregulation may play an important role in the shortened lifespan phenotype. “SMURF” assays performed to establish intestinal barrier permeability showed early “leaky” guts in the *mbl-1;exc-7* double mutants, supporting our immune dysregulation hypothesis. We are currently investigating further genes of interest (GOI) identified through our RNA seq analysis, and testing whether they modulate the lifespan phenotype of *mbl-1;exc-7* mutants. *mbl-1, fox-1,* and *exc-7* are neuronally-enriched genes and we are currently conducting experiments to test whether their expression in the nervous system is the critical tissue affecting whole-worm lifespan.

1927A A Role for UNC-45 in Maintaining Myosin During Aging *Courtney Christian*[1], Hiroshi Qadota[1], Guy Benian[1] 1) Department of Pathology, Emory University, Atlanta, GA.

UNC-45 is a chaperone required for the folding of functional myosin heads and the proper assembly of myosin into thick filaments. UNC-45 was first discovered in *C. elegans* and later found to be conserved in all animals. UNC-45 consists of a TPR domain that interacts with HSP-90, a central domain, and a UCS domain that interacts with and folds the myosin head. In addition to its essential role in muscle development, we hypothesize that UNC-45 has a role in mature muscle, to re-fold myosin heads damaged from physical, thermal, or oxidative stress. One type of stress is aging, as a popular theory is that the decline in cellular function found in aging is due to an accumulation of damage to macromolecules that occurs with time. Sarcopenia is the decrease in muscle mass and function seen in the elderly in the absence of any underlying disease. Herndon et al. (2002) showed that *C. elegans* can serve as a model for sarcopenia. Using immunostaining with antibodies to myosin heavy chain A (MHC A), we show that there is a gradual decline in the number of A-bands (a measure of thick filament assembly) beginning at day 8 adults. By day 12 and especially day 16, there is also disorganization of A-bands. This disorganization appears similar to that of *unc-45* ts mutants grown at the restrictive temperature. We have found that in *C. elegans* a decline in *hsp-90* mRNA (day 2) directly precedes a decline in HSP-90 protein (day3), which directly precedes a decline in UNC-45 protein (day4), which then in turn precedes a decline in Myosin B (day8), the main client of UNC-45. *unc-45, unc-54,* and *myo-3* mRNA decline immediately after reaching maturity (day 1) and remain stable, though low – which is expected for proteins assembled into the sarcomere. We also observe a decrease in UNC-45 protein, but not mRNA, in an *hsp-90* loss of function mutant, suggesting a role for HSP-90 in UNC-45 regulation and/or protein stabilization. We also observe early onset of sarcopenia when UNC-45 is lost during adulthood and delayed loss of UNC-45 (day 8) in a mutant with delayed onset of sarcopenia. This leads us to investigate the possibility that during aging a loss of HSP-90 leads to UNC-45 degradation, which then leads to a loss of muscle mass and function. A better understanding of how myosin and its chaperone proteins are regulated and affected by aging will lead to better preventative care and treatment of sarcopenia and, possibly, the age-related decline of heart muscle function.

1929C Memory of temperature experience shapes the oxidative stress response *Francesco Servello*[1], Nohelly Derosier[2], Javier Apfeld[1] 1) Northeastern University; 2) City College of New York.

Oxidation is a hallmark of many age-dependent diseases. Cells prevent and repair oxidative damage using many conserved mechanisms, but the extent to which these cellular responses are coordinated in multicellular organisms remains poorly understood. Here, we show that in the nematode *Caenorhabditis elegans* these cellular responses are coordinated by a single pair of thermosensory neurons. This regulatory system enables *C. elegans* to adjust its cellular oxidant defenses in response to the neuronal memory of previous temperature experience.

We found that genetic ablation of the AFD thermosensory neurons in *C. elegans* results in a three-fold extension in survival to lethal doses of the oxidant *tert*-butyl hydroperoxide; however, loss of thermosensory cGMP signaling within AFD and loss of many neuropeptides expressed in AFD does not alter survival at constant temperature conditions. As AFD is responsible for storing memory of previous temperature experience, we hypothesized that loss of AFD and therefore loss of this temperature memory would cause animals to chronically perceive all ambient temperatures as warm and preemptively induce oxidant defenses in target tissues. To address this possibility, we measured survival of animals under oxidative stress at 20°C with a warm (25°C) temperature history compared to that of animals with a chronically cool (20°C) temperature history. Warm temperature history extends survival by two-fold. Loss of cGMP signaling via GCY-8 within AFD abrogates this extension in survival. Furthermore, the effects on oxidant survival of both genetic ablation of AFD and warm temperature history are both dependent upon the action of DAF-16/FoxO and SKN-1/Nrf transcription factors in target tissues. Coordination of these transcriptional responses by AFD appears to require the action of CRH-1, the nematode homolog of CREB involved in memory formation.

Finally, we hypothesized that this regulatory system could be used by the nematodes in order to predict potential encounters with pathogens —that are the main source of exogenous oxidants— at different temperatures. Consistent with this view, worms with a genetic ablation of the AFD neurons show a three-fold extension in survival to killing by the pathogen *Enterococcus faecalis*.

We propose that similar sensory mechanisms may coordinate cellular oxidant responses in humans and may provide new approaches to combat age-related diseases.

1947C The longevity-promoting factor, TCER-1, widely represses stress resistance and innate immunity *Francis RG Amrit*[1], Nikki Naim[1], Ramesh Ratnappan[1], Julia Loose[1], Carter Mason[1], Laura Steenberge[1], Brooke T McClendon[2], Guoqiang Wang[3], Monica Driscoll[3], Judith L Yanowitz[2], Arjumand Ghazi[1] 1) Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh; 2) Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh; 3) Department of Molecular Biology and Biochemistry, Rutgers, The State University of New York, New Jersey.

A positive correlation exists between stress resistance and longevity, but emerging evidence suggests that lifespan and stress endurance are physiologically distinct. A major challenge in aging biology has been identifying factors that play distinct roles in these closely coupled processes because genes that promote longevity often enhance stress resistance. Here, we demonstrate that TCER-1, the *Caenorhabditis elegans* homolog of the human transcription elongation and splicing factor, TCERG1, has discrete and opposite effects on lifespan and stress resistance. We previously identified *tcer-1* as a gene that promotes longevity in germline-less *C. elegans* and reproductive fitness in wild-type animals. Surprisingly, *tcer-1* mutants exhibited exceptional resistance against multiple biotic and abiotic stressors, including infection by the human opportunistic pathogen *Pseudomonas aeruginosa*. Conversely, TCER-1 overexpression increased susceptibility to infection. TCER-1 acted cell non-autonomously to both enhance longevity and repress immunity. Interestingly, TCER-1 inhibited immunity only during the fertile stages of life and not in post-reproductive adults. Elevating its levels ameliorated the fertility loss that follows infection, suggesting that TCER-1 may repress immunity to augment fecundity. Mechanistically, TCER-1 acts through the inhibition of the conserved kinase, PMK-1, as well as through repression of PMK-1-independent, novel antibacterial factors critical for innate immunity. Recent RNA-seq studies have further highlighted the role of TCER-1 in influencing immunity under pathogenic challenge. Overall, our data establish key roles for TCER-1 in coordinating immunity, longevity and fertility, and reveal the molecular mechanisms that distinguish length of life from functional aspects of aging.

1951A Using Survival Assays and RNA-seq to Identify Strain-Specific Differences in the *Caenorhabditis elegans* Response to Microbial Pathogens *Patrick Lansdon*[1], Maci Carlson[1], Brian Ackley[1] 1) University of Kansas, Lawrence, KS.

Infection from microbial pathogens is a major threat to organismal survival. In its natural environment the microbivorous nematode, *Caenorhabditis elegans*, frequently encounters pathogenic bacteria. Although *C. elegans* possess physical barriers and exhibit coordinated behavioral responses to decrease the likelihood of infection, they must also recognize and respond to pathogens that have bypassed these defenses. This response is modulated through the innate immune system, a defense mechanism comprised of evolutionarily ancient components that are highly conserved across phyla. Yet, *C. elegans* do not exhibit obvious conservation of microbial defense pathways observed in arthropods and mammals, (*e.g.* Toll or NF-κB). Rather, pathogen detection occurs via many different systems that converge upon a core set of physiological responses as well as a set of pathogen-specific responses, some of which are conserved in other organisms (*e.g.* generation of reactive oxygen species, production of antimicrobial peptides, *etc.*). To investigate the evolutionary basis of innate immunity, we assessed survival of several *Caenorhabditis* strains infected with various pathogenic bacteria and found strain-specific responses to both *Pseudomonas aeruginosa* and *Enterococcus faecalis*. We are employing a two-pronged approach to identify molecular changes that may be responsible for these strain-specific differences in immunity. First, to detect transcriptomic changes we performed RNA sequencing of whole animals following 24 hours of pathogen exposure. This analysis yielded a large set of differentially expressed genes, some of which have been previously implicated in pathogen response. Currently we are curating a list of genes that are differentially expressed in both a pathogen-specific and strain-specific manner. Second, from our wild-type strains we generated crossbred F1s and compared survival of the cross-progeny to that of the wild-type strains following exposure to pathogenic *E. faecalis* and non-pathogenic *E. coli*. Genomic mapping of the F2 heterozygous offspring generated from these crossbred animals is underway to identify genetic loci overrepresented in animals exposed to *E. faecalis* relative to non-pathogenic *E. coli*. Ultimately, our study seeks to shed light on the evolutionary origins of innate immunity as well as reveal uncharacterized aspects of mammalian defenses against infection.

1952B A multi-organism genetic model for microbiota-driven parasite burden Ritika Das[1,2], Mericien Venzon[1,2], Ken Cadwell[1,2,4], E. Jane Hubbard[1,2,3] 1) New York Univ Sch Medicine; 2) Skirball Institute; 3) Department of Cell Biology; 4) Department of Immunology.

Intestinal microbes exert a wide range of effects in mammals, and they interact with pathogens including parasitic nematodes. In a multi-organism approach, we are using *C. elegans* to investigate mechanisms of interaction between the mammalian host microbiome and the parasitic nematode *Trichuris*.

Caenorhabditis elegans provides a convenient model for understanding how bacteria, both in the diet and in the environment, influence animal physiology. To systematically investigate how bacteria influence the *C. elegans* fertility, we screened the Keio *E. coli* mutant library consisting of ~4000 *E. coli* K-12 isolates, each bearing a single non-essential gene deletion. We raised worms on individual *E. coli* mutants in liquid 96-well format. To sensitize the germ line, we used a mutant *C. elegans* background with a reduced germline stem cell pool. We also used fluorescent transgenes to facilitate the identification of gravid or non-gravid adult worms. From a first screen, we identified five *E. coli* mutants that, when fed to *C. elegans*, delay fertility. Of these, three delay germline development relative to somatic development. None of the five appear to act via *C. elegans* insulin signaling, and the effect of one of the mutants is partially dependent on the DAF-7 TGFß pathway. Bacterial metabolomic profiling and follow-up suggest that the effect of this latter mutant is sensitive to the concentration of arginine in the media.

Billions of people worldwide are infected by *Trichuris trichiura*. Existing anthelmintic drugs display insufficient efficacy, and they fail to eradicate the parasite from the environment leading to a continuous cycle of reinfection. *Trichuris* matures and reproduces in the large intestine of its mammalian hosts, thus spending a key portion of its life cycle alongside trillions of bacteria. Given that parasite burden and egg laying are linked to disease outcomes and transmission, insight into how microbes interact with the parasite may lead to novel strategies for controlling this significant human pathogen and associated disease. Our preliminary results indicate that among the five mutant *E. coli* from our screen that delay fertility when fed to *C. elegans*, one interferes with *Trichuris* hatching in vitro and three limit *Trichuris* infection and/or reproduction when introduced into the intestines of germ-free mice.

1958B *Caenorhabditis elegans* processes sensory information to choose between freeloading and self-defense strategies *Jodie Schiffer*[1], Francesco Servello[1], William Heath[1], Sean Johnsen[1], Natalie McGowan[1], Julian Stanley[1], Stephanie Stumbur[1], Abigail Vogelaar[1], Javier Apfeld[1] 1) Northeastern University.

Hydrogen peroxide is the preeminent chemical weapon that organisms use for combat. Individual cells rely on conserved defenses to prevent and repair peroxide-induced damage, but whether similar defenses might be coordinated across cells in animals remains poorly understood. Here, we identify in the nematode *Caenorhabditis elegans* a neuronal circuit that processes information perceived the ASI chemosensory neuron pair to control the induction of hydrogen-peroxide defenses in target tissues. We found that catalases produced by Escherichia coli, the nematode's food source, can deplete hydrogen peroxide from the local environment and thereby protect the nematodes. In the presence of E. coli, the nematode's neurons signal via TGFβ-insulin/IGF1 relay to target tissues that they need not induce catalases and other hydrogen-peroxide defenses. This adaptive strategy is the first example of a multicellular organism modulating its defenses when it expects to freeload from the protection provided by molecularly orthologous defenses from another species.

1959C Sleep-length differences are associated with altered body composition and longevity in the fruit fly *D. melanogaster*. Jacqueline Thompson[1], Oanh Oanh Su[1], *Johannes Bauer*[1] 1) California State University Sacramento.

Sleep deprivation has been shown to negatively impact health outcomes, leading to decreased immune responses, memory loss, increased activity of stress and inflammatory pathways, weight gain, and even behavioral changes. These observations suggest that sleep deprivation substantially interferes with important physiological functions, including metabolic pathways of energy utilization.
Many of those phenotypes are correlated with age, suggesting that disrupted sleep may also interfere with the aging process. However, little is known about how sleep disruption affects aging and longevity. Here, we investigate this relationship using eight representative fruit fly lines from the Sleep Inbred Panel (SIP). The SIP consists of 39 inbred lines that display extreme short- and long-sleep patterns, and constitutes a crucial *Drosophila* community resource for investigating the mechanisms of sleep regulation.
We obtained four long-sleep and four short-sleep lines from the SIP, and verified their activity patterns. We then analyzed the longevity of these short- and long-sleep lines. Interestingly, our data show that that both male and female flies with short-sleep periods have ~16% longer life span, as well as reduced aging rate, compared to flies with long-sleep. This increased longevity is accompanied by a ~10% reduction in body weight for male and female short sleep animals, compared to the long sleepers. This weight reduction is accompanied by fat and glucose storage reduction in short- versus long-sleep flies.
In contrast, when the circadian rhythm of flies with normal sleep patterns is disrupted by continuous exposure to light, fly longevity is decreased. These data suggest that the circadian clock system and the sleep system modulate longevity by distinct mechanisms. Interestingly, the SIP short-sleep lines show metabolic signs of Calorie Restriction (CR), a well-established longevity-increasing intervention. In addition, when exposed to CR, short-sleep lines do not display longevity extension. This data is consistent with previous reports that show that starvation increases fly activity levels and suppresses sleep. Together, these data suggest that, at least in these flies, mechanisms of CR overlap with those of sleep modulation.

1967B The role of TGF-Beta/activin and mTORC2 signaling in cardiac homeostasis *Kai Chang*[1], Hua Bai[1] 1) Iowa State University.

Cardiovascular disease is the worldwide leading cause of death, especially in elder population. Understanding the mechanisms underlying cardiac aging is crucial for developing effective therapeutic interventions to promote cardiac health. Impaired autophagy and mitochondrial quality control have been previously linked to age-related declines of cardiac function. Yet, how those quality control mechanisms are altered during cardiac aging still remains elusive. Here using *Drosophila* heart as a model system, we show that activin signaling, a member of TGF-beta superfamily, negatively regulates cardiac autophagy and cardiac health during aging. We found that cardiac-specific knockdown of daw, an activin-like protein in *Drosophila*, increased cardiac autophagy and prevented age-related cardiac dysfunction, including arrhythmia and bradycardia (slow heart rate). Inhibition of autophagy blocked daw knockdown-mediated cardio-protection. Intriguingly, the key autophagy regulator, mechanistic target of rapamycin complex 1 (mTORC1), was not involved in activin-mediated autophagy. Instead, activin signaling genetically interacted with rictor, the key subunit of mTORC2, to regulate autophagy and cardiac aging. Recently, we also found that mTORC2/rictor regulates cardiac mitochondrial fragmentation under dietary stress in *Drosophila*. Our findings highlight an emerging role of activin signaling and mTORC2 in the regulation of cardiac homeostasis during aging.

1979B *Drosophila* TRIM32 cooperates with glycolytic enzymes to promote cell growth SIMRAN BAWA[1], David S. Brooks[1], Kathryn E. Neville[2], Abdul Kader Sagar[3], Brian V. Geisbrecht[1], Jason M. Tennessen[4], Marla Tipping[2], Kevin W. Elicieri[3], Erika R. Geisbrecht[1] 1) Kansas State University; 2) Department of Biology, Providence College, Providence, RI 02918; 3) Laboratory for Optical and Computational Instrumentation, University of Wisconsin-Madison, Madison, WI 53706; 4) Department of Biology, Indiana University, Bloomington, Indiana 47405.

Control of tissue and organismal size requires the continual reprogramming of metabolic pathways to integrate biosynthetic and degradative signals. During cell growth and/or proliferation, one such mechanism that promotes the accumulation of cellular material is a switch from oxidative to glycolytic metabolism, whereby glycolytic intermediates are diverted towards anabolic pathways. How this switch is regulated in different tissues is not clear. Herein we identify a novel role for the tripartite motif (TRIM) family member, TRIM32, in the maintenance of glycolytic flux. Using a proteomics approach, we uncovered the glycolytic enzymes Aldolase (Ald) and Phosphoglycerate mutase 78 (Pglym) as TRIM32 interacting proteins. Loss of *Drosophila* TRIM32, encoded by the *thin (tn)* gene, showed a reduction in glycolytic activity and amino acid abundance. This altered metabolic profile caused a striking reduction in the overall size of two inherently glycolytic larval tissues – somatic muscles and the developing brain. Consistent with a role for metabolic intermediates in glycolysis-driven biomass production, nutritional supplementation of amino acids in *tn* mutants restored muscle mass. Many tumors favor glycolytic metabolism to maximize substrate production for uncontrolled cell growth and proliferation. Remarkably, wing disc-associated tumor growth is abolished upon loss of TRIM32. Our results reveal a novel connection between TRIM32 and the maintenance of glycolytic enzyme levels and upregulated pathway activity for the sustained growth of normal and cancerous tissue growth.

1980C Genetic Manipulation of the Cellular Redox Environment Alters Oncogenic Phenotypes in *Drosophila*. *leslie saucedo*[1], kate segar[1], chris andersen[1] 1) University of Puget Sound.

The paradigm of antioxidants serving a protective role in cancer has shifted dramatically in recent years. Cancers often aberrantly express enzymes that control the redox environment and administration of antioxidants has been shown to accelerate the progression of some cancers. Research in model organisms like *Drosophila* have the advantage of precisely controlling the location and timing of gene expression that alters redox signaling. We genetically manipulated the levels of NRF2 and Keap1; two conserved master regulators of the cellular redox environment, while simultaneously overexpressing the oncogenes Src or Ras. We found that increasing antioxidant activity counters Ras-induced proliferation in stem cells and MAPK signaling in epithelial cells. We also found that decreased antioxidant activity promotes a Src-induced tumorigenic phenotype in adult flies. However, because the tumors formed in conjunction with reduced apoptosis rather than increased proliferation, this suggests that Src activity was likely mitigated with a lessening of antioxidant activity. Inhibiting antioxidants as a therapeutic approach to cancer is gaining momentum, therefore we hope that work in *Drosophila* could serve as a powerful system to isolate discrete mechanisms regulated by redox signaling and subsequently, better inform treatment.

1996A Muscle-specific rescue of exercise tolerance in a *Drosophila* model of Barth Syndrome *Deena Damschroder*[1], Robert Wessells [1] 1) Wayne State University School of Medicine.

Cardiolipin (CL) is a mitochondrial phospholipid that helps maintain the normal structure of the inner mitochondrial membrane and stabilize the protein complexes of the electron transport chain to promote efficient ATP synthesis. The proper function of CL requires the action of the remodeling enzyme Tafazzin. Mutations in the *tafazzin* gene are associated with a human disorder known as Barth syndrome (BTHS). Barth Syndrome is an X-linked multisystem disease characterized by cardiomyopathy, skeletal myopathy, and exercise intolerance. Barth patients have identified exercise intolerance as one of the most impactful symptoms to their everyday routines and urged scientists to focus on elevating their exercise intolerance in order to boost their quality of life.

We previously determined that *Drosophila tafazzin* (*TAZ*) mutants exhibit exercise intolerance similar to human Barth patients. Here, we report the efficacy of overexpressing various genetic mimetics of exercise to restore the exercise capacity of *TAZ* mutants. Additionally, we show that mutations in *tafazzin* in muscle or neural tissue affects normal baseline exercise capacity and that rescue with full-length *tafazzin* in muscle can restore *TAZ* mutants' exercise capacity. Together, our results suggest possible therapeuptic targets that can help restore quality of life to Barth patients.

2001C A Drosophila model for Sanfilippo Syndrome *Freya Morgan*[1,2], David Kavanagh[2,3], Elizabeth Van Swol[2], Sneha Mokashi[2], Robert Anholt[2], Trudy Mackay[2] 1) University of Surrey, Guildford, UK; 2) Center for Human Genetics, Clemson University, Greenwood, SC, USA; 3) Trinity College, Dublin, Ireland.

Sanfilippo syndrome (Mucopolysaccharidosis Type III, MPS III) is a rare, autosomal recessive disorder, caused by a deficiency in enzymes involved in the breakdown of heparan sulfate. The disease is characterized by the accumulation of heparan sulfate in lysosomes, which leads to degeneration of the central nervous system. Patients exhibit developmental regression, hyperactivity, sleep irregularity, coarse features and a reduced lifespan. The genome of *Drosophila melanogaster* contains conserved orthologs of human disease genes. We obtained Drosophila lines with CRISPR-Cas9 generated deletions of fly orthologs of *SGSH* and *NAGLU*, mutation in which cause MPS IIIA and MPS IIIB. A deletion in *CG14291*, which is orthologous to *SGSH*, displayed phenotypes reminiscent of human Sanfilippo syndrome. Flies of this line showed reduced lifespan (-28.8%, Males; -15.1%, Females) and reduced productivity (-78.7% at 2 weeks old) compared to the control. Males of this line exhibited a significant increase in average activity ($p<0.0001$) and showed impaired phototaxis compared to the control. Thus, the *CG14291* deletion line can serve as a model for Sanfilippo syndrome. However, the same mutation in an independently obtained mutant did not replicate these phenotypes, suggesting the existence of genetic modifiers in the strain used to generate the deletions. Naturally segregating epistatic partners that affect penetrance of the mutation can be identified by crossing the *CG14291* deletion line to lines of the *Drosophila melanogaster* Genetic Reference Panel and screening for the observed phenotypes.

2014A Characterization of metabolic defects across multiple *Drosophila* models of ALS *Hannah Ball*[1], Suvithanandhini Loganathan[1], Ernesto Manzo[1], Abigail O'Connor[1], Gabe Birchak[1], Daniela Zarnescu[1] 1) University of Arizona, Tucson, AZ.

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease that disrupts muscle function and has no cure. TAR DNA Binding Protein (TDP-43) is an RNA binding protein associated with pathological aggregates in 97% of patients. Expressing wild-type or mutant human TDP-43 in Drosophila recapitulates several symptoms of ALS, including locomotor dysfunction and reduced survival. We recently found that glycolysis is upregulated in this model as a compensatory mechanism that improves locomotor function and increases lifespan. Increasing the availability of glucose was found to

improve several phenotypes in our model of ALS based on TDP-43 overexpression as well as SOD1 and C9orf72. We also used a recently generated CRISPR model of TDP-43 proteinopathy and found that a high glucose diet mitigates locomotor defects in adults. Finally, to begin understanding the role of phosphofructokinase (PFK), the rate limiting enzyme of glycolysis at neuromuscular synapses in ALS, we generated a PFK-GFP CRISPR line. Using this transgenic line we examined PFK localization at the neuromuscular junction (NMJ) in ALS Drosophila. Preliminary experiments indicate that PFK localization is altered in the context of TDP-43 compared to controls. However, because PFK is also highly expressed in muscle cells, it is difficult to clearly determine the PFK-GFP signal within the synaptic boutons from the muscle thus higher resolution microscopy is needed. Current experiments are aimed at using expansion microscopy to further determine the effect of TDP-43 proteinopathy on PFK levels and/or localization in synaptic boutons. This research might explain the synaptic deficits occurring in ALS and related neurodegenerative disorders.

2016C Altered expression of Drp1 and Rbf1 in selected neurons *Azra Hasan*[1], Brian E. Staveley[1] 1) Memorial University of Newfoundland.

Parkinson Disease (PD) and other neurodegenerative diseases can be influenced by mitochondrial dysfunction. Mitochondrial health depends, in part, upon the relationship between the mitochondrial fission protein Drp1, the transcriptional regulator Rbf1, the bcl-2 proteins (Buffy and Debcl), and the E3 ubiquitin ligase parkin. Here we explore the consequences of the altered expression of *Drp1* and *Rbf1* in selected Drosophila neurons upon development, longevity and locomotor activity throughout life.
In our experiments, we directed the expression and inhibition of *Drp1* and *Rbf1* in Drosophila eyes (via *GMR-Gal4*) and selected neurons (via *Ddc-Gal4, Th-Gal4, D42-Gal4*). Furthermore, related experiments were conducted in a *parkin* loss of function background (*Ddc-Gal4 park-RNAi*). While the directed inhibition of *Drp1* compromises climbing ability, the combined loss of function of *Drp1* and *park* results in an extended median lifespan and improved climbing ability throughout the lifespan when compared to the loss of either *Drp1* or *park,* independently. In contrast, the directed expression of *Drp1* in neurons can decrease lifespan and climbing ability; the inhibition of *park* does not have an effect upon these phenotypes. Thus, the compromised climbing abilities in flies with directed inhibition of *Drp1* have produced a new model of Parkinson Disease and can be used to further investigate the mechanism(s) underlying PD and other neurodegenerative diseases.
The directed expression of *Rbf1* reduces lifespan and climbing ability over time. However, in combination with *park* loss of function, directed expression of *Rbf1* results in increased lifespan and climbing ability. Loss of *park* does not affect the loss of *Rbf1* phenotype, therefore it is likely that loss of *Rbf1* does not control mitochondrial fission and mitophagy. However, directed expression of *Rbf1* does control mitochondrial health. Therefore, the precise control of *Rbf1* is crucial to mitochondrial health and cell survival.
The roles of *Drp1* and *Rbf1* in the control of mitochondrial dynamics are essential to the longevity and healthspan of flies. The results of our ongoing experiments will be presented in which altered expression of *Drp1* and *Rbf1* in combination with other genes that control mitochondrial viability. Overall our experiments are allowing us to contribute to our understanding of mitochondrial health and enhanced conditions of homeostasis. Funded by School of Graduate Studies Fellowship and by an NSERC Discovery Grant.

2019C Interactions among models of Amyotrophic Lateral Sclerosis, Parkinson Disease and Aging in *Drosophila melanogaster* *Emily Hurley*[1], Brian Staveley[1] 1) Memorial University of Newfoundland.

The neurodegenerative disease Amyotrophic Lateral Sclerosis (ALS) is one of the most common adult-onset motor neuron diseases. Characterized by the progressive loss of upper and lower motor neurons, ALS progression results in a communication loss between the brain and muscles, that eventually leads to muscle weakness and atrophy. Parkinson Disease (PD) is a neurodegenerative disease, which is characterized by loss of dopaminergic neurons within the *substantia nigra*. Both ALS and PD may result from mitochondrial dysfunction. The majority of ALS and PD cases are believed to be sporadic, while approximately 10% of cases of each are confirmed to be inherited. A number of genes are known to play a role in disease progression: in ALS, major genes are s*uperoxide dismutase 1* (*SOD1*), *Fused in Sarcoma* (*FUS*) and *TAR DNA Binding Protein (TARDBP*). Minor genes include *sequestosome 1* (*p62/SQSTM1*), *Tank Binding Kinase 1* (*TBK1)*, and *Valosin Containing Protein* (*VCP*). The primary PD genes include *alpha-synuclein* (*SCNA*), *parkin* and *Pink1*. These are linked to the autophagy pathway and its sub-type mitophagy: two cellular processes suggested to play a substantial role in the progression of both ALS and PD.
This study focuses on four ALS related genes: *TARDBP*, *p62/SQSTM1*, *TBK1* and *VCP*; these are all highly conserved between human and Drosophila, which allows for modelling of ALS in *D. melanogaster*. To discover potential interactions and similarities between ALS and PD, our aim is to investigate the consequences of altered expression of these ALS genes in combination with altered PD gene activities. To date, we have shown that both overexpression and inhibition of the Drosophila homologue of *TARDBP* (TBPH) in the motor neuron result in a reduced lifespan and motor function. When overexpressed in both the motor neuron and dopaminergic neuron, the Drosophila homologue of TBK1 (IK2) results in a reduced lifespan and motor function. On the other hand, loss of function of the Drosophila homologue of VCP (TER94) in both the motor neuron and dopaminergic neuron, results in a reduced lifespan and motor function. While the loss of function of the Drosophila homologue of *p62/SQSTM1* (Ref(2)P) in both the motor neuron and dopaminergic neuron, results in enhanced longevity but reduced motor function. Experiments involving the interactions that these candidate genes may have with the PD genes, *alpha-synuclein* and *parkin* are ongoing. Funded by an ARC-NL Fellowship and an NSERC Discovery Grant.

2047A High-fat diet-induces retinal degeneration in *Drosophila melanogaster* *Luke Sanchez*[1], Philip Yost[1], Marcela Martinez-Estrada[1], Jennifer Curtiss[1] 1) New Mexico State University.

Age related macular degeneration (AMD) is a leading cause of vision loss in elderly adults. Despite correlations between a Western diet (rich in processed meats, fried foods, dairy and refined grains), AMD, and other neurodegenerative diseases, the mechanisms remain largely unclear.
In comparison with the well-characterized mechanisms of light-dependent retinal degeneration, we aim to identify a potential mechanism for diet-induced retinal degeneration in *Drosophila melanogaster* fed on a high-fat diet (HFD) or a high-sugar diet (HSD). Newly emerged wild type adult flies were placed on standard food (ND) or on standard food supplemented with 4.5% coconut oil (HFD). Each group was further subdivided for exposure to constant darkness, cyclic light, or constant moderate light for 7 days. Retinal degeneration was measured in one-micron retina sections and defined as the percent ommatidia with fewer than normal rhabdomeres. Wild type HFD flies in constant darkness show a significant increase in retinal degeneration compared to controls on a ND. Retinal degeneration in wild type HFD flies exposed to constant moderate light approached levels for ND control flies. Interestingly, HFD flies exposed to constant moderate light did not show an increase in retinal degeneration compared to controls on a ND. Wild type flies fed on a ND supplemented with 30% sucrose (HSD) showed similar increases in retinal degeneration as flies on a HFD. One common mechanism involved in light-dependent retinal degeneration is ER-stress and activation of the unfolded protein response (UPR), and it has been linked to several age-related neurodegenerative diseases. Western blots of flies expressing the xbp1-EGFP fusion protein (a marker

for ER-stress) fed on ND or HFD in varying light conditions revealed similar levels of activation of the UPR in all experimental conditions regardless of diet or light. Our results suggest that a HFD or a HSD induces retinal degeneration in a light-independent mechanism. In future experiments, we aim to determine whether HFD or HSD causes retinal degeneration by effects on mitochondrial function or oxidative stress.

2064C Regulation of post-mating immune response in female *Drosophila melanogaster* *Kathleen Gordon*[1], Mariana Wolfner[1], Brian Lazzaro[1] 1) Cornell University, Ithaca, NY.

In *D. melanogaster* and many other species, female reproductive investment comes at a cost to immunity and resistance to infection. Within hours of mating, *D. melanogaster* females become more susceptible to bacterial infection. Previous studies showed that females were less resistant to bacterial infection at 2.5 and 26.5 hours after mating, but did not test whether a mated female would eventually recover virgin levels of immunity. We tested whether mated females could recover virgin levels of immunity when infected at 2, 4, 7, or 10 days after mating. We observed no recovery of immune capacity in mated females over time. We conclude that mating has a permanent suppressive effect on the female immune system. Knowing that females mate multiply, we tested whether a second mating further affected immune performance. We hypothesized that females who mated twice might become more susceptible to infection than females mated once. Instead, we found that females mated either once or twice before infection survived at equal proportions and both significantly lower than virgin females. This indicates that effects of a single mating are sufficient to suppress the immune response and a second mating does not compound the effect. During mating, the male transfers seminal fluid proteins, like Sex Peptide, that change female physiology and behavior. Sex Peptide induces the female to produce Juvenile Hormone (JH), which promotes egg development. We and others have previously shown that JH is immunosuppressive and decreases resistance to bacterial infection. We thus hypothesize that JH signaling might control resource allocation between reproduction and immunity. Future experiments will seek to determine whether limiting investment in reproduction can improve immune capacity.

2065A The impact of route of infection on the protective effect of chronic infection in *Drosophila melanogaster* *Abigail Wukitch*[1], Emily Van Beek[1], Frank Satriale[1] 1) Bucknell University.

Using the model system *Drosophila melanogaster* we explored the impact that chronic infection had on a subsequent lethal infection and whether the route of infection, injection or oral, impacted survival. Chronic infections, as defined in this study, are infections that persist in the host for extended periods of time without killing the host. To establish chronic infection, flies were either injected with bacteria in the abdomen or placed on bacteria saturated food. After one week, chronically infected flies were injected in the thorax to cause a lethal infection with infectious doses over a thousand fold range. Both survival over one week post-infection and bacterial load at 10 hours post-infection were assessed. Chronic infection had a strong protective effect when the infection was established via injection but no protective effect when established orally. Flies chronically infected with *Serratia marcescens* showed a particularly remarkable 60% survival rate even when subsequently injected with our highest infectious dose (approx. 100,000 bacteria), compared with 100% fatality in the control flies. Further work with higher infectious doses is being done to ascertain the limits of this protective effect. To distinguish whether this protection was due to altered resistance, the host's ability to constrain bacterial load, or tolerance, the ability of a host to withstand the infection while not altering pathogen load, we looked at the relationship between bacterial load at 10 hours post-injection and survival. This allowed us to determine how strength of resistance at the early stages of infection relates to survival during lethal infection. Preliminary results show that the mechanism of protection is dependent on the bacterial species of the chronic infection and that the relationship is not strictly linear or logistic, suggesting that more complex modeling may be needed.

2107A Trisomic dosage imbalance exhibits tissue, temporal and sex specific non-linear genetic expression in a Down syndrome mouse model *Randall Roper*[1], Laura Hawley[1], Charles Goodlett[2] 1) Department of Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN; 2) Department of Psychology, Indiana University-Purdue University Indianapolis, Indianapolis, IN.

Trisomic dosage imbalance results in the overexpression of genes in three copies and leads to phenotypic deficits. The average expression of many three-copy genes is typically reported as approximately 1.5 fold the normal level in tissues of interest in individuals with Trisomy 21 (Ts21). The "few critical genes" hypothesis states that certain dosage sensitive trisomic genes are more important in individuals with Down syndrome (DS) in causing adverse phenotypes. Using mouse models of DS, *Dyrk1a* was identified as a dosage sensitive gene important in cognitive and skeletal deficits. Yet, how and when the aberrant expression of trisomic *Dyrk1a* causes DS phenotypes and the extent to which these changes are similar in males and females have not been shown. We hypothesize that the developmental regulation of *Dyrk1a* expression will help identify developmental periods when abnormal and normal phenotypes diverge and provide potential temporal windows for phenotypic intervention necessary or sufficient to alleviate Ts21 phenotypes. Our analysis of trisomic *Dyrk1a* in mouse models of DS shows non-linear expression of *Dyrk1a* RNA and DYRK1A protein that includes the dosage level expression (~1.5 fold), amplification (up to 5 fold) and compensation (no difference between euploid and trisomic) at different tissues at different points of development, with profiles that differ between the sexes. Levels of *Dyrk1a* exhibit dosage level expression and dosage compensation when *Dyrk1a* copy number is reduced to normal levels in otherwise trisomic DS mouse models or to a single copy in otherwise euploid littermates. *Dyrk1a* dosage level gene expression and amplification correlates with important changes in both cognitive and skeletal developmental phenotypes. These results indicate that trisomic primary gene dosage expression may be modified by other trisomic or non-trisomic genes during critical points in development. These alterations may affect the occurrence of divergent phenotypes and influence the survival of organisms with gene dosage imbalance by limiting the negative effects of trisomic genes.

2118C Trends in Malignancies Involving Various Human Body Parts Based on A Survey in 18 Areas in the United States from 2000-2017 *Merin Jose*[1], Irin Jose[2] 1) Saint Peter's University Hospital; 2) Delphi Technologies.

Introduction - Malignancies are a major cause of morbidity and mortality in the United States. Analysis of trends in various malignancies involving different sites over several years and between different ethnic groups can help us identify the contribution of the genetic and environmental factors.
Method - In this study, we took the data from the SEER (Surveillance, Epidemiology and End Result Program) database (*www.seer.cancer.gov*). The information of the delay adjusted cancer incidence of 23 different organs of the human body published in SEER was collected.
Average annual percent change (AAPC) in prevalence was calculated over the years for malignancy at each site. Results are represented as forest plot with 95 % CI of the AAPC to detect the trends in the disease prevalence. The disease prevalence was also calculated and compared for different racial group. It was compared using ANOVA and diseases with significant racial differences were identified.
Results - Based on the analysis there were increasing and decreasing trends of the malignancies as seen in the forest plot. It was noted that the cancers of larynx, prostate, colon and rectum, lung and bronchus, ovary and cervix had a significant decreasing trend over the years.

Conclusions – Through this we would like to emphasize the fact that certain malignancies have an increasing trend while certain others have a significant decreasing trend. Also, significant changes in disease trends were noticed among different races. This opens the platform to identify the environmental vs genetic factors influencing the increasing or decreasing trends pertaining to the particular malignancy involving a particular organ and also genetic factors affecting the differences in prevalence noted based on ethnicity.

2124C Gastrointestinal Tumor Phenotypes in Offspring of Collaborative Cross Mice and a Sensitized Line *Elena Mogilyansky*[1], Leena M. Alameer[2], Israa Salem[2], Dean E. Ihemesie[2], Alexis J. Landau[2], Linda D. Siracusa[1] 1) Hackensack Meridian School of Medicine at Seton Hall University, Nutley, NJ, USA; 2) Thomas Jefferson University, Sidney Kimmel Cancer Center, Philadelphia, PA, USA.

Stomach, small intestine, colon, and rectal cancers affect hundreds of thousands of people worldwide each year. Gastrointestinal (GI) cancer initiation, growth, and progression depend on genetic and environmental factors. The adenomatous polyposis coli *(APC)* gene is one of the top 5 genes somatically-mutated in colon and rectal cancers. Germline mutations in the *APC*gene cause Familial Adenomatous Polyposis (FAP), an autosomal dominant disorder; patients with FAP develop hundreds to thousands of colon and rectal adenomas, which if left untreated, progress to cancer. Adenoma number and time of onset can vary between family members carrying the same mutation in the *APC* gene. The Collaborative Cross (CC) strains were developed as a powerful resource to dissect genetic factors affecting complex disorders. We designed a screen to determine whether the genomic diversity in selected CC strains could result in novel GI tumor phenotypes. Females from 10 CC strains were mated with males from the sensitized, double congenic 129.B6 $Apc^{Min}Atp5a1^{Mom2R}$/+ + line which we generated. These congenic mice have a long life span and develop few adenomas with a high probability of progressing to adenocarcinomas. Hybrid F1 offspring were aged and the GI tract evaluated for tumor phenotypes, including location, number, shape, and size. The results revealed a diversity of GI tumor phenotypes, which develop due to combinations of alleles in the CC strains and the double congenic line. These findings indicate the presence of tumor-suppressing and tumor-promoting modifier loci in the genomes of CC strains. This exploratory work builds a foundation for future studies to identify new modifier genes that effectively suppress polyposis and potentially serve as therapeutic targets for GI cancers. Research supported by NCI R21 CA202496 to LDS.

2132B Phenomic analysis of the influence and interactions of auxotrophy and nutrient availability on yeast quiescence and chronological lifespan Sean Santos[1], Samantha Laflin[1], Audrie Broadway[1], Haley Albright[1], John Rodgers[1], Daniel Smith[1], *John Hartman IV*[1] 1) University of Alabama at Birmingham, Birmingham, AL.

Yeast cells in stationary phase culture exhibit different developmental fates, including death, senescence, and quiescence. Quiescence is an adaptive transition to a stable cell cycle exit (G0, prior to G1-S transition) in response to nutrient depletion in stationary phase, which is defined functionally by colony forming capacity upon re-exposure to nutritive conditions. Thus, quiescence vs. time constitutes yeast chronological lifespan (CLS). Results from high-throughput CLS studies only weakly correlate, which could be partly due to influences of media composition and auxotrophy on quiescence. This hypothesis was investigated with quantitative high-throughput cell array phenotyping (Q-HTCP) to measure impacts of auxotrophy and media composition on quiescence / CLS, characterizing quiescence over longer-than-typical time periods (past 30 days), and in greater replicate so that high-resolution phenotypic distributions are obtained for aging interventions. In these regards, auxotrophic alleles in the genetic background of the S288C gene deletion library (*his3, leu2, lys2, met17, ura3*), along with glucose, ammonium sulfate, auxotrophic amino acid availability were systematically studied. Target of Rapamycin signaling and replication stress were also examined in this context. Media acidification was monitored in high throughput to assess its correlation with CLS. Previously reported influences of leucine, methionine or glucose availability were observed, and a novel effect of lysine auxotrophy characterized. By contrast, histidine and uracil perturbations had little or no effect on quiescence. Ammonium sulfate availability altered CLS, but this affect was dependent on TOR1 expression and methionine metabolism. The pH of conditioned media from aged cultures was dependent on interactions between auxotrophy and aeration; however, there was weak correlation between media acidification and CLS. In summary, interacting effects of auxotrophy, media composition, and aeration on yeast cell quiescence indicate a fundamental role of metabolism in yeast chronological aging. These factors interact and differentially influence cell quiescence or the pH of conditioned media, however CLS and media acidification are weakly correlated. Hormesis-like effects were sometimes observed, whereby early loss of CFU capacity was associated with later preservation. Gasping, defined as a transient increase in CFU capacity in stationary phase, was observed to be largely independent of establishment or maintenance of quiescence. Quiescence was typically dynamic in the first month, followed by either stable establishment or complete loss. Taken together, these results suggest CLS is a developmental process that is more complex than previously considered and highlight parameters that can potentially explain variability in high throughput studies of CLS.

2147B Optimizing Image Analysis to Verify the Accuracy of Measurement of Drug Responses of Patient Derived Xenografts in Zebrafish Embryos *Kamden Gray*[1], Shaila Mudambi[2], Jessica Miller[2], Eric Glasgow[2], Seray Er[1], Stephen Byers[2] 1) Georgetown University; 2) Georgetown University Medical Center.

Zebrafish (Danio rerio) serve as a key organism for cancer research. Our lab has developed a patient derived xenograft model in zebrafish embryos, Zevatars, in which we can identify the best therapy for a patient's tumor in a personalized medicine setting. Tumor fragments labelled with CM-dil (a non-toxic, intracellular fluorescent dye) are prepared from a single $1mm^3$ block of tumor tissue and implanted into 2dpf zebrafish embryos using tungsten needles. The implanted embryos are treated with drugs in 96 wells and imaged Day 1 of treatment and subsequently re-imaged at Day 3-5 at 4X magnification and 532/560 nm. Change in tumor size in the yolk is measured as change in area between Day 1 and Day 3-5. Others have devised high throughput imaging methods for drug screening in zebrafish but these have concentrated on examining changes in the characteristics of the zebrafish itself rather than the xenograft cells it is bearing. In this study we aimed to optimize image acquisition and analysis methods to enable high throughput and accurate assessment of tumor changes in Zevatars. We initially utilized two methods 1) Area measurements using 2D images from an epifluorescent microscope with a manual thresholding method using ImageJ. 2) Planar fluorescence tumor volume estimated measurements using pseudo Z-stacks from a Keyence fluorescence microscope using MATLAB. Our analysis revealed that there was no difference in tumor size when estimated from a 2D image compared to a pseudo Z -stack image. We have used multiple methods to validate our imaging analysis; 1) Direct cell counting by dissociating implanted embryos and counting dye I labelled cells. 2) Using whole mount immunohistochemistry to stain and count implanted human cells. 3) Using qPCR to estimate cell number by assaying for human target RNAse P in our implanted zebrafish. Although Zebrafish xenografts are becoming more widely used as a method to model cancer research, the imaging approach has not been extensively validated. Standardizing our imaging methodology allows us to apply Zevatars in the clinic for more accurate prediction of patient tumor response.

2149A Using zebrafish to guide a precision medicine approach towards the treatment of complex lymphatic anomalies *Christoph Seiler*[1], Dong Li[2],

Charlly Kao[2], Mark R. Battig[2], Michael E. March[2], Sarah E. Sheppard[2], Adele Donahue [1], Hakon Hakonarson[2] 1) Zebrafish Core Facility, The Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA.; 2) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA..

Complex Lymphatic Anomalies (CLA) present a devastating disease spectrum that can affect the central thoracic duct or cause localized micro and macrocystic lymphatic malformations. To date there is only one main medical therapy based on Rapamycin/Sirolimus with inconsistent responses in patients, suggesting genetic heterogeneity beyond the mTOR pathway. To develop targeted therapies based on genetic information we sequenced a large cohort of CLA patients and identified candidate disease genes. One mutation was a presumed activating (S214P) mutation in ARAF. We show that transgenic expression of the human mutant gene in the zebrafish lymphatic system resulted in similar dilation of the thoracic duct as observed in the patient. Experiments using both cell culture and zebrafish revealed that MEK inhibitors targeting downstream ARAF signaling can revert the phenotype in these models. Subsequent therapy of the patient with the MEK inhibitor Trametinib led to dramatic clinical improvement, with re-modeling of the patient's lymphatic system, resolution of the lymphatic edema and near normalization of daily activities. We have now established models for several other mutations in RASopathy genes using transgenic expression or CRIPSR/Cas9 based direct gene knock out in zebrafish and compared the effects of mTor and MEK inhibitors in these models. These studies provide the basis for further identifying molecularly targeted therapies to treat patients with lymphatic malformations and suggest MEK inhibitors as effective therapy for a wide spectrum of lymphatic diseases.

2158A Innate Immune Response to Influenza A Viral Infection in the Zebrafish *Benjamin King*[1,3], Brandy Soos[1], Lily Charpentier[1], Kodey Silknitter[1], James Seuch[1], Liz Saavedra[1], Con Sullivan[2], Melody Neely[1,3], Carol Kim[1,3] 1) Dept. of Molecular and Biomedical Sciences, University of Maine, Orono, ME; 2) University of Maine at Augusta, Bangor, ME; 3) Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME.

The World Health Organization has estimated that up to 650,000 deaths occur per year from respiratory diseases associated with seasonal influenza infections. Influenza A virus (IAV) causes severe disease in older adults and individuals with chronic health conditions. IAV vaccines against specific viral antigens are difficult to design because of constant changes in the viral genome. The long-term goal of our studies is to understand the molecular mechanisms of the innate immune response to IAV infection and find new antiviral therapeutic targets. Neutrophils have essential roles in innate immunity to bacterial and fungal infections, but their roles in antiviral responses are understudied. Recently, it was demonstrated that IAV infection can be established in zebrafish (*Danio rerio*) larvae. The zebrafish is a powerful vertebrate model system that has been used to study infection and innate immunity. We are using this model to study the roles neutrophils have in controlling IAV infection and how over-activation of neutrophils during IAV infection trigger a damaging hyperinflammatory response. First, we are examining pathways that control the respiratory burst function and how reactive oxidative species control neutrophil function during IAV infection. Ongoing studies of IAV-infected neutrophil cytosolic factor 1 (*ncf1*) morphants show no difference in survival with control morphants at six days post infection (dpi), but increased survival at 3-5 dpi. Survival studies of IAV-infected WHIM (*Tg1(-8mpx:cxcr4b-EGFP)*) mutants that overexpress a truncated *cxcr4b* transgene also show a differences in survival compared to sibling controls that underscores the roles of neutrophils. Second, we are investigating how hyperinflammation occurs during IAV infection so that therapeutic measures that preserve the antiviral response, yet contain the associated inflammation, can be developed. This work is supported by the National Institute of Allergy and Infectious Disease of the National Institutes of Health under grant number R15AI131202.

2165B Sensory induced hyperactivity in a *syngap1ab* zebrafish models of ASD *Sureni Sumathipala*[1], Suha Khan[1], Celine Rey[1], Robert Kozol[2], Julia Dallman[1] 1) University of Miami; 2) Florida Atlantic University.

Autism spectrum disorders (ASDs) are a collection of inherited neurodevelopmental disorders diagnosed by social and cognitive impairments, communication deficits, and repetitive behaviors. ASDs have heterogeneous causes with about 30% cases with a molecular diagnosis. High confidence ASD-linked genes include *SHANK3*, *CNTNAP2*, *NRXN, NLGN*3, and *SYNGAP1* which are important for nervous system development and function. However, the mechanisms by which these gene mutations produce symptoms associated with ASD remain unclear.
My project uses zebrafish to test how loss of function mutations in Synaptic Ras-GTPase Activating Protein 1 (*SYNGAP1*) impact neural circuit function. SYNGAP1 is a component of the postsynaptic density of glutamatergic synapses and plays an essential role in receptor trafficking and synaptic strengthening allied with learning and memory. In our lab, we have generated stable *syngap1ab* mutant zebrafish models of ASD using CRISPR/Cas 9 genome editing. Using these models, we are quantifying ASD-relevant behaviors with the ultimate goal of delineating the neural-circuit-level mechanisms underlying those behaviors.
Due to sensory-induced hyperactivity observed in humans with heterozygous *SYNGAP1* loss-of-function variants, we tested *syngap1ab*$^{+/-}$zebrafish mutants to see whether they would be similarly hyperactive in response to light and/or sound (tap) stimuli. We found that the *syngap1ab*$^{+/-}$ mutants do exhibit hyperactivity in response to both light and tap stimuli at six days post-fertilization. Our kinematic data from *syngap1ab* $^{+/-}$ larvae show a significant increase in swim velocities when tapped, and increased duration of swim bouts compared to WT larvae with no alterations associated with swim coordination. Taken together, these results suggest that possible involvement of Syngap1 in the early development of sensory-motor integration in the zebrafish.
Next, we will be using external-signal regulated kinase (ERK) phosphorylation as a proxy to understand the brain-wide activity associated with observed hyperactivity. This approach will help us gain more insight as to how underlying neurodevelopmental and behavioral phenotypes associated with ASD. Identifying these underlying neural circuits responsible for the hyperactive responses will be useful in the future to develop therapeutic strategies to alleviate hypersensitive symptoms associated with ASD and epilepsy.

2172C Temperature induced DNA damage is associated with increased transposon mobility in spermatocyte nuclei. Nicole Kurhanewicz[1], Devin Dinwiddie[1], *Zachary Bush*[1], Diana Libuda[1] 1) University of Oregon.

All tissues are susceptible to dramatic increases in temperature; however, developing sperm are particularly sensitive to small temperature fluctuations. In contrast to oogenesis and other developmental processes, mammalian spermatogenesis requires a narrow isotherm of 2-7°C below core body temperature. Although failure to thermoregulate spermatogenetic tissue and prolonged exposure to elevated temperatures are linked to male infertility in several organisms, the mechanisms of temperature-induced male infertility are largely unknown. Here we show that upon a one hour upshift to 34°C, only spermatocytes exhibit temperature-induced transposon mobilization and a 30 to 40-fold increase in double strand DNA breaks (DSBs) throughout the genome of spermatocytes, which lead to a decrease in male fertility. Using *Caenorhabditis elegans* to eliminate sex-specific bias, we found that these temperature-induced DSBs are SPO-11 independent and occur specifically in the spermatocytes of both males and hermaphrodites at all stages of meiotic prophase I. Moreover, these temperature induced DSBs in spermatocytes are repaired within 2-3 hours

post-heat shock, with early pachytene nuclei exhibiting increased kinetics of repair in comparison to late pachytene nuclei. Further, we found that in males, heat-shock specifically increased both Tc1 transposase expression and Tc1 excision and reinsertion, which causes DSBs in specific sites of the genome. Taken together, our data provide mechanistic insight on how increased temperatures compromise the genome integrity specifically in spermatocytes and impairs fertility in males.

2178C DNA damage response pathways are induced after exposure to various heavy metals in *C. elegans* Erick Moberg[1], Scarlett Koga[1], Christopher Marrinello[1], Sahil Parag[1], Jessica Gualdin[1], Cathryn Seymour[1], Jennifer Waldroupe[1], Toni Rockholt[1], Shawn George[1], Chezna Lee[1], Xuan Ou Yang[1], Katlin Campbell[1], Megan McMurray[1], *Julie Hall*[1] 1) Lincoln Memorial University.

A major route of exposure to various heavy metals is through contaminated soil and water. Research has shown that these substances play roles in the induction of various diseases such as cancer, neurodegeneration and birth defects. In the cell, proteins such as metallothioneins respond to heavy metal exposure and chelate the metal to prevent cellular damage. However, little is known about the cellular response in regards to DNA damage after heavy metal exposure. To provide a better understanding of this cellular response, the induction of both cell cycle arrest and apoptosis were investigated after exposure to copper, cadmium, iron, lead, nickel and silver in the nematode *C. elegans*. Growth assays were conducted to determine EC10 and EC50 concentrations, which were utilized to investigate if DNA damage response pathway(s), apoptosis and/or cell cycle arrest, were being induced upon exposure. For all metals, apoptosis was induced in the germline with copper, nickel and iron resulting in the greatest induction. To determine if the induction of apoptosis is p53 dependent and thus due to DNA damage, a *cep-1* mutant is being tested. Preliminary data shows that lead and cadmium but not silver, copper, and iron induce p53 dependent apoptosis suggesting DNA damage is occurring in the cell. Cell cycle arrest was also induced in the germline for all metals. Nickel, silver and iron resulted in the most arrested cells. Apoptosis and cell cycle assays for all metals tested will allow us to better understand the damage being caused by the metal exposure as well as mechanisms induced by the cell in response to exposure.

2181C Deciphering Roles of Bloom Syndrom Helicase (Blm) in Genome Stability *Evan Dewey*[1], Jeff Sekelsky[1] 1) University of North Carolina-Chapel Hill.

DNA repair is critical to the maintenance and longevity of multicellular organisms. The DNA damage response provides a mechanism by which cells can respond to and correct damage accrued from damaging agents such as UV radiation, carcinogens, and other toxins. Without proper repair, cells can acquire mutations leading to improper function within tissues, genome instability, and cancer. While progress has been made in discovering the players and pathways involved in alleviating DNA damage, there is still more to understand about how these components function. One such regulator, Bloom Syndrome Helicase (Blm), is key to resolving both damage to DNA during replication, and in preventing detrimental “mitotic crossovers” (mitCOs) arising from homologous recombination, which can lead to loss of heterozygosity, genome instability, and cancer. My objectives are to characterize mitCO mechanisms, elucidate Blm function in resolving DNA damage during replication, and better understand Blm roles in cell cycle progression and tissue growth. I will define Blm function in resolving stalled replication forks by performing structure function mutations, examining fork and cell cycle progression to assess parts of Blm critical to DNA replication fidelity. I will identify types of DNA structures at stalled forks upon which Blm acts by performing a novel ChIP-EM assay, purifying Blm-bound fork structures from *Drosophila* embryos and visualizing them via Scanning Electron Microscopy. While loss of Blm is harmful to proper DNA repair, Blm is additionally overexpressed in many cancers, possibly to facilitate cancer cell growth and persistence. I will model Blm overexpression in *Drosophila* tissues, visualizing effects on cell cycle progression, tissue growth, and DNA repair in this context to understand potential Blm roles in cancer growth and persistence. Deciphering mitCO mechanisms and the functions of Blm in ensuring DNA replication fidelity, cell cycle progression, and tissue growth will lead to better understanding of DNA repair mechanisms and potential application of this knowledge toward cancer prevention and treatment.

2187C Lack of Blm protein during Drosophila embryonic development reduces lifespan of surviving progeny *Nathan Anderson*[1], Abbey Roy[1], Karly Lacey[1], Eric Stoffregen[1] 1) Lewis-Clark State College, Lewiston, ID.

During the early stages of Drosophila embryogenesis, maternally loaded Blm DNA helicase is essential for proper DNA replication; embryos from Blm mutant females, who fail to provision Blm to their eggs, accumulate DNA damage and most do not survive this early developmental period. Despite this severe maternal effect lethality, a small percentage of embryos do survive in the absence of Blm. However, we hypothesize that survivors of this Blm-null embryonic environment experience sub-lethal DNA damage that may pose long-term biological consequences such as decreased lifespan. To test this hypothesis, the lifespan of adult flies that developed with or without Blm during early stages of development was compared. Flies that developed without Blm had a reduced lifespan compared to those that developed with Blm. This data implicates Blm in preventing lasting biological impacts which may be caused by DNA damage accumulation during early development.

2196C A whole-genome approach to defining the meiotic recombination model in *Drosophila melanogaster* *Carolyn Turcotte*[1], Susan McMahan[1], Jeff Sekelsky[1] 1) University of North Carolina at Chapel Hill.

During meiosis, correct number and placement of crossovers ensures accurate homolog segregation and is thus vital to propagation of species. Crossovers originate from repair of double-strand breaks via homologous recombination (HR), and repair pathway choices within HR are traceable in meiotic products via distinguishable heteroduplex DNA (hDNA) signatures. hDNA is normally obscured by mismatch corrections that generate restorations of the original sequence or gene conversions. In most organisms, attempts to retain hDNA by eliminating the canonical mismatch repair (MMR) pathway result in discontinuous hDNA tracts. Whether discontinuous tracts occur due to an alternative form of mismatch correction or reflect actual hDNA patterns is unclear, obscuring interpretation of results. Our lab has shown that mutating MMR and nucleotide excision repair in *Drosophila melanogaster* results in continuous hDNA tracts, making *Drosophila* the only animal thus far in which discontinuous hDNA tracts can be eliminated. Accordingly, our lab mapped hDNA at recombinants of a test locus in *Drosophila*. hDNA signatures that are not expected from the classical meiotic recombination model were observed, demanding revision of this model. However, very few recombinants were recovered in this study, and unequal SNP distribution across the test locus generates bias, as hDNA not containing a SNP is undetectable. Testing our new recombination model therefore requires much more extensive analysis of hDNA than is possible with this methodology. I am developing whole-genome analysis of hDNA to test and revise our new meiotic recombination model. This technique uses a genetic tool exclusive to *Drosophila* that allows sequencing of exclusively the maternal genome in progeny, enhancing ability to detect hDNA. Using this genetic tool, I am pioneering “hetSeq,” a whole-genome sequencing technique in which hDNA tracts are recovered by sequencing exclusively maternal DNA. I will use hetSeq to construct a meiotic recombination model from genome-wide heteroduplex signatures. Once optimized, hetSeq will be further employed to study how different meiotic

proteins contribute to HR pathway choice.

2203A Polyploid Cell Cycles in Development, Genome Instability, and Cancer *Brian Calvi*[1], Michael Rotelli[1], Robert Policastro[1], Kelly Hartsough[2], Gabe Zentner[1], Claire Walczak[1,2], Mary Lilly[3] 1) Indiana University, Bloomington IN; 2) Indiana University School of Medicine, Bloomington, IN; 3) National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

The endocycle is a common cell cycle variant that entails periodic genome duplication without cell division (G / S cycle), producing large, polyploid cells. In a wide array of organisms, including humans, specific cell types switch from mitotic cycles to endocycles during normal development. These developmental endocycles (devECs) contribute to tissue growth through an increase in cell size rather than cell number. Cells also switch to endocycles in response to conditional inputs. These induced endocycling cells (iECs) can be beneficial by promoting wound healing and tissue regeneration. Evidence suggests, however, that iECs can also be detrimental by contributing to therapy resistance and genome instability of cancer cells. Despite the importance of endocycles for development and disease, their regulation and impact on genome integrity remain incompletely defined.
We will report our progress in using *Drosophila* and human cells as model systems to define how endocycles are regulated and how they contribute to genome instability. We have used integrated genetic-transcriptomic and other approaches in *Drosophila* to determine how cell cycle remodeling fosters the switch from mitotic cycles to endocycles. We found that downregulation of a Cyclin A / CDK1 – Myb-MuvB – Aurora B pathway promotes the switch to endocycles in both devECs and iECs. Pharmacological repression of CDK1 or Aurora B activity in human cells was also sufficient to induce endocycles. These human iECs were more resistant to several chemotherapeutic agents, analogous to our previous findings in *Drosophila* that both devECs and iECs repress the p53 apoptotic pathway. Unlike most devECs, both fly and human iECs can switch back to mitotic cell cycles. This return to mitosis (RTM) is associated with highly-error prone mitotic chromosome segregation, which leads to high levels of chromosome instability, DNA damage, and aneuploid daughter cells. Altogether, the data from our labs and others suggest that transient iECs may contribute to cancer therapy resistance and progression. I will discuss how our recent genetic, transcriptomic, and cell biological data in *Drosophila* and human cells is revealing a rewiring of multiple networks in endocycling cells and suggesting strategies for improved cancer therapies.

2209A Constraints on horizontal gene acquisition in bacteria: genetic analysis of a novel restriction-modification system Julie Zaworski[1], Oyut Dagva[1], Alexey Fomenkov[1], *Elisabeth Raleigh*[1] 1) New England Biolabs.

Bacterial lineages respond to changes in their physical and biotic environment by acquisition of niche-adaptive functions via Horizontal Gene Transfer (HGT). They are specified in lineage-variable segments called genome islands. For about half of these islands, the RecA-independent mechanism of assembly and dissemination is obscure, while site-specific recombinases and transposases play a role in circulation of others. We study an island region bearing highly variable restriction-modification (RM) systems. The island (dubbed the Immigration Control Region, ICR) varies within and between enteric species to protect against exogenous DNA entrance. In addition, unidentified site-specific HGT mechanisms may act here.
To initiate study of the mechanism of intergeneric transfer between *E. coli* and *Salmonella enterica* sv Typhimurium LT2, we chose a restriction-disabled derivative often used for molecular genetic constructions (LB5000). Sequencing of this strain allowed identification of the mutations that potentially result in restriction-deficiency in three RM systems: two well-studied (SenLT2I (LT, StyLT in the early literature) and SenLT2II (SB, StySB)) and one poorly characterized, SenLT2III (SA, StySA). Surprisingly, in the genetic region expected for the StySA system, multiple mutations were found in domains of two separate genes. These identified homologs of a BREX-like architecture for the StySA system. Mutational states of the 8 gene cluster were tested for site-specific methylation level (PacBio RSII) and bacteriophage restriction. We compared wild type alleles with engineered deletions of individual genes and with the multiply mutated conserved domains of two genes. In addition, we performed transcriptomic analysis (Cappable-Seq and RACE) in order to unravel the operon structure of this gene cluster. This work should contribute to understanding the role played by this recently-discovered but widespread family of prokaryotic genome defense activities.

2242A Involvement of the INO80 and SWR1 Complexes in Chromosome Segregation *Ines Pinto*[1], Jesus Moreno[1], Jacob Ginter[1], Md Riajul Hossain[1] 1) Department of Biological Sciences, University of Arkansas, Fayetteville, AR.

We are interested in understanding the function that chromatin and chromatin remodeling complexes have during mitotic chromosome segregation. Chromosome loss and ploidy maintenance are essential aspects of genome integrity. In an effort to evaluate the proteins that are involved in ploidy maintenance, we carried out a genetic screen of the non-essential haploid deletion library for genes that when mutated caused ploidy increase. Among the mutants that increased ploidy, we encountered members of the INO80 and the SWR1 chromatin-remodeling complexes. These large multi-subunit ATP-dependent remodeling complexes participate in a variety of biological processes including transcription, DNA repair, DNA replication, and chromosome transmission. SWR1 replaces the canonical histone H2A in nucleosomes with H2A.Z. Its counterpart, INO80 catalyzes the eviction of the H2A.Z histone variant replacing it with H2A. The INO80 complex has been implicated in the maintenance of ploidy through the characterization of mutations of the genes encoding the Ies6 and Ino80 subunits, which result in a clear ploidy increase. To evaluate the contribution of each subunit to chromosome segregation, we tested deletion mutants of all the non-essential subunits of both complexes, as well as a temperature-sensitive allele of the common *ARP4* subunit, for benomyl sensitivity and increase-in-ploidy phenotypes. We found that only some deletions of each complex caused increase-in-ploidy and increased benomyl sensitivity, and that a shift of *arp4*^{ts} to restrictive temperature also led to cells with increased ploidy as well as aneuploidy. Interestingly, the rate of diploidization varied among the subunits tested. We also analyzed genetic interactions between the alleles causing ploidy-increase and shugoshin (*SGO1*), required for sensing tension and mitotic chromosome stability. High levels of *SGO1* showed suppression of the *ino80Δ* phenotypes, suggesting that *ino80* defects can be alleviated by restoring bi-orientation. In contrast, double mutants carrying *sgo1Δ* and SWR subunit deletions (*swr1Δ* or *arp6Δ*) exacerbated the ploidy and benomyl sensitivity of the single SWR1-subunit mutants, indicating that shugoshin is necessary, although not sufficient, for the maintenance of normal ploidy. We are currently investigating the effect of various deletions on the pericentromeric localization of the Ino80 subunit, as well as the levels of H2A.Z and their relationship with shugoshin function and chromosome segregation.

2244C Regulation of Protein Kinase A (PKA) activity to mediate chromosome segregation in *Saccharomyces cerevisiae* *Hana Alsufyani*[1], Sameer Shah[1], John Choy[1] 1) The Catholic University of America.

Chromosome segregation results in the partitioning of sister chromosomes into two new cells with an equal number of chromosomes. This process occurs during mitosis and meiosis and errors can lead to aneuploidy, a karyotypic state in which cells carry the incorrect number of chromosomes. Most of the intrinsic factors that regulate the chromosome segregation process such as the multi-subunit kinetochore complex that connect chromosomes to the mitotic spindle are highly studied and virtually all of the protein subunits have been identified in yeast, flies, worms, and humans.

However, much less is known about how extrinsic factors, which include nutrients, temperature, and pH might act on the intrinsic chromosome segregation machinery. Recently, the major glucose signaling pathway (Ras/Protein Kinase A) was shown by our lab to phosphorylate the outer kinetochore subunit, Dam1, and contributes to chromosome segregation fidelity in the budding yeast, *S. cerevisiae*. While this is the first description of a molecular pathway connecting glucose to chromosome segregation many questions remain. In particular, what is the mechanism regulating PKA-mediated phosphorylation of the kinetochore. Is the known Ras/PKA pathway that responds to glucose also directing PKA toward the kinetochore or are there additional signaling components that are specific to PKA's role in kinetochore phosphorylation. As a first step toward determining the mechanism of PKA regulation we measured the expression and activity of PKA at different cell cycle stages. Our initial results show that PKA activity increases through the cell cycle although protein levels remain constant. Associated with the increase in PKA activity we also observe increasing levels of Dam1 phosphorylated at the PKA site. Our next step is to determine if the known Ras/PKA pathway components are required for the cell cycle regulation of PKA activity and Dam1 phosphorylation. These studies will bring new insights into how extrinsic factors such as glucose can directly modulate kinetochore function and chromosome segregation fidelity.

2259C Quantification of cellular and tissue phenotypes of a pleiotropic mutant zebrafish is enabled by X-ray histotomagraphy *Alex Lin*[1], Georgia Thomas[1], Casey Smallwood[1], Kai Ang[1], Victor Canfield[1], Damian van Rossum[1], Keith Cheng[1] 1) Penn State Hershey College of Medicine.

A histology-based zebrafish forward genetic screen for mutations causing cytological abnormalities yielded a mutant, *huli hutu* (*hht*), whose pleiotropic phenotype includes atypical nuclei in gastrointestinal cells and nuclear fragmentation in the retina and central nervous system. We have used a new, 3D form of histology based on micro-CT, X-ray histotomography, to reveal diminished heart volume and shape, and dysplastic cartilage and skin. We were also able to definitively establish the absence of a swim bladder and pneumatic duct, which is challenging to determine usig histology. Cell death shown by karyorrhexis was associated with DNA damage identified by ɣ-H2AX stainng in the brain, eyes, and spinal cord. These phenotypes were caused by a frameshift-based premature stop codon at the 38th amino acid position of the 600-amino acid Pola2 protein, or B subunit of DNA polymerase α (Pol α). An extended 120-168 hpf survival of the *hht* fish stands in striking contrast to the immediate cell cycle arrest of the corresponding mutants in yeast and *Arabidopsis.* We found that the prolonged lifespan of these mutants can be explained by the presence of wild-type maternal *pola2* mRNA in the fertilized embryos. The gradual disappearance of wild-type mRNA in homozygous mutant embryos appears to cause diminished DNA synthesis, increased DNA damage, cell death, and tissue-dependent cytological deformities. The breadth of cytological, tissue, and organ phenotypes of *hht* mutants can be used as a model for developing computational tools for quantitative 3D characterization of pleiotropy, including cytological and tissue phenotypes across organ systems.

2267B Identifying New Sleep Genes in *C. elegans* *Clarissa Nassar*[1], Cheryl Van Buskirk[1] 1) California State University, Northridge, Northridge, CA.

Although sleep is a behavior that all animals appear to engage in, its cellular function is unknown. Because sleep has been conserved throughout evolution, it can be studied in simpler organisms such as the roundworm *Caenorhabditis elegans* (*C. elegans*). Intriguingly, this worm sleeps when it experiences cellular damage from environmental stressors such as UV, heat, and bacterial toxins. This form of sleep is known as stress-induced sleep (SIS), and failure to engage in SIS following noxious exposure is associated with decreased survival. These observations suggest that cellular damage drives sleep need and that a key function of sleep is to repair cellular damage. In support of this notion, zebrafish show increased sleep after UV-induced DNA damage, and this sleep is associated with increased cellular repair. Thus, the identification of novel sleep genes in nematodes may help us to better understand the fundamental role of sleep in vertebrates. To identify additional sleep genes in *C. elegans*, our lab has undertaken a genetic screen for SIS-defective mutants. The goal of this project is to characterize one of these sleepless mutants and to identify the associated sleep gene, with the ultimate goal of uncovering a deeply conserved element of sleep regulation.

2268C Do Male *C. elegans* Sleep? *Kostantina Orselli*[1], Cheryl Van Buskirk [1] 1) California State University Northridge, Northridge, CA.

The nematode *Caenorhabditis elegans* (*C. elegans*) is a model organism ideal for the study of conserved behaviors, including sleep. Human sleep is regulated by circadian cues (sleep timing) and by homeostatic control (need-based regulation); however, sleep in *C. elegans* is not under circadian control, and thus ideal for the study of sleep need. Interestingly, these worms sleep following exposure to damaging conditions, a phenomenon known as stress-induced sleep (SIS), indicating damage causes sleep need. Populations of these animals consist mainly of hermaphrodites, with males arising rarely that are capable of mating with hermaphrodites. As a result, previous studies have overlooked male sleep behavior. To determine if sleep is sexually dimorphic in *C. elegans* we used a special male-enriched population to examine SIS. We found that males were sleepless. To determine if this sleeplessness was due to a competing drive to mate, we examined sleep in males that had been isolated from hermaphrodites, and we found that males were still sleepless. Our genetic analyses have narrowed down the possible mechanisms by which this difference arises. Our results have broader implications for the emerging understanding of behavioral plasticity in sleep regulation across species.

2269A Hunger drives behavioral changes through inter-tissue signaling in *C. elegans* *Molly Matty*[1], Anupama Singh[1, 2], Hiu Lau[1], Karina Kono[1], Zachary Cecere[1], Sreekanth Chalasani[1] 1) Salk Institute for Biological Studies, San Diego, CA; 2) Sanford Burnham Prebys Medical Discovery Institute, San Diego, CA.

While much is known about how external cues affect neural circuits, less is known about how internal states modify animal behavior and neuronal function. Here, we acutely food-deprive populations of *C. elegans* and analyze their responses in integrating attractant and repellent signals simultaneously. We show that food deprivation reversibly reduces repellent sensitivity, allowing animals to engage in a risky behavior. We show that a glucose-response element binding protein, MondoA, acts within intestinal cells to detect the lack of food. The intestine, in turn, relays this information using peptides released from dense core vesicles. These peptides are received by chemosensory neurons using the DAF-2 insulin receptor and downstream non-canonical insulin signaling. Together, these studies suggest that there is a link between metabolism, neuronal function, and animal behavior.

2277C dentification of mRNA targets of ETR-1/CELF that regulate Q neuroblast migration in muscle cells using fluorescence-activated cell sorting and RNA-seq *Matthew Ochs*[1], Rebecca McWhirter[2], David Miller[2], Erik Lundquist[1] 1) University of Kansas, Lawrence KS; 2) Vanderbilt University, Nashville Tennessee.

Migration of neuroblasts during the development of the nervous system is a critical process to ensure proper function of the nervous system. The Q neuroblasts are a great model to study migrating neuroblasts during development. The Q neuroblasts are bilaterally symmetrical, undergo

similar divisions to generate three functional neurons. QR is born on the right side of the animal and migrates towards the anterior. QL is born on the left side of the animal and migrates towards to posterior. Here we show that mutating the gene *etr-1* results in migration defects of Q neuroblast descendants, AQR and PQR, but not the initial migrations of QR and QL. *etr-1* encodes a CELF-type RNA binding protein and has been previously shown to be essential for proper muscle development. CELF-type proteins have been shown to regulate RNA processing such as alternative splicing in other systems. We show that *etr-1* acts non-autonomously in muscles to regulate the migration of AQR and PQR. We believe that ETR-1 is processing RNAs in the muscles to regulate the migrations of AQR and PQR. Since *etr-1* encodes an RNA binding protein we are interested in identifying potential targets of *etr-1*. To identify these targets we used fluorescent-activated cell sorting and RNA sequencing of body wall muscles from wild-type and *etr-1* animals. We identified potential targets of *etr-1,* some of which have been previously been shown to be involved in Q neuroblast migration. Candidate genes were identified and knocked down by RNAi to screen for AQR and PQR migration defects. Of the genes tested, RNAi knockdown of *unc-52* resulted in severe migration defects. We are interested in discovering how these candidate genes interact with *etr-1* in controlling the proper migrations of AQR and PQR.

2280C Identifying regulators of directed neuroblast migration in *Caenorhabditis elegans* *Vitoria Paolillo*[1], Erik Lundquist[1] 1) University of Kansas, Lawrence, Kansas.

Guided neuronal migration is an essential process during nervous system development. The Q cell neuroblasts in *Caenorhabditis elegans* provide a simple and experimentally tractable model system for studies of directed neuronal migration. The Q neuroblasts are born in the same region of the animal and have similar differentiation patterns, yet undergo left-right asymmetric migration, with QR on the right migrating anteriorly and QL on the left migrating posteriorly. QL descendants encounter a posterior EGL-20/Wnt signal, which activates a canonical Wnt signaling pathway to induce expression of the Hox gene *mab-5* in QL and QL descendants, but not in QR and QR descendants. MAB-5 is both necessary and sufficient for posterior Q cell descendant migration, as QL descendants migrate anteriorly in *mab-5* loss-of-function (LOF) mutants, and QR descendants migrate posteriorly in *mab-5* gain-of-function (GOF) mutants. However, it is unknown what genes are regulated by MAB-5 in the Q cells to drive posterior migration. We isolated Q cells from wild-type and *mab-5* LOF animals via fluorescence-activated cell sorting and completed RNA-seq to generate novel Q cell transcriptomes. We identified 222 genes that were differentially expressed in the *mab-5* LOF Q cells versus wild-type Q cells. We predict that MAB-5 might affect Q cell migration by regulating other transcription factors, RNA processing factors, the cytoskeleton, and cell signaling factors. Thus, we have prioritized candidate transcription factors for functional studies, and identified 13 putative transcription factors that show decreased expression in the *mab-5* LOF Q cells versus wild-type Q cells. We predict that these 13 transcription factors require MAB-5 for their expression, and thus may regulate posterior Q cell migration. Indeed, our preliminary functional studies have revealed that several of these putative transcription factors regulate Q cell migration. We anticipate that further functional studies of these candidate *mab-5* targets will reveal new insights into directed Q neuroblast migration.

2289C The role of the limk1 gene on short-term memory formation in *Drosophila melanogaster* *Ekaterina Zalomaeva*[1,2,3], Varvara Falina[1,2], Georgiy Andreev[1,2], Ekaterina Nikitina[1,2], Elena Savvateeva-Popova[1], Alexander Zhuravlyov[1] 1) Pavlov Institute of Physiology RAS; 2) The Herzen State Pedagogical University of Russia; 3) Saint Petersburg Institute of Bioregulation and Gerontology.

Nowadays one of the topical problem of neurobiology is the research of the etiology and progression of different neurodegenerative diseases. One of the causes of neurodegenerative diseases is disturbance of actin remodeling cascade whose key enzyme is LIMK1. *Drosophila* constitutes a convenient model for studying the link between genome organization and chromosome architecture observed in cognitive disorders. We analyzed the formation and dynamics of short-term memory in *D. melanogaster* stocks with *limk1* gene polymorphism: *Canton-S (CS), Berlin (Ber)* and *Oregon-R (Or-R)*. *CS* stock hasn't any defects in the gene structure. *Ber* stock characterized by disturbances in primer binding in intron 2 and near introns 3 and 4. *Or-R* stock hasn't fragment between primers are limited the region with both LIM-domains and part of the PDZ-domain. The revealed association between *limk1* gene's mutational damage, changes in its expression and activity as well as cognitive impairment allows to use this model for the study of neurodegenerative and genomic diseases. Conditioned courtship suppression paradigm was used to asses learning ability and short-term memory formation. A 5-day-old virgin male was put in a special box with a fertilized female *CS* and was left for 30 minutes. To assess the formation and dynamics of short-term memory, courtship behavior was analyzed in naive males and in males after 0, 15, 30 and 60 minutes after training. To assess the effectiveness of training was calculated the learning index (LI). Randomization test was used to statistical analysis. Research demonstrated that LI *CS* and *Ber* stocks on all points after training significantly different from zero, but not different from each other within the same stock. The LI *CS* stock was significantly greater than LI *Ber* after 15 and 60 minutes after training ($p \leq 0,05$). The LI *Or-R* stock on all points after training are not different from zero and from each other, but was significantly less than LI *CS* at similar points($p \leq 0,05$). Also, LI *Or-R* was significantly less than LI *Ber* after 30 minutes after training ($p \leq 0,05$). Thus, *CS* and *Ber* stocks capable of formation short-term memory and to preserve it, while *Or-R* incapable of learning and formation short-term memory. Polymorphisms in the *limk1* gene apparently contribute to changes in the content and ratio of *limk1* isoforms in the analyzed stocks, and that influence in processes of learning and formation short-term memory.

2290A Single-cell transcriptional responses to cocaine exposure in the Drosophila brain *Sneha Mokashi*[1], Vijay Shankar[1], Brandon Baker[1], Rachel Hannah[1], Robert Anholt[1], Trudy Mackay[1] 1) Clemson university, Clemson, SC.

Previous studies identified polymorphisms in candidate genes associated with variation in consumption of cocaine among lines of the Drosophila Genetic Reference Panel, and RNAi-mediated targeted gene disruption implicated dopaminergic projections to the mushroom bodies. To identify specific cell populations that respond to acute cocaine exposure, we analyzed single-cell transcriptional responses in duplicate samples of flies that consumed fixed amounts of sucrose or sucrose supplemented with cocaine, sexes separately. After exposure, 20 brains for each sample were dissected, pooled and dissociated. Cells were separated and lysed, and cDNA was synthesized using Chromium 10x microfluidics followed by sequencing on an Illumina Novaseq. The integration of all eight samples distributed across sexes, conditions and replicates resulted in a dataset of 86,224 cells. Unsupervised clustering of this population yielded 36 distinct clusters. Annotation of clusters based on their gene markers revealed that all major cell types (neuronal and glial), as well as neurotransmitter types from most brain regions, were represented (including the optic lobe and the mushroom body). Differential expression analysis within individual clusters indicated cluster-specific responses to cocaine. Specifically, clusters corresponding to glia, T1 and T4/T5 neurons of the optic lobe, Kenyon cells, and photoreceptor cells showed dramatic transcriptional responses following cocaine exposure. Some clusters also showed significantly divergent responses across the sexes. Additionally, transcriptional responses to cocaine in most clusters were considerably more pronounced in male than in female brains. Thus, cocaine exposure elicits sexually dimorphic transcriptional responses in both glia and neurons in multiple compartments of the Drosophila brain. Supported by1U01-DA041613.

2292C Neural and Genetic Mechanisms of Cold Sensation in *Drosophila Melanogaster* *L. Amanda Xu*[1,5], Elizabeth A. Ronan[1,2], Limei Zhu[1], Gun-Ho Kim[3], Bing Ye[1,4] 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA; 2) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA; 3) Department of Mechanical Engineering, Ulsan National Institute of Science and Technology, South Korea; 4) Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA; 5) Huron High School, Ann Arbor, MI, USA.

All animals must sense temperature in order to survive. Temperature cues are detected in the nervous system by sensory neurons, and the basics of these mechanisms are likely conserved from invertebrates to humans. The ability for sensory neurons to sense temperature depends on genes that encode temperature receptors. While heat sensation has been extensively studied, much less is known about cold sensing, due to the lack of technologies that can lower temperatures rapidly and precisely. In order to address this issue, we have engineered devices that deliver cold stimuli in a rapid and precise manner without mechanosensory input. Here we report that the larvae of *Drosophila melanogaster* exhibit several behavioral phenotypes in response to cold temperatures, independent of known cold sensors. Our results show that *Drosophila* larvae sense and respond to cold temperatures through previously uncharacterized neural and genetic pathways which may be evolutionarily conserved in higher organisms.

2315B Functional assessment of *de novo* missense variants associated with Autism Spectrum Disorders through an overexpression-based screen in *Drosophila* *Jacob Harnish*[1], Samantha Deal[1], Paul Marcogliese[1], Jonathan Andrews[1], Brooke Hull[1], Hillary Graves[1], Sharayu Jangam[1], Hemanjani Bhavana[1], Michael Wangler[1,2], Shinya Yamamoto[1,2] 1) Baylor College of Medicine, Houston, TX; 2) Neurological Research Institute-Texas Children's Hospital, Houston, TX.

Whole-exome sequencing (WES) is becoming less expensive, more widespread tool for researching human diseases. WES identifies hundreds of rare variants in an individual's genome that must be interpreted by geneticists and clinicians. However, current *in silico* tools are insufficient to predict the pathogenicity of many missense variants. This becomes especially problematic when undertaking large sequencing efforts to uncover rare variants that contribute to the pathology of more common conditions. For example, numerous recent efforts have uncovered rare gene variants associated with intellectual/developmental disabilities and Autism Spectrum Disorder (ASD). Thousands of these are *de novo* missense variants that are of unknown significance. Here, we utilize an overexpression-based screen in *Drosophila* to investigate several such *de novo* missense variants identified in the Simons Simplex Collection (SSC), a large cohort of over 2,500 ASD simplex families. Using this method, we prioritized 79 genes and generated UAS-human cDNA transgenic lines for exogenous expression of reference or variant alleles in fly. By expressing these transgenes using ubiquitous (Tubulin-), wing (nubbin-) and eye (GMR-) GAL4 drivers, we found phenotypic differences in 25% of cases (n=20). Interestingly, experiments overexpressing human orthologs of essential fly genes identified 7 loss-of-function (LOF) alleles (total n=45), while overexpressing human orthologs of non-essential fly genes revealed 4 LOF, 8 gain-of-function (GOF) and 2 complex alleles (total n=33). We are further focusing on a strong GOF variant in the gene that encodes Pyruvate Carboxylase and addressing how this variant affects protein function or abundance to assess a possible link between ASD and metabolism. Overexpression screens using human cDNA transgenic *Drosophila* strains serve as a high-throughput, multidimensional, *in vivo* assay to rapidly assess the potential functional impact of rare variants identified in ASD and other human disease patients.

2318B A Comprehensive Analysis of SWI/SNF Complex Function in *Drosophila melanogaster* as model for Coffin-Siris Syndrome. *Scott Barish*[1,2], Alfredo Valencia[3], Nazar Mashtalir[3], Cigall Kadoch[3], Daryl Scott[1], Michael Wangler[1,2,4], Shinya Yamamoto[1,2,4,5], Hugo Bellen[1,2,4,5,6] 1) Baylor College of Medicine, Houston, TX 77030, USA; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX 77030, USA; 3) Pediatrics, Harvard Medical School, Cambridge, MA 02115, USA; 4) Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, USA; 5) Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA; 6) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA.

Coffin-Siris syndrome (CSS) is a rare but severe neurodevelopmental disorder that is characterized by developmental disability, hypoplasia of the fifth digit, coarse facial features, and a variety of other rarer symptoms. Mutations in the members of the SWI/SNF chromatin remodeling complex are thought to cause CSS, although there is little to no functional data characterizing the patient variants. Here we characterize the expression and function of several members of the SWI/SNF complex as well as analyze the impact of patient variants on protein function. We show that the majority of SWI/SNF proteins are broadly expressed within the nervous system with Snr1 and Polybromo having much more restricted expression domains. We demonstrate that the non-canonical BAF (Brahma Associated Factors) complex controls position effect variegation (PEV) at telomeres while the PBAF (PolybromoBAF) and BAF complexes control PEV in gene rich regions. We also observe a range of phenotypes upon knockdown of SWI/SNF genes in both the wing and eye, suggesting that individual complex members may have more functional subdivision that was previously understood. Finally, we show that variants associated with CSS in *SMARCB1* display strong loss-of-function phenotypes, whereas variants associated with less severe developmental disorders have weak loss-of-function phenotypes in the fly. Together, we present a broad-based analysis of SWI/SNF complex function and highlight the ability to understand how chromatin remodeling factors contribute to disease in the fly.

2319C Developmental switching of nicotinic acetylcholine receptor subunits supports central cholinergic synapse maturation *Justin Rosenthal*[1,2], Jun Yin[1], Anupama Sathyamurthy[1], Chengyu Sheng[1], Emma Spillman[1], Quan Yuan[1] 1) National Institutes of Health. Bethesda, MD; 2) University of Maryland. College Park, MD.

Developing neural circuits employ cell-type-specific mechanisms to establish unique connectivity patterns and mediate experience-dependent modifications. In particular, neurotransmitter receptor signaling strongly influences synapse formation, maturation and maintenance and has been investigated extensively in the excitatory glutamatergic synapse of the vertebrate central nervous system (CNS), where the ratio of AMPA/NMDA receptors regulates synapse strength and contributes to the physiological maturation of the glutamatergic synapse. In contrast, the majority of excitatory synapses in the Drosophila CNS use acetylcholine as the neurotransmitter, where the molecular composition of the postsynaptic acetylcholine receptor (AchR) complex remains unknown. To understand how synapse development and plasticity is regulated by neurotransmission in the fly central cholinergic synapse, we study the ventral lateral neurons (LNvs) in the *Drosophila* larval visual circuit, where LNvs receive excitatory cholinergic input from presynaptic photoreceptors. Using RNA-seq analyses, morphological screens and calcium imaging studies, we identify *Drosophila* nAchRa1 (Dα1) and nAchRa6 (Dα6) as the main functional nAchR subunits in LNvs and demonstrate their distinct roles in supporting synaptic transmission and dendrite morphogenesis. Furthermore, our analyses reveal a transcriptional upregulation of Dα1 and downregulation of Dα6 during larval development, indicating an association between the developmental substitution of nAchR subunits and the maturation of the central cholinergic synapse. Taken together, our study supports the transcriptional control of neurotransmitter receptor expression as a core element of developmental and activity-dependent regulation of synapse development in both vertebrate and invertebrate systems.

2345B Robust olfactory responses in the absence of odorant binding proteins *Shuke Xiao*[1], Jennifer Sun[1], John Carlson[1] 1) Department of Molecular, Cellular, and Developmental Biology, Yale University.

Odorant binding proteins (Obps) are expressed at extremely high levels in the antennae of insects, and have long been believed essential for carrying hydrophobic odorants to odor receptors. Previously we found that when one functional type of olfactory sensillum in Drosophila was depleted of its sole abundant Obp, it retained a robust olfactory response (Larter et al., 2016). Here we have deleted all the Obp genes that are abundantly expressed in the antennal basiconic sensilla. All of six tested sensillum types responded robustly to odors of widely diverse chemical or temporal structure. One mutant gave a greater physiological and behavioral response to an odorant that affects oviposition. Our results support a model in which many sensilla can respond to odorants in the absence of Obps, and many Obps are not essential for olfactory response, but that some Obps can modulate olfactory physiology and the behavior that it drives

2352C Parasitoids and predators: How *Drosophila* mothers assess and respond to hymenopteran threats *Shaun Davis*[1], Gregory Chism[2], Anna Dornhaus[2], Todd Schlenke[1] 1) Department of Entomology, University of Arizona, Tucson, AZ; 2) Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ.

Organismal fitness depends on the correct recognition of environmental threats. A major threat to juvenile Drosophila in nature are Hymenopterans. For example, parasitoid wasps lay their eggs inside fly larvae, and once hatched, the wasp larvae grow inside the fly larvae and eventually consume the flies from the inside out. Adult flies are not themselves attacked, but when females recognize wasp presence they alter their behavior by reducing oviposition, which reduces the potential for offspring infection. We are interested in how flies are able to detect these environmental threats. Blind and anosmic flies both fail to reduce oviposition, suggesting multimodal integration is necessary for the correct behavioral response. At the visual level, we found that the motion vision is required, and that a group of object detecting lobular columnar 11 (LC11) neurons are partially responsible for recognizing wasps and reducing oviposition. We are currently determining what other visual neurons mediate wasp detection and how the visual circuits interact with the olfactory system to regulate oviposition rates. In addition to parasitization, Drosophila eggs are also preyed on by ants. We found that when adult female flies recognize ant presence they alter their oviposition behavior in a different way, by burying their eggs more deeply in the food substrate, which results in dramatically reduced egg predation. This oviposition depth behavior is regulated by olfactory stimuli only. We are currently using gas chromatography and genetic tools to determine the odor compounds and fly odorant receptors responsible for ant detection. Whether there is any overlap in the neural circuitry responsible for wasp versus ant detection, and for oviposition rate versus oviposition depth behaviors, is the subject of ongoing work.

2355C Identification of a genetic locus and environmental factors influencing initial cocaine sensitivity in C3H substrains *Christiann Gaines*[1], Sarah Schoenrock[1], Colton Linnertz[1], Ian Carroll[1], Martin Ferris[1], Fernando Pardo-Manuel de Villena[1], Lisa Tarantino[1] 1) University of North Carolina at Chapel Hill.

Quantitative trait locus (QTL) mapping in inbred mice has been used for decades to identify genetic loci contributing to complex traits. These studies have been extremely successful in identifying regions of the genome associated with drug response and reward. However, the transition from QTL to quantitative trait genes (QTGs) and causal polymorphisms has been less successful due to issues with low mapping resolution and the presence of numerous polymorphisms among the more commonly used and divergent inbred mouse strains. More recently, the identification of polymorphic single nucleotide polymorphisms in inbred mouse substrains and tools for efficiently assessing substrain genotypes has opened up a new avenue for rapid identification of QTGs. In order to take advantage of these new tools, one needs only to identify phenotypic differences in inbred mouse substrains and conduct QTL mapping. Genetic complexity in substrain crosses is greatly reduced, limiting the number of causal loci (usually one) and the number of polymorphisms that must be interrogated at the identified QTL. We have identified a significant difference in cocaine-induced locomotor activation in C3H/HeJ and C3H/HeNTac substrains. We will present mapping data from an F2 cross between these substrains that identifies a locus on Chr 19. We will describe our use of whole-genome sequencing and phenotype data from two additional C3H substrains to identify the most likely causal polymorphism. We will also discuss potential environmental factors that could influence behavioral differences in these two substrains including maternal effects and differences in the gut microbiome.

2360B Does atypical cadherin CELSR1 suppress Wnt-mediated chemoattraction of branchiomotor neurons? *Devynn Hummel*[1], Derrick Glasco[1,2], Whitney Pike[1], Anand Chandrasekhar[1] 1) University of Missouri - Columbia MO; 2) Bob Jones University - Greenville SC .

During development of the central nervous system, differentiating neurons often migrate extensively prior to forming the circuits vital to cognitive and motor function. Within the vertebrate hindbrain, Facial Branchiomotor (FBM) neurons migrate caudally from rhombomere 4 (r4) to r6 to assemble the circuits that drive feeding and respiratory behaviors required for survival. Many components of the Wnt/PCP (Planar Cell Polarity) pathway are needed to initiate FBM neuron migration, however, much less is known about the mechanisms regulating the direction of migration.

Our lab discovered that in mice lacking the Wnt/PCP component CELSR1, many FBM neurons inappropriately migrate rostrally suggesting that *CELSR1* regulates the directionality of migration. Tissue-specific knockout of *Celsr1* in r3 and r5 was sufficient to generate the rostral migration phenotype, suggesting that the phenotype is due to the loss of a local chemotactic cue. Intriguingly, *Celsr1* and a known chemoattractant gene, *Wnt5a*, are expressed in overlapping domains along the midline rostral to r4. Furthermore, the rostral migration phenotype observed in *Celsr1*-deficient animals is suppressed upon deletion of *Dvl2* demonstrating that rostral migration of FBM neurons is dependent on *Dvl2*, and possibly *Wnt,* function. Based on these findings, we hypothesize that *Celsr1* suppresses WNT5A activity within r3 to block rostral migration. In this model, WNT5A activity is not suppressed in *Celsr1* mutants, and as a result, FBM neurons move towards the rostral source of WNT5A.

We are currently testing three predictions of this model. First, we are testing whether ectopic migration of FBM neurons towards WNT5A-coated beads is dependent on *Dvl2* function. Second, we will determine whether FBM neurons fail to migrate rostrally in *Celsr1 Wnt5a* double mutants, as this would indicate that the rostral migration of FBM neurons in *Celsr1* mutants is a result of chemoattraction towards *Wnt5a*-expressing cells in the rostral hindbrain. Third, we will overexpress *Wnt5a* in r3 of *Celsr1* mutants to determine whether the rostral migration of FBM neurons seen in mutants could be greatly enhanced by excess WNT5A. These proposed studies will provide critical insight into a novel role for *Celsr1* functioning as a chemotactic cue, rather than as a Wnt/PCP component, during FBM neuron migration.

2378B Neuroepigenetic regulation of a tunable behavioral circuit *Joy Meserve*[1], Michael Granato[1] 1) University of Pennsylvania.

Our ability to learn relies on neural circuits being flexible and adaptive. At the same time, neurons need to maintain essential synaptic connections throughout the entire lifespan of an organism, striking a fine balance between flexibility and stability. One mechanism by which cells achieve this balance is through chromatin mediated transcriptional regulation, ensuring stable, long term gene expression while allowing transcriptional flexibility. Mutations in chromatin regulators are predicted to disrupt the balance of neuronal flexibility and stability and indeed have been identified in patients with neurological and neuropsychiatric disorders. Exactly how these mutations affect gene expression and ultimately cause disease is not well understood. We have recently begun to investigate this question using the larval zebrafish. Larval zebrafish exhibit a variety of quantifiable behaviors, including the acoustic startle response, which is modified by experience and controlled by relatively small sets of neurons. Importantly, regulation of the startle response is disrupted in several neuropsychiatric disorders, making this an ideal model to understand the role of chromatin modifications in regulating behaviors. We are taking two parallel approaches to investigate chromatin regulation of behavior. First, we will examine how disruption of chromatin modifiers affects startle circuit development and, in turn, startle behavior. We will use both an epigenetic drug library and targeted mutations in chromatin modifiers associated with neurological disorders to screen for defects in the startle response. Secondly, we aim to understand how open chromatin and histone marks change in neurons during development and how these changes relate to gene expression, morphological changes, connectivity, and neuron activity. We will isolate specific neuron populations at different points in their development and profile chromatin and gene expression using high-throughput sequencing techniques. Ultimately, we aspire to combine our two approaches to understand how disruption of chromatin regulators disrupts specific chromatin sites, leading to changes in gene expression, neuron development, circuit function, and ultimately, behavior.

2382C Using Zebrafish as a Model System for Studying the Autism Risk Gene *ADNP* *William Theune*[1], Carter Takacs[1] 1) University of New Haven.

Autism Spectrum Disorders (ASD) are a classification of developmental disorders which are characterized by intellectual disability, difficulty with social interaction, and impairment in verbal and nonverbal communication. ASD affects as many as 1 in every 59 children worldwide and is an incredibly complex and genetically diverse group of disorders.
One of the most common forms of ASD is associated with *de novo* mutations in the *adnp* gene (Activity-dependent neuroprotector homeobox; accounting for 0.17% of ASD individuals). Termed ADNP syndrome, this disorder is characterized by intellectual disability, facial dysmorphia, and congenital heart defects. A clinical study of patients with ADNP syndrome found that frame-shift mutations in *adnp* lead to a loss of the C-terminus of the protein, believed to be responsible for recruiting components of the BAF chromatin remodeling complex.
We set out to use zebrafish as a model organism to gain mechanistic insights into ADNP function. Zebrafish have two paralogs of *adnp*; *adnpa* and *adnpb*. Our work suggests that ADNPA/B may play a role in cardiac, as well as, brain development. Specifically, CRISPR/Cas9-mediated knockout of *adnpa* leads to reduced blood flow and the development of cardiac edema at 48 hours-post fertilization(hpf). Approximately 24% of embryos within single clutches of *adnpa* F1 embryos exhibit this phenotype in addition to displaying a reduction in head size and severe deficiencies in motor function and sensory responses. *Adnpb* F0 embryos display a similar phenotype beginning at 24 hpf. At 6 days post-fertilization all affected embryos develop fatal hemorrhaging around the heart. These findings are consistent with the prevalence of cardiac deficits observed in patients with ADNP syndrome. Future work aims to determine the affected genetic regulatory pathways that underlie cardiac and neuronal phenotypes observed in the zebrafish mutant line.

Monday, April 27 12:00 PM - 3:00 PM

New Technology and Resources/Genomics and Systems Biology 1 - Poster Q&A 504C Upgraded tools for tissue-specific mutagenesis by CRISPR/Cas9 in *Drosophila* *Gabriel Koreman*[1], Yineng Xu[1], Bei Wang[1], Chun Han[1] 1) Weill Institute for Cell and Molecular Biology, Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

CRISPR/Cas9 is a powerful tool with the potential to remarkably advance genetic analysis. We previously developed CRISPR-TRiM (CRISPR-mediated tissue restricted mutagenesis), a system for efficient tissue-specific mutagenesis in *Drosophila*. Here, we report new tools that significantly enhance the CRISPR-TRiM system. First, we further improved the design of tRNA-based multiplexed gRNAs by optimizing tRNA choices and the gRNA scaffold, resulting in even more efficient transgenic gRNAs. Second, we developed new gRNA vectors to genetically label mutant cells based on co-CRISPR of parallel reporters. We demonstrate that this parallel labeling system is effective for epithelial cells when proper Cas9 lines are used. Lastly, we generated transgenic flies for converting existing Gal4 drivers into tissue-specific Cas9 lines using homology assisted CRISPR knock-in (HACK). This method opens the door for CRISPR-TRiM analysis in diverse tissues and broader developmental stages. Overall, these improvements have made CRISPR-TRiM a more versatile, reliable, and effective tool in *Drosophila* research.

506B Drosophila Genomics Resource Center: Generation of cell lines with attP sites and serum-free insect cell culture *Daniel Mariyappa*[1], Arthur Luhur[1], Kris Klueg[1], Danielle Overton[1], Johnny Roberts[1], Andrew Zelhof[1] 1) DGRC, Indiana University, Bloomington, IN .

Here we report new resources for the research community. Several *Drosophila* lines with attP sites are now available for researchers to generate transgenic flies. Nevertheless, replicating *in vivo* experiments in *Drosophila* cell lines currently is limited to transient transfection with the gene of interest. To aid a more controlled experimental setup, we are creating clonal *Drosophila* cell lines with attP integration sites using CRISPR/Cas9 gene editing. The insert has attP sites flanking Act::dsRed, thus enabling the positive selection of cells with attP sites. The genomic loci 25C6 (2L) and 99F8 (3R) were chosen based on: 1. The transgenic efficiency in flies, 2. non-coding loci and 3. the usage of the loci amongst researchers. We will present data outlining the creation and verification of the attP sites at these loci in S2-DGRC, Kc167, DmBG2-c2 and DmBG2-c2 cell lines. To test the functionality of the inserted attP sites, we created two GFP expressing constructs flanked by attB and successful cassette exchange was achieved. In the long-term, we aim to generate S2R+, S2-DGRC, Kc167, DmBG2-c2, DmBG3-c2 and OSS cell lines carrying the attP>>Act::dsRed<
Historically, the inability to generate large amounts of insect hemolymph led researchers to supplement insect cell culture media with fetal bovine serum (FBS). While *Drosophila* cells grow well with FBS supplemented culture media, it is not physiological, is expensive and prone to batch variations. To circumvent these issues, we investigated the possibility of serum-free *Drosophila* cell culture by supplementing the media with fly extract (FEX, an extract from Oregon R adult flies). We have now successfully adapted Drosophila cells to grow in the absence of FBS and will report our results on adapting *Drosophila* cells to serum-free growth conditions, their freeze-thaw conditions and suitability for transient transfections, in con-

junction with transcriptomic and metabolic analyses. These experiments will help characterize the serum-free cultured cells enabling researchers to further utilize them.

510C The *Drosophila* Research and Screening Center-Biomedical Technology Research Resource (DRSC-BTRR) at the DRSC/TRiP *Stephanie Mohr*[1], Yanhui Hu[1], Jonathan Zirin[1], Raghuvir Viswanatha[1], Shannon Knight[1], Jonathan Rodiger[1], Enzo Mameli[1], Justin Bosch[1], Jun Xu[1], Aram Comjean[1], Ben Ewen-Campen[1], Baolong Xia[1], Ah-Ram Kim[1], Ryan Colbeth[1], Cooper Cavers[1], Gabriel Birchack[1], Grace Zhang[1], Emily Stoneburner[1], Norbert Perrimon[1] 1) Harvard Med Sch.

The *Drosophila* RNAi Screening Center and Transgenic RNAi Project (DRSC/TRiP)-Functional Genomics Resources is an established platform for high-throughput screening in *Drosophila* cultured cells, fly stock production, and bioinformatics resources for the community. We recently founded the NIH NIGMS P41-funded *Drosophila* Research and Screening Center-Biomedical Technology Research Resource (DRSC-BTRR). The goal of the DRSC-BTRR is to develop new technologies, including in the area of CRISPR/Cas-based perturbation in *Drosophila* cells or *in vivo*. DRSC-BTRR technologies are developed in close partnership with collaborating laboratories engaged in specific biomedical research projects that can benefit from technology innovation by our group. Here, we will present an overview of the DRSC-BTRR and associated technologies and projects, including how the DRSC-BTRR enrolls and interacts with collaborating labs. In addition, we will present on our development of pooled CRISPR knockout screening for mosquito cells, including single guide RNA design, and present new CRISPR technologies for *Drosophila* cell-based and *in vivo* studies.

566B *Caenorhabditis* nematodes, population suppression, and gene drives: an emerging story *Stephen Von Stetina*[1], John Min[1], Alex Hong[1], Kevin Esvelt[1] 1) Media Lab, MIT.

CRISPR-mediated genetic engineering has the potential to help humanity in a multitude of ways, such as improved gene therapies, the ability to easily mimic disease-causing mutations in model organisms, and the potential to reduce vector-borne diseases or invasive species. Our group is proposing to encode a CRISPR system in the male germline that will target a gene essential to XX carrying embryos but has no effect on XY progeny, inducing a decline in total population size (see abstract by Kamau et al, this meeting, for rationale). Before releasing such a system in the wild, however, we need to ensure that this genetic control method will: 1. Work over a short timescale to effectively reduce a local population density; 2. Be stable over multiple generations; and 3. Does not induce any unintended alterations. We aim to test this sex-biasing population control method in *Caenorhabiditis* nematodes. Jonathan Hodgkin (2002) developed an "XY" system of sex-determination in *C. elegans*, allowing us to model XY-based population suppression. We will make use of the *peel-1/zeel-1* toxin/anti-toxin system (Seidel et al 2011). "Daughterless" males will carry both *peel-1* and *zeel-1* while females will have neither. Upon mating, the toxin will be delivered by sperm to all the oocytes, but only the resulting male embryos will express the antitoxin and thus survive embryogenesis. The males and females will also express specific fluorescent proteins, allowing us to use fluorescence as a means of track the ratio of males to females over many generations. However, this type of a genetic system requires continued release of "daughterless" males in order to keep the population suppressed. A more powerful system would use a CRISPR-gene drive. When a drive-carrying organism mates with a wild-type, super-Mendelian inheritance is observed, as all the progeny become homozygous CRISPR-carrying organisms. However, with nothing to stop CRISPR-activity, the potential exists for every organism within a species to become edited, a very undesirable outcome. The Sculpting Evolution group is dedicated to solving this problem by inventing and testing self-exhausting gene drives. We have dubbed one form of self-exhausting drive a daisy drive (Noble et al, 2019), as the elements required to drive are separated into modules (example: parts A, B, C) that depend on each other for complete drive. Part C drives B which drives A, but nothing drives C. This means that when C is diluted out, B is no longer driven. When B is diluted out, A is no longer driven and the gene drive stops. I will also discuss our efforts to model such a gene drive in *Caenorhabiditis* nematodes, including testing an alternative nuclease that is reported to work at lower (e.g. worm-rearing) temperatures, Lba Cas12a (Zetsche et al 2015).

580A Perturbing proteomes at single residue resolution using base editing *Philippe Després*[1,2,3], Alexandre K Dubé[1,2,3,4], Motoaki Seki[5,6,7], Nozomu Yachie[5,6,7], Christian Landry[1,2,3,4] 1) Département de Biochimie, Microbiologie et Bio-Informatique, Université Laval, Canada; 2) PROTÉO, Université Laval, Canada; 3) Centre de Recherche en Donnés Massives (CRDM), Université Laval, Canada; 4) Département de Biologie, Université Laval, Canada; 5) Research Center for Advanced Science and Technology, Synthetic Biology Division, University of Tokyo, Japan; 6) Department of Biological Sciences, Graduate School of Science, the University of Tokyo, Japan; 7) Institute for Advanced Biosciences, Keio University, Japan.

Base editors derived from CRISPR-Cas9 systems and DNA editing enzymes offer an unprecedented opportunity for the precise modification of genes, but have yet to be used at a genome-scale throughput. Here, we test the ability of an editor based on a cytidine deaminase, the Target-AID base editor, to systematically modify genes genome-wide using the set of yeast essential genes. We tested the effect of mutating around 17,000 individual sites in parallel across more than 1,500 genes in a single experiment. We identified over 1,100 sites at which mutations have a significant impact on fitness. Using previously determined and preferred Target-AID mutational outcomes, we predicted the protein variants caused by each of these gRNAs. We found that gRNAs with significant effects on fitness are enriched in variants predicted to be deleterious by independent methods based on site conservation and predicted protein destabilization. Finally, we identify key features to design effective gRNAs in the context of base editing. Our results show that base editing is a powerful tool to identify key amino acid residues at the scale of proteomes.

590B The establishment and characterization of a zebrafish genetic mosaic system for phenotypic analysis of gene functions at single-cell resolution *Bing Xu*[1], Sarah Kucenas[2], Hui Zong[1] 1) Department of Microbiology, Immunology, and Cancer Biology, University of Virginia, Charlottesville, VA; 2) Department of Biology, University of Virginia, Charlottesville, VA.

Conditional knockout animal model is developed to study gene functions in a specific cell type. However, field of mutant cells prevent one from studying interactions between mutant and WT cells. To achieve gene inactivation in rare cells, genetic mosaic animal models, including mosaic analysis with a repressible cellular marker (MARCM) in Drospholia, mosaic analysis with double markers (MADM) in mouse, were developed. Through Flp/FRT or Cre/loxP mediated inter-chromosomal mitotic recombination, sporadic mutant cells can be generated and unequivocally labeled by fluorescent protein, allowing phenotypic analysis at single-cell resolution. Although these models led to many groundbreaking discoveries, dynamic analysis of mutant and wild type cells behavior in vivo is still difficult due to the non-transparent nature of these model organisms. Here, we report the establishment of a zebrafish Mosaic Analysis with Double Markers (zMADM) system, to take advantage of the resolution provided by MADM and embryonic transparency of zebrafish. The two zMADM cassettes were knocked into the pre-selected genomic region with CRISPR/Cas9, respectively, to create two stock lines. After breeding two lines, the injection of Cre mRNA or plasmid produced MADM-labeled cells, confirming the successful establishment of the zMADM system. We quantified fluorescently labeled cells and determined that the labeling efficiency with

Cre mRNA in zMADM was ~0.5%. To demonstrate the application of zMADM system, we showed the Cre controlled by cell type-specific promoters can sparsely label those cell types faithfully. Next, we used live imaging to witness the birth of two sibling cells, and traced the development and migration of them in zMADM zebrafish. In particular, we discovered varied symmetric and asymmetric cell division patterns during the formation of optic tectum neuronal column. Finally, we showed that the labeling of zMADM reveals fine processes of neurons, validating the power of zMADM for morphogenesis studies at single-cell level. In summary, we have successfully established and characterized zMADM system, which can be used to study genetic determinants of lineage development in vivo, and to model human diseases for both basic and translational studies.

592A Generation of large genomic deletions to remove zebrafish *rca2.1* *Chon-Hwa Tsai-Morris*[1], Gennady Margolin[1], Emily Katz[1], Ryan Dale[1], Daniel Chauss[2], Claudia Kemper[3], Ben Afzali[2], Benjamin Feldman[1] 1) NIH/NICHD; 2) NIH/NIDDK; 3) NIH/NHLBI.

CD-46 plays a crucial role in the human immune system. We wish to illuminate the role of zebrafish *rca2.1*, a CD-46 ortholog, in development and health. The sequence and expression of *rca2.1* suggests it is a better orthologue to human CD-46 than murine candidate genes. Located on chromosome 23, *rca2.1* spans 25 kb and the latest genome assembly predicts three alternative splice forms. Our initial goal is to generate *rca2.1* null zebrafish using CRISPR/Cas9. Based on concerns that novel translational start sites and/or decay of otherwise non-functional RNAs can elicit genetic compensation, we sought to disrupt all *rca2.1* transcription. To identify the transcriptional start site (TSS) and to determine which predicted alternate transcripts are expressed during early development, we established a private UCSC track featuring valuable CAGE and RNAseq data from public sources. We used this track to select gRNA combinations aimed at cooperatively deleting the TSS target and downstream spans of *rca2.1*. Each candidate gRNA was pre-tested for cutting efficiency. Fertilized embryos were then co-injected with either (#1) three gRNAs targeting the TSS, start codon and termination codon, or (#2) two gRNAs targeting the TSS and termination codon. With the intention of stimulating precise joining of the deletion gap, a 100 nt oligonucleotide was included in the second co-injection, with hybrid sequence from upstream of the 5' cut site and downstream of the 3' cut site. Fragment analysis indicated the presence of diagnostic peaks arising from the desired 20 Kb deletion in close to 20% of F_0 embryos injected by either approach. Germ-line transmission from 7 out of 8 F_0 adults representing both approaches has subsequently been achieved. Thus, using multiple gRNAs with or without a facilitating oligo, we have efficiently recovered large genomic *rca2.1* deletion alleles. Unlike classic CRISPR/Cas9 in-del alleles, interpretation of phenotypes observed should be uncomplicated by concerns of extant or novel alternative transcripts or genetic compensation linked to nonsense-mediated decay.

628A It's about time: a secure web-based multi-omics method to uncover complex regulatory mechanisms over time *Ashley Conard*[1,2], Nathaniel Goodman[1, 2], Claire Hu[3,5], Norbert Perrimon[3,4], Charles Lawrence[1,6], Erica Larschan[1,7] 1) Center for Computational and Molecular Biology, Brown University, Providence, RI; 2) Computer Science Department, Brown University, Providence, RI; 3) Department of Genetics, Harvard Medical School, Boston, MA; 4) Howard Hughes Medical Institute, Harvard Medical School, Boston, MA; 5) Director of Bioinformatics DRSC/TRiP Functional Genomics Resources, Harvard Medical School, Boston, MA; 6) Applied Math Department, Brown University, Providence, RI; 7) Department of Molecular Biology, Cellular Biology and Biochemistry, Brown University, Providence, RI.

Uncovering how transcription factors (TFs) and their targets communicate at the DNA, RNA and protein levels is critical to understand signaling cascades in normal and pathogenic cells. RNA-seq has become the *de facto*standard to detect gene regulatory networks (GRNs) using an established set of analysis steps. Although RNA-seq analysis pipeline methods comparing two conditions have been studied, methods for interpreting ordered data (in time or space) are still in their infancy and are essential to assign cause and effect relations. Most current pipeline methods treat order as a categorical variable, thus disregarding the ordered relationship between genes, and thus not accurately reconstructing GRNs. Moreover, RNA-seq data do not provide direct evidence of interaction. Thus, methods integrating ordered RNA-seq and ChIP-seq data are urgently needed. To date there is no accessible and adaptive time course multi-omics pipeline method supporting reproducibility.
Here we present TIMEOR: Trajectory Inference and Mechanism Exploration using Omics data in R to fill this gap. This is a secure interactive web and command line time course multi-omics pipeline method for differential gene and isoform expression (DE). It takes raw .fastq files and performs all analysis from quality control and DE to GRN reconstruction. TIMEOR has five unique features: 1) adaptive default analysis methods given an experimental design; 2) multiple method comparisons for alignment and DE (for distant and close timepoint); and 3) statistical, graphical and interactive results for data exploration. Within each cluster, 4) TIMEOR performs automated gene enrichment, pathway, network and motif analysis, and optional ChIP-seq analysis for binding and epistasis relations. Lastly, merging gene networks, time course RNA-seq and ChIP-seq data 5) TIMEOR reconstructs GRNs with directed edges by labeling the interaction type between genes and gene products.
We validated TIMEOR's GRN reconstruction with both simulated and real data. Using a previously published ten close timepoint *Drosophila* RNA-seq dataset, TIMEOR recapitulates past results and finds novel regulatory effects on circadian rhythm regulators after insulin stimulation. Moreover, TIMEOR reconstructs the GRN between perturbed and putative TFs by establishing high confidence interactions using ChIP-seq data for each TF. Overall, TIMEOR is the first integrative, web-based method to predict GRNs from time course RNA-seq and ChIP-seq data.

654C Copy number variants in discontinuous landscapes of heterozygosity in the mouse genome *Hailie Pavanel*[1], Nicholas Boehler[1], Bin Luo[2], R. J. Kulperger[2], C. B. Dean[3], Kathleen A. Hill[1] 1) Department of Biology, University of Western Ontario, London, ON, Canada; 2) Department of Statistical and Actuarial Science, University of Western Ontario, London, ON, Canada; 3) Department of Statistics and Actuarial Science, University of Waterloo, Waterloo, ON, Canada.

Along the chromosome sequence, the rate of mutation is not constant and the mechanisms for this are not fully understood. Heterozygosity has been associated with increased mutagenesis in *Arabidopsis* but this phenomenon has yet to be fully explored in a mammalian model. Inbred mice are a common model for mutation research but this genetic background precludes analysis of the impact of heterozygosity. Two commercially-available stocks of outbred mice, CD-1 (Caesarian Derived-1) and NMRI (Naval Medical Research Institute) provide the opportunity to examine mutations across a discontinuous chromosomal landscape of heterozygosity. CD-1 and NMRI mice were inbred approximately 15 and 51 generations, respectively, producing different patterns of clustered homozygosity and heterozygosity. These mouse genetic backgrounds provide different spatial distributions of clustered heterozygosity for study of colocalization of mutations such as copy number variants (CNVs). This study examines the association between heterozygosity and CNVs in CD-1 and NMRI mice using publicly available Mouse Diversity Genotyping Array (MDGA) data. Single nucleotide polymorphism (SNP) genotyping and CNV detection were performed for all autosomes of 99 CD-1 mice and 279 NMRI mice. SNP genotypes were surveyed to identify localized heterozygosity and CNVs assayed as a measure of associated mutations. In a sample of 16 inbred mice, genomic heterozygosity is 0.25%. Genomic heterozygosity at SNP loci is 10.7 and 6.2% with, on average, 17 and 14 CNVs per mouse in the CD-1 and NMRI mice, respectively. A spatial statistical tool was used for detection of clusters of SNP heterozygosity in inbred mice and both mouse stocks and

CNVs were examined for proximal association. Chromosomal percent heterozygosity at SNP loci is on average, 0.25 ± 0.02, 11.04 ± 0.60, and 6.49 ± 0.43 in inbred, CD-1 and NMRI mice. The relationship between increasing chromosomal heterozygosity and CNV occurrence was examined and both CD-1 and NMRI mice show an increasing trend, with R^2 values of 0.14 and 0.09, respectively. J statistic values were analyzed for all chromosomes containing CNVs in CD-1 and NMRI mice and 41.0% and 21.8% of these chromosomes, respectively, display proximal association between SNP heterozygosity and CNVs. CD-1 mice display more proximal than distal associations. Yet to be determined are the attributes of the clusters of heterozygosity that are associated with proximal CNVs and what, if any, other types of mutations are associated with heterozygosity as well as the underlying mechanisms of this phenomenon.

687C Robustness of gene expression with respect to the dosage of Dorsal morphogen *Prasad Bandodkar*[1], Hadel Asafen[1], Sophia Carrell-Noel[1] 1) North Carolina State University, Raleigh, NC.

In a developing tissue, proper spatial and temporal placement of gene expression domains determines the fate of a cell. Positional information is disseminated in the form of morphogen gradients across the length of a tissue. Gene expression patterns are remarkably robust to a variety of factors, including the dosage of the morphogen. In this work, we investigate the robustness of gene expression in the dorsal-ventral (DV) axis of the precellular *Drosophila* embryo, with respect to perturbations in the dosage of maternally deposited morphogen Dorsal, an NF-κB homolog. Dorsal, which is initially uniformly distributed in the embryo, forms a ventral-dorsal gradient as development proceeds. We found that the boundaries of genes regulated by Dorsal are robust to changes in dosage of the morphogen, which is paradoxical in itself, as by definition gene expression must be sensitive to morphogen concentration. An empirical description of the Dorsal gradient results in unacceptably high sensitivities and predicts unviable embryos. In order to explain this discrepancy, we developed a mechanistic model drawing on extensive experimental and modeling work done on the Dorsal signaling system. We found that, Cactus, an inhibitor of Dorsal, which is generally believed to be only cytoplasmic, must also be present in the nucleus. Furthermore, Toll receptors, responsible for dissociating Cactus from Dorsal in the cytoplasm, must be saturated. Also, the model overwhelmingly predicts facilitated diffusion of Dorsal by Cactus complexes from dorsal to ventral regions of the embryo. In our previous work, we have shown that these mechanisms aid in proper development in wildtype embryos. In this work, we find that these three mechanisms are critical for robust gene expression when the dosage of *dorsal* is compromised. Our work highlights the need for a quantitative understanding of biophysical mechanisms of morphogen gradients to understand emergent phenotypes, such as robustness.

689B Determining the Chemistry and Functionality of the *Caenorhabditis* Disordered Proteomes *William McFadden*[1], Judith Yanowitz[1] 1) Magee-Womens Research Institute.

A classical paradigm in biology is that structure leads to function; however, it has been found that many proteins that lack rigid secondary or tertiary structures are responsible for critical cellular functions. Certain amino acid compositions have an increased tendency to favor a disordered state rather than a highly-packed peptide. Over 40% of proteins in *Caenorhabditis elegans* are predicted to contain an intrinsically disordered region (IDR) of at least 30 amino acids in length. Further, there are peptides that contain little-to-no structured regions, which are known as intrinsically disordered proteins (IDPs). IDPs and IDRs have been previously implicated in many of biology's greatest questions such as the origin of life, emergence of multicellularity, and evolution of sexual reproduction. A commonly used model to study reproduction is *C. elegans*, so we wished to characterize *C. elegans* and its genus further to understand the evolutionary history of these fascinating proteins.

We utilized previously described programs to determine the intrinsically disordered proteomes within the *Caenorhabditis* genus. These programs were SLIDER, RAPID, and IUPred. We further analyzed the chemical properties and the cellular functions of these IDPs/IDRs at a proteome-wide scale. An increased level of disorder correlated with an increased median isoelectric point (pI). GO term analyses of the *C. elegans* disordered proteome indicate that ordered proteins containing an IDR were enriched in functions related to nucleic-acid binding and regulatory processes. IDPs, which are considerably disordered along the entire peptide, were enriched in heterodimer and nucleic-acid binding functionality, as well as nucleus, nucleolus, and cytoskeleton localization. The IDPs were also enriched in the reproductive system and in male worms. This study will be extended to Drosophilids to determine if what we have seen in *Caenorhabditis* is conserved. These findings lay the groundwork for the continual progress of this study to investigate the *C. elegans* disordered proteome and assist future disorder-based studies.

693C Inferring TE haplotype markers from population genomics data using hierarchical clustering *Iskander Said*[1], Michael McGurk[1], Andrew Clark[1], Daniel Barbash[1] 1) Cornell University.

Transposable elements (TEs) are genetic parasites that invade genomes and manipulate their host's molecular machinery to replicate. Just as organisms must compete for resources and evade predators in their ecological setting, TEs do the same, but within their own genomic ecology. TEs must compete with each other for limited genomic space while evading the genome's immune system, piRNAs. And just like their macro-organismal counterparts, these selective forces may be driving a radiative genetic diversity of these TEs. We aim to characterize this diversity by developing new methods to infer TE haplotype markers from unassembled short-read data. We define TE haplotype markers as sets of SNPs that are physically linked with each other on the same TE sequence. To infer these markers, we reason that the copy number of SNPs on the same TE sequences should be correlated. We take short reads and align them to TE consensus sequences using the ConTExt pipeline and estimate the copy number of alleles. We then employ a Hierarchical Clustering approach on the correlations of allele copy number to infer the degree of genetic linkage between SNPs. The result are sets of SNPs inferred to be linked in sequence and represent markers that distinguish TE variants. We benchmarked this approach by using simulations of short-read data, and then used this method to characterize the genetic variation of recently active TEs within 85 strains of the Global Diversity Lines, a population genomics resource of *Drosophila melanogaster* genomes. To verify these TE haplotype markers we aligned TE consensus sequences to PacBio assemblies and compared the full length TE haplotypes in the PacBio sequences to our inferred TE haplotype markers. Our analysis of the GDL revealed a great diversity of TE haplotype markers, many of which are enriched for specific geographic populations. Signatures of population structure for these TEs can be largely attributed to the expansion of only a small number TE variants distinguished by their haplotype markers. The TE variants that expanded are mostly found in low frequency globally, suggesting that they are ancestral variants that rose to higher copy number in a sub-divided population. We also find that some variants are replacing the major allele variants in these populations, showing that TE subfamilies compete for limited space in the genome.

694A Characterization of Polymorphic SINE Insertions and Genes in Dog Retrotransposon Free Regions *Yun Seok Lee*[1], Sara Kalla[2], Allison Seebald[3], Jessica Choi[1], Nathan Sutter[1] 1) La Sierra University, Riverside, CA; 2) Baylor College of Medicine, Houston, TX; 3) Cornell University, Ithaca, NY.

Retrotransposons are mobile genetic elements that have played a major role in mammalian genome evolution. For example, retrotransposon inser-

tions in the dog genome have introduced novel open reading frames and splice acceptor sites, and caused phenotypes ranging from narcolepsy and other diseases to the merle coat pattern selected within some breeds. One dog retrotransposon in particular, SINEC_Cf, is so young that thousands of insertions have not yet gone to fixation. Despite the presence in the dog reference genome of 1,351,940 LINEs and 1,134,572 SINEs (of which 171,386 are SINEC_Cf), we have identified 1375 "free regions" that are at least 10,000 bp long and contain no SINEs, LINEs, or assembly gaps. There are 16,901 free regions at least 5000 bp long, many of which span over gene upstream or downstream ends. We have analyzed the genes found in these dog SINE+LINE free regions because transposon free regions in the human and mouse genomes were previously shown to be rich in genes crucial for early development and transcriptional regulation. We have also analyzed patterns of polymorphic SINE insertion into our free regions to check whether SINEs in these loci have lower than average insertion frequencies or tend to insert at free region edges. To make this possible we Illumina sequenced 434 libraries created by extending into flanking non-repeat sequence from a primer hybridizing to conserved SINEC_Cf sequence. The libraries represent 356 dogs from 125 breeds.

697A Degradation of the repetitive genomic landscape in a close relative of *C. elegans* *Gavin Woodruff*[1], Anastasia Teterina[1,2] 1) University of Oregon, Eugene, OR, USA; 2) Severtsov Institute of Ecology and Evolution RAS, Moscow, Russia.

The abundance, diversity, and genomic distribution of repetitive elements is highly variable among species. These patterns are thought to be driven in part by reproductive mode and the interaction of selection and recombination, and recombination rates typically vary by chromosomal position. In the nematode *C. elegans*, repetitive elements are enriched at chromosome arms and depleted on centers, and this mirrors the chromosomal distributions of other genomic features such as recombination rate. How conserved is this genomic landscape of repeats, and what evolutionary forces maintain it? To address this, we compared the genomic organization of repetitive elements across five *Caenorhabditis* species with chromosome-level assemblies. As previously reported, repeat content is enriched on chromosome arms in most *Caenorhabditis* species, and no obvious patterns of repeat content associated with reproductive mode were observed. However, the fig-associated *Caenorhabditis inopinata* has experienced repetitive element expansion and reveals no association of global repeat content with chromosome position. Patterns of repeat superfamily-specific distributions reveal this global pattern is driven largely by a few repeat superfamilies that in *C. inopinata* have expanded in number and have weak associations with chromosome position. Additionally, 15% of predicted protein-coding genes in *C. inopinata* align to transposon-related proteins. When these are excluded, *C. inopinata* has no enrichment of genes in chromosome centers, in contrast to its close relatives who all have such clusters. Forward evolutionary simulations reveal that chromosomal heterogeneity in recombination rate alone can generate structured repetitive genomic landscapes when insertions are weakly deleterious, whereas chromosomal heterogeneity in the fitness effects of transposon insertion can promote such landscapes across a variety of evolutionary scenarios. Thus, patterns of gene density along chromosomes likely contribute to global repetitive landscapes in this group, although other historical or genomic factors are needed to explain the idiosyncrasy of genomic organization of various transposable element taxa within *C. inopinata*. Taken together, these results highlight the power of comparative genomics and evolutionary simulations in testing hypotheses regarding the causes of genome organization.

Monday, April 27 12:00 PM - 3:00 PM

New Technology and Resources/Genomics and Systems Biology/Neurogenetics 1 - Poster Q&A 518B WGS and bioinformatics analysis combined with genetic mapping of EMS mutants in *Drosophila melanogaster* with balancer chromosomes *Kayla Bieser*[1], Richard Tillett[2], Jacob Kagey[3] 1) Nevada State College; 2) University of Nevada Reno; 3) University of Detroit Mercy.

A Flp/FRT EMS screen was conducted in the *Drosophila* eye in the context of blocked apoptosis to screen for conditional mutants that altered control of cell growth and development. Undergraduate genetics students participating in the Fly-CURE consortium, characterized and conducted deficiency mapping of mutants from this screen to a genomic region on 2R. However, the gene and molecular location of some mutants have proven difficult to map utilizing these techniques. Some of these mutants run into a mapping dead end via genetic mapping by not failing to complement any known lethal alleles. As such, in an effort to identify the mutation, genomic DNA was extracted from stocks of heterozygous EMS mutated flies to conduct whole-genome sequencing and bioinformatics analysis to identify SNPs. A bioinformatics workflow has been successfully established and verified utilizing four EMS mutants identified in the initial screen and a control stock representing their pre-EMS genetic background. For our proof of concept, the sequences were analyzed blindly without reference to any suspected gene(s) but the genetically mapped region that failed to complement was utilized. As crossing over has been blocked in these mutants due to stocks being kept over balancer chromosomes, potential EMS mutations were instead identified by heterozygosity and strain-exclusivity. From the WGS analysis, a list of approximately 50-130 SNPs was generated for each mutant on 2R, but when combined with deficiency mapping data this list could be refined to just 1-3 SNPs identified per mutant. With this technique we successfully identified the *Cos2* and *Egfr* mutant alleles as proof of concept. The *Cos2* mutation was confirmed by PCR analysis and Sanger sequencing, identifying heterozygous base signal at the predicted SNP, providing confirmation for the validity of the methods utilized. Using this same technique in mutants for which no gene had been identified by deficiency mapping, we here report the first known genomic mutation in the gene *Hyccin*, demonstrating that this technique can be used to complete the mapping of genes from our screen. Additional experiments are ongoing to verify other unknown mutant stocks and curriculum is being developed from our methodology for use in an upper-level undergraduate student biology course as an extension to the Fly-CURE.

534C Whole GenomeΩ Imaging for Detection of Structural Variants in Constitutional Disease Jill C Lai[1], Alex Hastie [1], Joyce Lee[1], Ernest T Lam[1], Ben Clifford[1], Sven Bocklandt [1], Steffen Oeser[1], Thomas Anantharaman [1], Henry B Sadowski [1], Andy W C Pang[1] 1) Bionano Genomics, San Diego, California, United States of America .

Majority of the human diseases have a genetic underlying component and structural changes to genome often contribute to the complexity of studying genetic disorders. Understanding these structural changes is critical to develop appropriate diagnostic methods as well as for developing therapeutic options. For example, facioscapulohumeral muscular dystrophy (FSHD), is caused by a collapse of a tandem repeat array with unit sizes of 3.4 kilobases each. This repeat array generally can only be measured by southern blot, a labor intensive and low-resolution approach. Another disease family that is difficult to detect is triplet expansion diseases such as fragile X syndrome and myotonic dystrophy, in which repeat arrays can expand to many kilobases. Microdeletions and microduplications, which cause diseases such as DiGeorge syndrome and other syndromes, are detectible by microarrays as well as whole genome sequencing but large rearrangement cannot be detected with conventional technologies or whole genome sequencing.

Here, we describe Bionano Whole Genome Imaging which is quickly becoming established as a key technology for detecting complex genome-wide structural changes. The Bionano Saphyr workflow can accurately assemble and assay relevant regions for each of above-mentioned disease classes, even those involving very large segmental duplications. Bionano has developed bioinformatics tools to effectively prioritize the ~6000 genome wide structural variants based on the estimated frequency in a control population, whether it's inherited or *de novo*, whether it's somatic and also in proximity to a gene. We provide several examples of pathogenic variants found through Bionano whole genome imaging that enhance the understanding of genetic disorders.

569B A roadmap to low-coverage whole genome sequencing for population genomics *Runyang Lou*[1], Arne Jacobs[1], Aryn Wilder[2], Nina Therkildsen[1] 1) Department of Natural Resources, Cornell University, Ithaca, NY; 2) Institute for Conservation Research, San Diego Zoo, San Diego, NY.

Low-coverage whole genome sequencing (LC-WGS), by being able to generate genome-wide data at low costs, has become an increasingly popular sequencing strategy in population genomic studies, especially with non-model systems. Due to the low sequencing coverage per sample, individual genotypes cannot be called with high confidence based on LC-WGS data, so most population genomic inference methods developed for this type of data take individual genotype uncertainties into account by basing their computations on genotype likelihood. Many of such inference methods have been developed recently and some of them have similar functionalities, but there has not been a comprehensive review of them to date. In addition, depending on the biological system and the types of population genomic inference, the optimal sequencing design (i.e. sample size, sequencing coverage per sample) is likely to differ, but it is not yet clear how we can best balance between inference accuracy and budget with LC-WGS. In this project, we first provide an extensive overview of existing methods developed to process and analyze LC-WGS data, particularly focusing on the methods based on genotype likelihood. When doing so, we also compare and contrast different methods with similar functionalities. Then, we combine forward genetic simulation simulation together with empirical data to quantify the accuracy of different types of population genomic inference (e.g. allele frequency estimation, population structure in spatial populations, selection scan) in different biological systems under different sequencing strategies. Lastly, using simulation, we compare genotype-likelihood-based methods with genotype imputation methods developed for LC-WGS data, and further compare LC-WGS with other sequencing strategies commonly used in population genomics, including restriction-site-associated DNA sequencing (RAD-seq) and pooled sequencing (pooled-seq). With these, we hope to provide a practical guide for researchers interested in using LC-WGS for population genomic studies, and to inspire future development of population genomic inference methods for LC-WGS data.

608B Utilizing the Genetic Diversity of *C. elegans* for Unknown Metabolite Identification *Amanda Shaver*[1], Pamela Kirby[2], Goncalo Gouveia[3], Brianna Garcia[4], Lauren McIntyre[5], Erik Andersen[6], Arthur Edison[1,2,3] 1) Department of Genetics, University of Georgia; 2) Complex Carbohydrate Research Center, University of Georgia; 3) Department of Biochemistry and Molecular Biology, University of Georgia; 4) Department of Chemistry, University of Georgia; 5) Department of Molecular Genetics and Microbiology, University of Florida; 6) Department of Molecular Biosciences, Northwestern University.

C. elegans has been and remains a valuable genetic model organism utilized to study conserved mechanisms in regulating metabolism, longevity, and age-related diseases. The extensive genetic work and tools now available for *C. elegans* make it an ideal candidate organism to help develop, test, and validate a pipeline to identify unknown metabolites. Today the field of metabolomics has the ability to collect tens of thousands of metabolite features within a single sample with great resolution, however, most of these features remain unknown as identifying them has proven challenging.

We are utilizing the existing wealth of genetic and phenotypic data to use *C. elegans* as a model system for characterization of complex metabolomes and in turn a pipeline for semi-automated compound identification. We are utilizing available genetic mutants from the Caenorhabditis Genetics Center (CGC) and the fully sequenced natural isolates available through the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR) to cover much of the genetic diversity in the species.

To generate a high-throughput growth assay suitable for downstream metabolomic analysis, we developed a new method to cleanly culture millions of mixed stage *C. elegans* within a given sample. Our protocol allows us to spend minimal handling time on each culture while generating a sample to be utilized for both phenotypic assays (i.e. population distribution and body size) and metabolic workflows at multiple institutions (i.e. LC-MS/MS). Utilizing this workflow, we can extract *C. elegans* stage-specific information from each strain that will allow us to uncover detailed growth and phenotypic data previously not described and allow us to associate specific metabolic features with a given life-cycle stage. This method can be utilized in many experiments across the *C. elegans* field to enhance the phenotypic data extracted from a given sample.

614B Interrogating the role of the histone mark H3K9me3 in D. melanogaster Genome Organization and Gene Regulation *Alexis Stutzman*[1], Jill Dowen[2,3,4,5], Daniel McKay[2,4,6] 1) Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC; 2) Integrative Program for Biological and Genome Sciences, University of North Carolina, Chapel Hill, NC; 3) Department of Biophysics & Biochemistry, University of North Carolina, Chapel Hill, NC; 4) Department of Biology, University of North Carolina, Chapel Hill, NC; 5) Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC; 6) Department of Genetics, University of North Carolina, Chapel Hill, NC.

Organization of chromatin in three-dimensional (3D) space impacts DNA-templated events by controlling the accessibility of transcriptional machinery to the underlying DNA sequence. Despite its importance in regulating genome function, the mechanisms controlling 3D architecture remain poorly understood. Here, we examine the contribution of histone post-translational modifications (PTMs), a feature of chromatin that is highly correlated with 3D genome organization, but whose role has not been directly tested. In particular, we interrogate the role for the heterochromatin-correlate, histone H3 lysine 9 trimethylation (H3K9me3), in governing 3D organization of the D. melanogaster genome. We directly test the contribution of H3K9me3 by using a genetic platform in which the endogenous histone gene locus has been deleted and replaced with transgenic versions encoding non modifiable histone H3K9. H3K9me3 provides a binding site for heterochromatin protein 1 (HP1), which has been implicated in liquid-liquid phase separation, a biophysical property that separates molecules in 3D space. Given this property of HP1 and it's localization to H3K9me3, we hypothesize H3K9me3 demarcates regions of the genome that separate from the rest of the genome in 3D space. We perform a comprehensive analysis of genomics data, including HiC and RNA-seq, from H3K9me3-deficient D. melanogaster to assess the direct impact of H3K9me3 loss to 3D organization.

641B A k-mer query tool for visualizing pan-genomes *Ziwei Chen*[1], Hang Su[1,2], Maya Najarian[1], Fernando Pardo Manuel de Villena[3], Leonard McMillan[1] 1) Department of Computer Science, University of North Carolina, Chapel Hill, NC 27514, USA; 2) Curriculum of Bioinformatics and Computational Biology, University of North Carolina, Chapel Hill, NC 27514, USA; 3) Department of Genetics, University of North Carolina, Chapel Hill, NC 27514, USA.

As the assembly of multiple intra-specific genomes becomes available, the trend is to build pan-genomes, which incorporate multiple genomes in a single reference representation. However, few bioinformatics tools have been developed for visualizing and analyzing the sequence content of pan-genomes. We previously constructed a graph-based pan-genome called the Collaborative Cross Graphical Genome (CCGG) to provide a better genomic reference representation for a widely-used genetic reference mouse population known as the Collaborative Cross (CC). The CCGG includes 83 mouse genomes (8 founders and 75 CC strains) in a single graph. Here, we presented a new visualization tool for the CCGG that allows the exploration of homologous segments called *paths* between unique and conserved sequences called *anchors* in the CCGG. Our method characterized samples based on the frequencies of k-mers derived from the CCGG. We first generated all 45-mers on paths between anchors in order to find sets of 45-mers that are distinct on every path between a pair of anchors. We then queried those k-mers in sequenced CC samples using a Multi-String Burrows-Wheeler Transform (msBWT). The selection of k-mers is designed to be unbiased relative to any of the sequences represented in the graph and covers a variant on 98% alternative paths. The complete set of all k-mers from eight founders is only twice as large as that in the standard reference. The result was organized and compressed to speed up online queries. This CCGG visualization tool contains results for 96 sequenced CC strains and 8 sequenced founders. It has been used to further resolve the recombination boundaries of CC. This tool is available online and can be accessed at http://devel.csbio.unc.edu/GraphicalGenome/viewer/.

662B Principles of the 3D genome organization in malaria mosquitoes Varvara Lukyanchikova[1,2], Miroslav Nuriddinov[2], Nariman Battulin[2], Alexander Tyapkin[2], Polina Belokopytova[2], Veniamin Fishman[2], *Igor Sharakhov*[1] 1) Virginia Tech; 2) Institute of Cytology and Genetics.

The spatial organization of the genome plays an important role in cell function. The 3D chromosome folding in eukaryotes have been studied using Hi-C – a groundbreaking technology that exploits *in vivo* chromatin proximity information. This method can also yield dramatically improved genome assemblies. The main goals of this study were 1) to apply the Hi-C approach to improving the fragmented genome assemblies for *Anopheles* species and 2) to understand the main principles of spatial genome organization in medically important malaria vectors. We performed a Hi-C protocol on 15-hour Anopheles eggs to generate Hi-C libraries, including two biological replicas for four *Anopheles* species, which were sequenced with Illumina 150-bp paired-end sequencing. We obtained new accurate chromosome-level genome assemblies for *Anopheles albimanus, An. atroparvus, An. coluzzii*, and *An. stephensi*. In accordance with the Rabl-configuration model, Hi-C revealed strong centromere-centromere, telomere-telomere interactions, and long-distance interactions between chromosome arms. We identified topologically associating domains (TADs), A/B compartments, and high-frequency long-distance contacts in the *Anopheles* genomes. TAD boundaries often coincide with A/B-compartments transitions, separating regions with active/repressed transcriptional states. Heterochromatin lacks typical TADs and has random interactions across the entire region. Some of long-distance chromatin contacts are conserved across anophelines. We performed 2D and 3D FISH to validate the interactions between the putative loop anchors in nuclei of follicle cells and ovarian nurse cells of *An. coluzzii, An. stephensi*, and *An. atroparvus*. Overall, we validated most of the long-range interactions by FISH. We observed cell-by-cell variability suggesting that 100% formation of the loops in not essential for the function of follicle cells. In case of ovarian nurse cells, we found that colocalization correlates with the low-levels of chromosome polyteny. We suggest that the high polyteny likely creates specific mechanical properties of chromosomes that hinder specific interaction between the loop anchors. An alternative explanation is that highly-polytenized chromosomes do not require such interactions. We also demonstrate that Hi-C is a robust tool for visualization and discovery of chromosomal inversions in several *Anopheles* species. Our results provide new facts for understanding of how architectural genome folding carries into effect within the nuclear space in malaria vectors.

712A Investigating the non-linear association of multiple genome-wide factors on cellular aging through network permutations Haobo Guo[1], Ruofan Yu[2], Yu Sun[2], Weiwei Dang[2], *Hong Qin*[1] 1) University of Tennessee at Chattanooga; 2) Baylor College of Medicine.

Cellular aging can be viewed as a system-level property of gene networks. As an effective model for cellular aging, the budding yeast is the first organism with genome-wide lifespan measurements for all single-gene-deletion of non-essential genes. We developed a network permutation algorithm that can efficiently generate network null models from the yeast protein interaction networks (yPIN) with power-law features. By comparing the connecting patterns of multiple genomic scale factors in the observed yPIN and network null models, we are able to investigate the non-linear associations of replicative lifespan with multiple genome-wide genotypic and phenotypic measurements. Our results support the view that yeast replicative aging is a stochastic process in a rugged Waddington landscape. We also found distinctive ridges and valleys in the aging landscapes which may shed light on some well-conserved lifespan extension mechanism, such as the calorie restriction.

714C Mistranslation elicits different cellular responses based on the amino acid substitution *Matthew Berg*[1], Yanrui Zhu[1], Bianca Ruiz[2], Joshua Isaacson[1], Julie Genereaux[1], Raphael Loll-Krippleber[3], Bryan-Joseph San Luis[3], Charles Boone[3], Grant Brown[3], Judit Villen[2], Christopher Brandl[1] 1) Department of Biochemistry, University of Western Ontario, London, ON, Canada; 2) Department of Genome Sciences, University of Washington, Seattle, WA, USA; 3) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada.

Life does not require a perfectly accurate proteome. In fact, errors occur at a rate of one mis-incorporated amino acid in every 10^4 to 10^5 codons. Mistranslation, or the mis-incorporation of an amino acid that differs from what is specified by the "standard" genetic code, can also occur due to mutations in the translation machinery. Cells therefore have mechanisms to cope with the resulting errors in protein folding and aggregation. Defects in these pathways may contribute to disease due to a loss of proteostasis. Our goal was to examine how different types of mistranslation affect cells. Using three tRNA variants that mistranslate the genetic code, we investigated genetic interactions and effects of mistranslation on the proteome in *Saccharomyces cerevisiae*. The tRNA variants mistranslate alanine at proline codons, serine at proline codons or serine at arginine codons with frequencies of 2.9%, 4.7% and 2.8% respectively. The alanine at proline and serine at arginine mistranslating tRNAs cause ~10% increase in doubling time as measured by growth in liquid media, while the more severe serine at proline mistranslating tRNA causes ~20% increase. All mistranslating tRNAs induce a heat shock response. Synthetic genetic array analysis of the tRNAs against the yeast temperature sensitive collection revealed that all the tRNAs had negative genetic interactions with genes involved in protein folding. Interestingly, however, we found distinct differences in the genetic interactions of each tRNA. Similarly, proteome analysis using mass spectrometry identified different subsets of up and down regulated proteins, depending on the type of mistranslation. We conclude that while protein quality control mechanisms are required for all

types of mistranslation, the specific amino acid substitutions effect cells in different ways. We previously found variants in human tRNAs that have the potential to mistranslate. Based on the unique genetic and proteomic responses observed for different mistranslating tRNAs, we believe that in addition to exacerbating diseases caused by protein mis-folding, naturally occurring mistranslating tRNAs have the potential to negatively influence a wider range of diseases, depending on the specific amino acid substitution caused by the mistranslation.

717C Learning from evolution and applying humanized yeast in the quest for new drugs *Riddhiman Garge*[1], Hye Ji Cha[1], Chanjae Lee[1], Jimmy Gollihar[1,3], Aashiq Kachroo[2], John Wallingford[1], Edward Marcotte[1] 1) Center for Systems and Synthetic Biology, Department of Molecular Biosciences, Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX; 2) Concordia University, Montreal, Canada; 3) US Army Research Laboratory, Biotechnology Branch, The University of Texas at Austin, Austin, TX.

Model organisms have repeatedly served as valuable surrogates in understanding the molecular and genetic basis of human disease. This mainly stems from the evolutionary conservation of genetic traits between distant organisms even after vast timescales of divergence. While cellular modules functionally diverge through the course of evolution, their native environments also tend to do so leading to orthologous modules often involved in non-obvious, seemingly unrelated cellular processess. At TAGC 2020, I'll describe how our interest in uncovering phenotypic associations across deeply conserved gene modules led us to systematically humanize yeast cells coupled with synthesizing ~30 years of agricultural field studies of pesticide resistance helped repurpose a novel class of FDA-approved antifungals as vascular disrupting agents.

739A Zebrafish Genome Resources at the National Center for Biotechnology Information (NCBI) *Nuala O'Leary*[1], Terence Murphy [1], Francoise Thibaud-Nissen[1], Valerie Schneider[1], Eukaryotic Genome Annotation Group[1], RefSeq Curation Group[1] 1) National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA .

Genomic research on model organisms such as zebrafish (Danio rerio) relies on the availability of high-quality annotated reference genomes to facilitate consistent reporting and mapping of genetic data. The Reference Sequence (RefSeq) project (https://www.ncbi.nlm.nih.gov/refseq/) at the NCBI provides a comprehensive annotation of the zebrafish Tuebingen strain reference genome assembly (GRCz11) maintained by the Genome Reference Consortium (GRC). The zebrafish RefSeq dataset is generated by a combination of computational analysis and manual curation that results in an annotation that focuses on representation of all full-length, non-redundant transcripts. The primary sources of data used in this annotation pipeline include mRNAs, expressed sequence tags (ESTs), protein data, RNA-seq datasets, and protein homology. Zebrafish is one of a select group of vertebrates that are the major focus of RefSeq's manual curation efforts, which involves the in-depth review of sequence data to define new transcript variants, resolve sequence errors, and remove inaccurate information. We also collaborate with expert groups, including the Zebrafish Information Network (ZFIN) and UniProtKB, to provide appropriate annotation and nomenclature for both genes and proteins. In addition to zebrafish, NCBI provides stable reference genome annotation for other fish species with high-quality genome assembly data submitted to NCBI's Assembly resource (https://www.ncbi.nlm.nih.gov/assembly/). To date, 80 fish species have RefSeq annotated genomes, providing a valuable resource for comparative genomic research. We have recently expanded our gene ortholog dataset for fish along with a new visualization tool for comparing homologous gene sets. In this poster presentation we will provide an overview of NCBI's zebrafish genome resources and highlight the utility of these resources to the zebrafish research community. We will also provide practical guidance on how to access RefSeq data and tools for analysis of individual genes as well as whole genome datasets.

2287A Glutamate clearance in the glia-deprived C. elegans synaptic hub *Joyce Chan*[1,2], Irving Estevez[1], Itzhak Mano[1,2] 1) CUNY School of Medicine at City College; 2) The Graduate Center of the City University of New York.

As the major excitatory neurotransmitter in the mammalian brain, Glutamate (Glu) is critical for normal neuronal physiology. Normal processing depends on Glu clearance and the ability to separate synapses in order to maintain specificity of neuronal circuits: failure in ensuring this results in hyper-stimulation of glutamatergic circuits. The canonical model of brain connectivity describes glutamatergic synapses as well insulated and enveloped by glia expressing Glu Transporters (GluTs), which work to clear Glu following synaptic activity. However, only a third of glutamatergic synapses in the hippocampus and other critical brain areas are isolated from other synapses by glia. Poor synaptic isolation may facilitate Glu spillover between adjacent synapses, resulting in loss of circuit specificity. Recent findings also show that brain extracellular space (ECS) is larger than previously believed, suggesting a role for perfusion and bulk flow in robust synaptic clearance and spillover avoidance in critical brain areas. How accurate signal transmission is achieved in such areas remains unclear. The C. elegans model is a powerful system to study circuit specificity in absence of glia, with detailed connectome information and animal transparency that allows optogenetic recording of neuronal activity in the intact animal. We suggest a dual mechanism for Glu clearance in absence of glial isolation: 1) a complement of differentially localized GluTs, paired with mechanical agitation of body fluids, facilitating clearance by bulk flow; 2) Robust clearance is achieved by cooperation between GluTs with different physiological properties, which maintain low ambient Glu concentrations. We hypothesize that the combined function of these two mechanisms separate neuronal signals, compensating for lack of glial isolation. We demonstrate a combined role of synaptic location and pharyngeal pulsatility in Glu clearance. I find that distal GluTs exert preferential clearance on some synapses, while a perisynaptic GluT preferentially clears other synapses. Synaptic responses in both cases are inhibited when agitation of intracellular fluids ceases. These studies will provide novel insights to mechanisms of robust Glu clearance in the absence of glia, a feature shared between nematodes and vital areas of the mammalian brain.

2369B Octopamine is required for female modulation of sperm competition in *Drosophila melanogaster* *Dawn Chen*[1], Andrew Clark[1], Mariana Wolfner[1] 1) Cornell University.

In polyandrous species, competition between rival male sperm for opportunities to fertilize a female's eggs (i.e. sperm competition) is an important aspect of postmating sexual selection. In *Drosophila melanogaster*, sperm properties and seminal fluid proteins have been shown to influence a male's competitive ability, but the mechanisms by which females sense and respond to male and ejaculate qualities are less well understood. We previously showed that when three genes (*caup, hid* and *Rab2*) were each knocked down in octopaminergic *Tdc2*$^{+}$ neurons in doubly-mated females, the outcome of sperm competition was altered. Since these genes serve basic developmental or neural functions, we hypothesize that their knockdown affects *Tdc2*$^{+}$ neurons and octopaminergic (OA) signaling, and that these defects in turn affect sperm competition. Our current investigation focuses on dissecting the role of the female's OA signaling in mediating sperm competition. We find differences in sperm storage dynamics in females with mutations in OA synthetic pathway compared to control females, offering the first-mating male a higher paternity share: mutant females store more first-male sperm and less second-male sperm, and retain more sperm over the course of a ten-day assay. We observe that although the sperm storage dynamics in the female's three sperm storage organs are qualitatively similar, only the seminal receptacle (SR)

sperm paternity ratio correlates with the progeny paternity ratio, consistent with previous reports that SR sperm form the immediate fertilization set. Two postmating processes that influence the outcome of sperm competition are mating plug ejection timing and sperm displacement dynamics. We are currently examining the role of OA signaling in mating plug ejection and the precise timing of OA's effects in sperm displacement and storage. A better understanding of the female nervous system's role in sperm competition will inform understanding of male-female communication of ejaculate quality, and of how reproduction evolves in the context of sexual conflict and selection.

Monday, April 27 12:00 PM - 3:00 PM

New Technology and Resources/Genomics and Systems Biology/Neurogenetics 2 - Poster Q&A 496A Toward an EM Time Series: Automated Spatial Single Cell Identification in a Developmental Context *Anthony Santella*[1], Irina Kolotuev[2], Zhirong Bao[1] 1) Sloan Kettering Institute, NY, NY; 2) Université de Lausanne, Lausanne, Switzerland .

Though automation makes EM data more accessible, navigation and identification of structures remains a bottleneck for interpretation and analysis in large tissues. We present a robust, general method for assigning single cell identities from a template to a sample despite differences in the cells present and their spatial configuration, validating this in the context of the C. *elegans* embryo.
We introduce **neighbor graph constraints** to model the invariant spatial structure of a labeled samples and use this to assign a global quality score to a labeling based on its internal consistency with expected cell-cell contacts. This score is used in a novel **gradient descent optimization** of the template sample which removes cells whose presence cause neighbor constraint violations and are therefore hypothesized to be missing in the unlabeled sample. Our final answer is produced by an **instance-based learning** like approach where the sample is independently matched against each example in the **ensemble** of reference data sets and a **consensus** identity is assigned.
We apply this method to identifying all cells in Electron Micrographs of two *C. elegans* embryos at ~300min and 350min p.f.c. Imaging with Focused Ion Beam SEM and a serial array method provides an undistorted image of the worm simplifying the problem of alignment. Time lapse fluorescence microscopy provides the reference atlas data set of cell positions, identities, and division timings. For validation, identities were independently and manually assigned to a subset of cells in the two EM data sets based on position and cell morphology. Predicted identities were 88% and 91% accurate in the early and late data sets on the cases confirmed by morphology suggesting cell identities can be reliably assigned based on position alone. This approach has potential in other organisms where establishing single cell alignment between individuals is critical for understanding the extent of single cell consistency. Weaker prior information presents a challenge since in general individual identities cannot be established by lineaging. However, the large strokes of our method are universal, though tissue level labels based on anatomy require a different objective function based on counts of expected tissue-tissue contacts. Though we can permute our template absent single cell identities we lose the use of priors for which cells may divide or die, possibly necessitating more advanced optimization methods.

519C A single-cell survey of *Drosophila* blood *Sudhir Gopal Tattikota*[1], Yanhui Hu[1], Yifang Liu[1], Bumsik Cho[2], Victor Barrera[3], Michaeal Steinbaugh[3], Sang-Ho Yoon[2], Aram Comjean[1], Fangge Li[1], Franz Dervis[1], Ruei-Jiun Hung[1], Jin-Wu Nam[2], Shannan Ho Sui[3], Jiwon Shim[2], Norbert Perrimon[1,4] 1) Harvard Medical School, Boston, MA; 2) Hanyang University, Seoul, Korea; 3) Harvard TH Chan Bioinformatics, Boston, MA; 4) Howard Hughes Medical Institute, Boston, MA.

Mechanisms controlling hematopoiesis are highly conserved in the fruit fly *Drosophila*. However, unlike in vertebrates, the fly blood system is composed of only three distinct hemocyte populations: 1. plasmatocytes (PM) 2. crystal cells (CC) and 3. lamellocytes (LM). These cell types have been extensively characterized based on the expression of a few marker genes and cellular morphologies, which are inadequate to classify the complete hemocyte repertoire. Thus, an unbiased approach is warranted for a comprehensive characterization of additional blood cell types. In this study, we used microfluidics-based single-cell RNA sequencing (scRNA-seq) technologies to map the *Drosophila* larval blood cell types and their diverse states across various inflammatory conditions such as wounding and wasp infestation. Our scRNA-seq analysis recovered all known hemocyte populations and resolved PMs into diverse states based on the expression of novel marker genes related to cell cycle, antimicrobial response, and metabolism in addition to the identification of intermediate states within CCs and LMs. Further, we discovered rare subsets within CCs and LMs enriched in components of the fibrablast growth factor (FGF) signaling pathway. **We report that the FGF ligand *branchless* (*bnl*) and its receptor *breathless* (*btl*) are expressed in rare subsets of CCs and LMs, respectively. We demonstrate that both *bnl* and *btl* are required for mediating effective immune responses against parasitoid wasp eggs *in vivo*, highlighting a novel role for FGF signaling in inter-hemocyte cross-talk.** In summary, our scRNA-seq analysis reveals the diversity of hemocyte populations and provides a rich resource of gene expression profiles for a systems-level understanding of their functions.

558C A high-throughput microbial single-cell RNA sequencing method reveals gene expression states in bacteria. Anna Kuchina[1], *Leandra Brettner*[1,2], Luana Paleologu[1], Charles Roco[1,3], Alexander Rosenberg[1,3], Alberto Carignano[1], Ryan Kibler[1], Matthew Hirano[1], R. William DePaolo[1], Georg Seelig[1] 1) University of Washington, Seattle, WA; 2) Arizona State University, Tempe, AZ; 3) Split Biosciences Inc., Seattle, WA.

Here, we present microSPLiT (microbial split-pool ligation transcriptomics), a high-throughput single-cell RNA sequencing method tailored specifically for bacterial communities. A technology based on combinatorial barcoding, microSPLiT easily scales to process hundreds of thousands of cells in a single experiment without need for specialized equipment. We show that microSPLiT can be used simultaneously on gram-positive and gram-negative bacteria and provides single-cell transcriptomic data that resolves heterogeneous functional states within bacterial communities.

ScRNA-seq methods have become a mainstream tool for profiling cell types and states in eukaryotes. However, because of technical challenges, these methods so far have not been compatible with bacteria. microSPLiT is uniquely suited to overcome these challenges since it does not require isolation of individual microbes in droplets or wells and allows sample multiplexing for parallel screening of experimental conditions. Scaling exponentially with the number of barcoding rounds, microSPLiT enables a massive increase in the number of cells that can be sequenced, which makes it uniquely suitable for profiling large and diverse microbial communities.

We validated microSPLiT to detect stress responses in a mixed-species consortium of gram-positive and gram-negative model organisms *E. coli and B. subtilis*, and profiled transcriptional changes in *B. subtilis* cultures transitioning from logarithmic to stationary phase of growth. We showed that microSPLiT can be used to resolve heterogeneous subpopulations of cells activating specific transcriptional programs in different conditions, providing a first to date unbiased single-cell transcriptomic overview of metabolic and developmental shifts in gene expression. Next, microSPLiT

will be applied to resolve heterogeneous functional cell states and cell-to-cell variation in the complex multi-species natural communities such as the human microbiota.

601A Transcriptional profiling in zebrafish using optimized photoswitchable MEK *Aleena Patel*[1], Eyan Yeung[1], Sarah McGuire[1], Andrew Wu[1], Jared Toettcher[1], Stanislav Shvartsman[1,2], Rebecca Burdine[1] 1) Princeton University, Princeton NJ ; 2) Flatiron Institute, New York NY.

Delivering short, targeted pulses of signaling pathway activation with optogenetic tools enables us to study immediate responses in rapidly developing embryos. A photoswitchable version of the MEK1 enzyme (psMEK) directly triggers the Extracellular Signal-Regulated Kinase (ERK)- pathway by short-circuiting signal transduction at the most proximal step of effector kinase activation. This optogenetic tool, however, is only weakly activating *in vivo* because it uses suboptimal phosphorylation-mimicking substitutions in the activation loop of MEK. We optimize psMEK by adding gain-of-function, missense mutations associated with human diseases into the kinase domain. Simply adding mutations, chosen for their activating potential, tunes and enhances photoswitchable MEK activity. We show that optimized photoswitchable MEK is the first tool for optogenetic ERK activation in zebrafish embryos and can be used to control expression of the mesodermal fate regulator, notail, with light. We are now using optimized photoswitchable MEK perturbations combined with sequencing technologies to study the propagated effects of disrupted gene regulatory networks involved in germ layer specification in gastrulating zebrafish embryos.

647B Impact of internal polyphosphate in mammalian cells *Emma Bondy-Chorney*[1,3], Iryna Abramchuk[2,3], Charlotte Holinier[1,3], Rawan Nasser[1,3], Alix Denoncourt[1,3], Kanchi Baijal[1,3], Mathieu Lavallee-Adam[2,3], Mireille Khacho[2,3], Michael Downey[1,3] 1) Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5; 2) Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5; 3) Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5.

Polyphosphates (PolyP) are highly anionic, linear polymers composed of long chains of inorganic phosphates linked together by phosphoanhydride bonds that can range anywhere from a few to thousands of residues in length. Found in all kingdoms of life, PolyP plays roles in phosphate homeostasis, cell growth, infection, and blood coagulation. Recently, PolyP was shown to function as a PTM through a covalent attachment to lysine residues on proteins within a poly-acidic serine lysine-rich (PASK) motif. Interestingly, the mechanisms for synthesizing and degrading PolyP are best understood in bacteria and yeast, the enzymes responsible for these pathways in mammals are unknown. In order to study the role of PolyP in mammalian cells, we have developed an innovative system wherein we express the enzyme responsible for synthesizing PolyP in *E. coli*, Ppk1, in the human cell line HEK293T. This is the first system with the capability to increase internal PolyP inside mammalian cells. We have already successfully used this system to identify the first polyphosphorylated proteins in humans. We hypothesize that overproduction of PolyP via Ppk1 expression in HEK293Ts will allow us to uncover cellular and molecular pathways that are regulated by PolyP in mammals. We carried out large-scale, unbiased, transcriptome and proteome-wide studies to uncover RNA and proteins with differential expression during PolyP overproduction. Results from the RNA-sequencing revealed >100 RNAs with differential expression in Ppk1 expressing cells, as compared to control conditions (2-fold, $p<0.05$, 79% validation rate by qPCR). GO-term analysis showed enrichment in nuclear and nucleolar localization, DNA related processes and functions, and metal ion binding function. Global measure of proteins via mass spectrometry revealed changes in the amounts of several proteins in Ppk1 expressing cells, including targets previously identified in our RNA-sequencing. Follow up by Western blotting analysis of one target, early growth response 1 (EGR1), uncovered that an upstream process that regulates *EGR1* mRNA transcription, the phosphorylation of the Erk1/2 pathway, was also activated by PolyP overproduction. Cell fractionation experiments showed the PolyP generated in Ppk1 expressing cells was unevenly distributed in all cellular compartments, with large amounts observed in the cytoplasm. Finally, we have uncovered that one protein target of polyphosphorylation, DEK, is mislocalized from the nucleus into the cytoplasm and membrane/organelle fractions following PolyP overproduction. Taken together, these results suggest that increasing internal PolyP in mammalian cells influences multiple pathways and results in changes in RNA and protein expression and localization.

685A Identification of *STE12*-regulated long non-coding RNAs in the methylotrophic yeast *Ogataea polymorpha* Juliana C. Olliff[1], Sara J. Hanson[1] 1) Molecular Biology, Colorado College, Colorado Springs, CO.

Due to its inherent risks, sexual reproduction a highly regulated process that requires a convergence of signaling mechanisms that ensure reproduction occurs efficiently and with high fidelity. In budding yeast, haploid cells of opposite mating-types mate to form diploids that can then proceed through meiosis and form spores. When a haploid yeast cannot find a mating partner of the opposite type, a programmed DNA rearrangement termed mating-type switching allows cells to change their mating-type to facilitate mating. In the methylotrophic yeast *Ogataea polymorpha*, switching occurs through inversion (flip/flop) of a 19-kilobase region containing the mating-type genes that allows for their cell-type specific expression and repression to establish mating-type. In *O. polymorpha*, switching is induced by the transcription factor *STE12* in response to nitrogen starvation conditions. Here we investigated the downstream effectors of *STE12*-mediated switching in *O. polymorpha*. We induced switching in haploid cells and performed total RNA-seq analysis to identify long noncoding (lnc)RNAs regulated by *STE12*. Through this work we identified 19 previously unannotated transcripts in *O. polymorpha* that are upregulated by *STE12*, of which eight appear to be lncRNAs. These putative lncRNAs may play a role in the regulation of switching in this species, providing us with novel targets for better understanding the mechanism of flip/flop mating-type switching.

719B Yeast genomics reveals spill chemical impact on cellular processes *Michael Ayers*[1], Amaury Pupo[1], Zachary Sherman[1], Jennifer Gallagher[1] 1) West Virginia University.

In 2014, the coal cleaning chemical 4-methylcyclohexanemethanol (MCHM) spilled into the water supply for 300,000 West Virginians. Initial toxicology tests showed relatively mild results, but the underlying effects on cellular biology were underexplored. The existing resources of the yeast genetics community create a model system for extensive exploration of the effects of such chemicals. Treated wild type cells grew poorly, but viability experiments showed only a small decrease, leading us to believe yeast cells arrested in response to MCHM. Cell cycle analysis via flow cytometry of asynchronous cells revealed a complete absence of cells in S phase within thirty minutes of treatment. Cells accumulated in G1 over a six hour time course. A genetic screen of all haploid knockout mutants from the BY4742 collection revealed 330 genes required for optimal growth in MCHM. According to GO term analysis, these knockout strain genes belong to three major cell processes: mitochondrial gene expression/translation, the vacuolar ATPase, and aromatic amino acid biosynthesis. The accompanying RNA-seq dataset for MCHM treated cells showed an increase in expression of pleiotropic drug response genes and amino acid biosynthetic genes, and a decrease in ribosome biosynthesis. Analysis of the two genomic datasets in combination with observed cell cycle arrest revealed that the environmental stress response (ESR) was activated upon treatment. Both datasets

agreed that the aromatic amino acid genes *ARO1*, *ARO3*, and four of the five *TRP* genes, were required for response to MCHM. This implicated nutrient deprivation as the cause of the ESR activation. However, yeast were grown in rich media, so the source of nutrient deprivation was elusive. We hypothesized that the rich media may be lacking in the necessary amounts of aromatic amino acids, so rich media was further supplemented with excess tryptophan, tyrosine, and phenylalanine to compensate. However, excess supplementation did not improve growth on MCHM. Previous metabolomics analysis also showed that amino acid levels increased on MCHM, so the source of nutrient deprivation signal and the function of these pathways in response to MCHM is still unclear. The combined datasets also implicated the importance of mitochondria and the vacuole in treated yeast. Previous work showed that petite yeast lacking mitochondria were sensitive to MCHM. We hypothesized that these observations were all related to reactive oxygen species (ROS) homeostasis. Flow cytometry with ROS-reactive dye revealed an increase of ROS in treated cells. A comet assay detected DNA damage as well with treatment. MCHM appears to cause cell cycle arrest and DNA damage through the accumulation of ROS. We propose that arrested cells survive through implementation of robust ESR and ROS homeostasis pathways, but there are unknown homeostatic roles of nutrient biosythetic genes to be elucidated.

2274C Novel factors regulating ventral nerve cord pioneer axon navigation in *C. elegans* Abigail Feresten[1], Jaffar Bhat[2], Harald Hutter[1] 1) Simon Fraser University, Burnaby, BC; 2) University of Nevada, Reno, NV.

During nervous system development, axons navigate complex environments to reach synaptic targets. Growth cones of early extending axons interact with guidance cues in surrounding tissue, while later extending axons can interact directly with earlier “pioneering” axons, “following” their path. This is how the ventral nerve cord (VNC) is formed in *C. elegans*. The first axon to extend along the VNC is that of the AVG neuron, which pioneers the right VNC axon tract. Approximately 50 axons extend after AVG, including the motoneuron and interneuron axons of the motor circuit. When AVG is absent, some of these axons are dramatically misguided. Through an enhancer screen, we identified several genes needed in conjunction with *nid-1* for correct AVG axon extension. NID-1 is a basement membrane component enriched along the VNC. In the absence of *nid-1*, AVG is misguided at 15% penetrance.

Previously, we linked AVG axon guidance to mutations of *aex*-3, a guanine exchange factor for *rab-3*. AEX-3 and RAB-3 regulate transport of UNC-5, a receptor for the guidance cue UNC-6/netrin. We recently identified a new gene in this pathway; F52H2.7 is a putative CDK-5 binding partner. *cdk-5* and F52H2.7 genetically interact with *rab-3* in the context of AVG axon guidance, further expanding this regulatory pathway. I have also identified three IgCAMs that regulate AVG axon navigation; *wrk-1, rig-5,* and *rig-6*. Unexpectedly, animals lacking functional *nid-1, rig-5,* and F52H2.7 show a modest but significant rescue phenotype when compared to *nid-1, rig-5* and *nid-1,* F52H2.7 double mutants. This suggests that F52H2.7 may also regulate RIG-5 localization, though the mechanisms for this remain unclear. We will explore these and other questions using GFP fusion constructs and cell-specific rescue constructs in coming months.

2279B Embryonic asymmetry and Development of Functional Neuronal Connectivity *Megan Bone*[1], Tempalyn Wiggins[1], Katarzyna Rosikon[1] 1) Delaware State University.

The relationship between laterality and functional asymmetry has particular relevance for the development of an organism. Most animals, including humans appear symmetrical externally, but display visceral lateral asymmetry with a bias for one enantiomeric body plan over the other. The nematode lab model *Caenorhabditis elegans* also shows predominantly bilaterally symmetric external anatomy, but with clear internal L/R asymmetry that is established during early embryogenesis. The point at which the sperm enters the embryo during fertilization becomes the posterior end of the embryo. Dorso-ventral polarity is established during the second cleavage of the anterior cell, AB, into the anterior blastomere posterior and anterior blastomere anterior cells (ABp and ABa) defining the dorsal-ventral axis of the animal. This division yields two spindles which are initially parallel to the L/R axis and shift at an angle of 20° in an anti-clockwise manner (Wood, 1997). It has been proposed that the foundations of neuronal LR asymmetry in adult worms are laid in early embryonic decisions.
We have examined mutants in the *C. elegans gpa-16* gene which is likely to be involved in the determination of handedness. A temperature-sensitive *gpa-16* mutant (*it143*), when reared at non-permissive temperature of 25° C, yields close to 70% unviable embryos, but of the survivors 40% are sinistral (Bergmann et al., 2003). Previous studies have clearly shown the reversed spindle orientation of sinistral animals during their 4-6 cell developmental stage. The 70% embryonic lethality observed at non-permissive temperature has not been examined, though it may hold a clue to genetic mechanisms governing L/R establishment. In order to unravel the fate of these embryos at the critical stage for L/R axis establishment we have examined the *gpa-16 t.s (it143)* and the *gpa-16 deletion (ok2349)* mutant embryos at their 4-6 cell stages. Additionally, we are working towards defining gene expression profiles, along with chromatid and centromeric segregation during early embryogenesis in efforts to uncover the mechanisms underlying asymmetric neural organization.

2282B The Role of Basement Membrane Proteins for Proper Q Neuroblast Migration in *C. elegans* *Angelica Lang*[1], Erik Lundquist[1] 1) University of Kansas, Lawrence KS.

Neuronal migration is crucial for proper nervous system development. In *C. elegans*, neuronal migration can be modeled in the two Q neuroblasts, QR and QL. They originate in approximately the same location in the worm but, during development, QR migrates anteriorly and QL migrates posteriorly. The transmembrane proteins UNC-40 and PTP-3 have been previously shown to work in two separate molecular pathways to control this initial migration. DPY-17, a collagen protein found in the cuticle and basement membrane of the worm, also appears to influence the direction of Q cell migration. Our data indicates it is the structural orientation of DPY-17 within the basement membrane that provides directional information to migrating cells and that it is working within the PTP-3 pathway. The involvement of DPY-17 suggests a broader role for basement membrane proteins in Q cell migration. We found that other structural proteins in the basement membrane, such as the laminin protein EPI-1, have a permissive role in migration but do not influence direction. This is an important finding as it shows that disrupting the structure of the basement membrane is not enough to alter the direction of migration, as with DPY-17. Other basement membrane proteins may also influence migration direction, such as the perlecan protein UNC-52. These findings set up a model for neuronal migration in which migration is in part directed by proteins in the basement membrane of the worm.

2314A Regulation of *engrailed* and *invected* expression in the *Drosophila* central nervous system *Fountane Chan*[1], Yuzhong Cheng[1], Judith Kassis[1]

1) NICHD, NIH, Bethesda, MD.

The neighboring *invected* (*inv*) and *engrailed (en)* genes encode homeodomain proteins important for segmentation during early development in *Drosophila.* These co-regulated genes are expressed in various cell types, including a subset of cells in the central nervous system (CNS). We are interested in deciphering the regulation of their expression in the CNS. We found that *inv* and *en* are co-expressed in the majority of the *wild-type* CNS. To examine expression in a transgenic context, we are utilizing a 79kb *HA-en* transgene that includes all the elements necessary to rescue *inv-en* double mutants to viable and fertile adults. Although many cells in the CNS express both Inv and HA-En, a subset of cells only express one or the other throughout development and this misexpression appears to be stage-dependent in embryos. This finding suggests the 79kb HA-*en* transgene lacks some regulatory elements for CNS expression. In addition, deletion of imaginal disc enhancers within the transgene increases misexpression of HA-En in the CNS. This misexpression is further exacerbated in lines containing a mutation in a component of PRC1, a Polycomb group protein complex. We are also analyzing expression from a large *inv* transgene that contains nervous system enhancers. Despite misexpression in the CNS, these transgenic lines are still viable and fertile, though they may have disrupted development of the nervous system and behavioral defects. We hope our analysis in *wild-type* and transgenic flies will elucidate the regulatory mechanism of *en* and *inv* expression in the *Drosophila* CNS.

2320A Lysine Demethylase 5 (KDM5) as a key regulator of early neurodevelopment and cognitive function in a *Drosophila* model of Intellectual Disability *Hayden Hatch*[1], Helen Belalcazar[1], Owen Marshall[2], Julie Secombe[1] 1) Albert Einstein College of Medicine, Bronx, NY; 2) Menzies Institute for Medical Research, University of Tasmania, Australia.

Intellectual disability (ID) disorders affect 2% of the population and are characterized by an IQ score less than 70 with deficits in adaptive functioning. Our research focuses on the KDM5 family of transcriptional regulators, mutations in which account for 1-3% of inherited ID ranging from mild to severe. Although recent advances in comparative genomic hybridization and whole exome sequencing have revealed over 75 KDM5 mutations segregating in families with inherited ID, the molecular mechanisms by which KDM5 proteins impact neuronal function remain largely unknown, leaving patients without effective treatment strategies. Thus, the overarching goal of this project is to understand how KDM5 contributes to neuronal and transcriptional outputs that influence cognition, and how these processes are altered by mutations associated with ID.

Here, we utilize *Drosophila*, which encodes a single *kdm5* ortholog, to investigate neuronal morphology, transcriptional outputs, and behavioral phenotypes of flies bearing conserved patient-derived KDM5 missense mutations. We have demonstrated that fly strains bearing patient mutations in an A/T Rich Interacting Domain (ARID) that has previously been implicated in KDM5 DNA binding *in vitro*, as well as a C_5HC_2 zinc finger domain of unknown function, present with profound defects of the Mushroom Body (MB), a paired neuropil-rich structure required for the acquisition, consolidation, and retrieval of long- and short-term memory. Interestingly, fly strains bearing patient mutations that disrupt KDM5's histone demethylase activity do not present with morphological MB defects. This is significant, as the prevailing model linking KDM5 dysfunction to ID assumes that altered demethylase activity of KDM5 is largely responsible for such deficits. We additionally demonstrate that KDM5 is required within immature, but not mature, postmitotic MB neurons for proper neuronal development. We have additionally utilized **Ta**rgeted **Da**mID (**TaDa**) to identify differentially-expressed KDM5 target genes specifically within a subclass of immature MB neurons of *kdm5* null mutants. This approach has revealed a number of candidate transcriptional regulatory networks through which KDM5 may function to promote proper neuronal morphology and cognitive function, several of which we are currently investigating.

2328C Notch signaling regulates neural stem cell entry into quiescence in *Drosophila* *Chhavi Sood*[1], Virginia Justis[1], Sarah Siegrist[1] 1) University of Virginia.

During development, neural stem cells divide symmetrically to expand the stem cell pool and asymmetrically to generate different types of progeny. In response to intrinsic and extrinsic factors, neural stem cells also decide between quiescence versus proliferation states. We are interested in understanding how quiescence versus proliferation decisions are controlled during development. We use *Drosophila* as a model system because of the availability of genetic tools and because the population of neural stem cells, known as neuroblasts (NBs) in *Drosophila*, is relatively simple and defined. Most NBs in the central brain enter and exit quiescence in a nutrient-dependent and PI3-kinase regulated manner. To better understand how NB proliferation decisions are regulated we carried out a large-scale RNAi screen. From this screen, we identified components of the Notch pathway. Notch is an evolutionarily conserved juxtacrine cell signaling pathway that allows for cross-talk between NBs, their daughters and their glial niche in *Drosophila*. To activate Notch signaling, Notch binds to its ligand, Delta. Here we investigate how Delta/Notch signaling regulates NB entry and exit from quiescence during the embryonic to larval transition. We found that Notch signaling in NBs is activated in a nutrient-dependent manner and once on, it remains on in a nutrient-independent manner. Next, we used RNAi to knockdown Notch signaling in NBs. After 24hrs of feeding, we found no difference in NB proliferation compared to control. However, in freshly hatched larvae before animal feeding, we found ectopic neuroblast proliferation when Notch or Delta was knocked down. This suggests that Notch/Delta could be required for NB entry into quiescence. To investigate this possibility, we assayed NB proliferation prior to NB entry into quiescence. We found that NB proliferation continued longer when Notch and Delta were knocked down in NBs compared to control. Next, we assayed the expression of Notch signaling components as NBs entered into quiescence. We found that Notch and Delta expression was downregulated as NBs enter quiescence. Next, using Fly-Fucci we found that most NBs in the central brain were arrested in G_0 during quiescence and knocking down Notch in NBs resulted in a decrease in the number of NBs arrested in G_0. We conclude that Notch signaling is required for NB entry into quiescence by regulating arrest in G_0 and that active Notch signaling correlates with NB proliferation.

2367C Translating time across humans and model organisms from transcriptional and structural variation Christine J Charvet[1], Sharee Mcgriff[1], Chiaowen J Hsaio[2], Emi Takahashi[3] 1) Delaware State University, Dover, DE; 2) Moderna Therapeutics, Cambridge, MA; 3) Boston Children's Hospital, Boston, MA.

We previously implemented a general model of neural development derived to fit 18 mammalian species, including humans, macaques, and several rodent species (http://www.translatingtime.net). Here, the timing of transformations (e.g., when axons myelinate) collected over the course of development and across species are used to find corresponding ages across humans and model organisms. The goal of this work is to identify developmental sources of changes in brain structure and function and to provide an empirical basis with which to recognize equivalent ages across species. Currently, the translating time resource only extends up to 2 year after birth in humans and its equivalent in model organisms. Although there is no resource with which to find corresponding time points across model organisms and humans during postnatal ages, such a resource would enhance translating findings from model organisms to humans. We amass data on the timing of transformations from behavioral, anatomical,

structural MR imaging and gene expression from RNA sequencing over the course of postnatal development. We capture the timing of transformations from peaks in gene expression across species, and we measure the growth of the cortical white matter at successive timepoints during postnatal development to find corresponding ages across species. In total, we collect 319 transformations to find corresponding agesup to 30 years of age in humans and their equivalent in other species. Capturing the timing of transformations from neuroimaging and gene expression permits identifying corresponding ages during postnatal development across species, which will permit identifying deviant developmental programs leading to the emergence of the human brain.

2386A Using the zebrafish lateral line to characterize the HOPS/CORVET complex in axon development. *Serena Wisner*[1], Amrita Mandal[1], Catherine Drerup[1] 1) Eunice Kennedy Shriver National Institute of Child Health & Human Development, NIH, Bethesda, MD.

The transport of cargos along neuronal processes is important for maintaining neuronal health and function. This transport occurs in two directions; anterograde (towards axon terminals) and retrograde (towards the cell body). Anterograde transport is accomplished by a super family of kinesin motor proteins while retrograde transport is accomplished by a single motor protein complex, Cytoplasmic dynein. While we know that dynein is responsible for all retrograde transport, it is unknown how it attaches to specific cargos and moves them to the right place at the right time. To address this gap in our knowledge, our lab uses forward genetics to identify novel modulators of retrograde transport, using the zebrafish lateral line as a model system. In our forward genetic screen, we identified a mutant *vps18* zebrafish line. This mutant has axon terminal swellings, a sign of disrupted retrograde transport due to loss of the Vps18 protein. VPS18 (Vacular protein sorting-associated protein18) is a core component of the HOPS and CORVET complexes. HOPS and CORVET have been shown to be critical for membrane fusion in the endolysomal pathway in yeast and cultured mammalian cells; however, their function in neurons is unknown. To determine if *vps18* defects were due to loss of the HOPS and/or CORVET complexes, we used a G0 crispant screen. Guide RNAs against individual HOPS components resulted in axon terminal swellings as seen in our *vps18* mutants. Conversely, knockout of CORVET-specific proteins had no axonal phenotype. This indicates that HOPS, rather than CORVET, has a role in axonal physiology. To determine if defective axonal transport underlies the morphological defects, we labeled various cargos and analyzed their localization and transport in axons. Strikingly, almost all cargos were dramatically reduced in axons. Additionally, when observing transport dynamics of autophagosomes, early and late endosomes, and dynein, we found that the transport of all of these cargos was significantly disrupted in *vps18* mutants. This data suggests a role for Vps18 in vesicle transport in addition to a potential role in vesicle production. Together, our work on the *vps18* mutant will define the function of this protein and the HOPS complex in axonal health and function.

2387B Forward genetics reveals a potential regulator of axonal transport *Katherine Klier*[1], Catherine Drerup[1] 1) Eunice Kennedy Shriver National Institute of Child Health & Human Development, NIH, Bethesda, MD.

Transport of cellular cargos along axons is critical for neuronal health and function. Perhaps not surprisingly, disruptions in cargo transport are causal in many neurodegenerative diseases. While we know that axonal transport is indispensable in neurons, we know relatively little about how it is coordinated to move cargos including organelles, proteins, RNA, and structural elements in a spatial and temporally specific manner. In order to identify modulators of cargo transport, we conducted a forward genetic screen using the zebrafish lateral line as a model system. Through our screen, we identified a novel mutant line, *lln3* (line designation; gene unknown), which contains axon terminal swellings, a phenotype indicative of cargo accumulation and defective transport. Analyses of dynein localization in *lln3* revealed gross disruptions to the movement of this retrograde (cell body directed) motor in axons. In order to determine the causative mutation in the *lln3* strain, we used RNAseq based approaches. This mapping data narrowed the region of interest to the distal arm of chromosome 10. Analysis of single nucleotide changes revealed missense mutations in three genes of interest. Currently, we are using CRISPR/Cas9 mutagenesis to determine if loss of function in these proteins underlies the *lln3* phenotype. Results of these experiments will provide insight into what gene causes the transport defect observed in *lln3*. Future studies will focus on further characterizing the *lln3* mutation and determining the cellular and molecular mechanisms that are disrupted by the mutation in this strain.

Monday, April 27 12:00 PM - 3:00 PM

New Technology and Resources/Genomics and Systems Biology/Neurogenetics 3 - Poster Q&A 524B FlyMet: an online metabolomics atlas and resource for *Drosophila* *Sue Krause*[1], Karen McLuskey[1], Ronan Daly[1], Shireen Davies[1], Karl Burgess[2], Julian Dow[1] 1) University of Glasgow, Glasgow, Scotland; 2) University of Edinburgh, Edinburgh, Scotland.

Untargeted mass spectrometry-based metabolomics is a powerful technique which attempts to identify all the metabolites in a system (the metabolome). It produces large and complex data sets, creating a need for compact and comprehensible analysis tools.

Based on previous work demonstrating the feasibility of metabolomic analysis of whole flies and individual tissues, our lab is now developing FlyMet.org, a comparable atlas of 19 reference tissue metabolomes, obtained by separately microdissecting tissues of adult (male and female) and larval *Drosophila melanogaster*. Our database and web application (FlyMet.org) provide information on peaks, their identification and confidence levels, and relative abundances. Metabolites will be linked to metabolic pathways and gene identifiers where available, and thus to FlyAtlas.org and FlyAtlas2.org.

Through the development of FlyMet.org, we will use metabolomics to help characterize putative enzymes as well as to add phenotypic information to uncharacterized genes.

606C Global Gene Expression Analysis in the Absence of a Non-Receptor Tyrosine Kinase During Post-Embryonic Development of the Nematode C. elegans. *Ryan Bax*[1], Tu Hoang[1], Gabriella Bulman[1], Suhail Rajah[1], Kent Jones[1], Aaron Putzke[1] 1) Whitworth University, Spokane WA.

Fer-related kinase -1 (FRK-1) is a non-receptor tyrosine kinase that regulates cell proliferation and differentiation during embryonic development of the nematode Caenorhabditis elegans. During the embryogenesis, the movement of hypodermal cells allows for enclosure of the embryo. Furthermore, FRK-1 limits asymmetric Wnt signaling dependent for specification of a subset of hypodermal cells called seam cells and for endoderm proliferation.
A deletion of genomic frk-1, allele (ok760), results in larvae that are severely uncoordinated and fail to progress developmentally, arresting in the first larval stage. Our previous studies have shown that deletion of the frk-1 gene results in an excess number of seam cells, changes in cell morphol-

ogy and alters expression of heterochronic regulators. Due to the severity of the frk-1(ok760) mutant phenotype, we sought to investigate FRK-1 influenced signaling pathways by measuring global gene expression levels.
Using RNA Seq, the relevance of genes that are differentially expressed in the absence of FRK-1 were analyzed using Functional Enrichment Analysis (FEA). FEA identified twenty-one gene clusters of significantly affected genes of which seven were chosen for further analysis. Once identified, we confirmed gene expression data in vivo, examined associated interactions of differentially expressed genes, and began examining novel gene signaling in association with FRK-1 activity. Here we present our RNA Seq analysis and results that show FRK-1 is required as a master regulator for proper expression in specific pathways associated with developmental growth, extra-cellular matrix cuticle formation, cell motility, cell adhesion and cellular localization.

622A Gene regulatory network evolution during *Drosophila melanogaster* and *Aedes aegypti* nervous system development *Isabella Schember*[1], Marc Halfon[1,2,3] 1) University at Buffalo; 2) NYS Center of Excellence in Bioinformatics and Life Sciences; 3) Roswell Park Comprehensive Cancer Center.

Development proceeds through coordinated gene expression programs governed by specific gene regulatory networks (GRNs). Understanding how GRNs evolve over a large evolutionary range is challenging due to a lack of known transcription factor interactions, *cis*-regulatory modules (CRMs), and gene expression patterns for related organisms. We are studying GRN evolution during development of the central nervous systems (CNS) of *Drosophila melanogaster* and its ~250 Mya diverged relative the disease vector mosquito *Aedes aegypti*. Despite similar CNS morphology, both species show divergence of a set of genes co-expressed in the ventral midline of the *D. melanogaster* CNS, including the master regulator *single minded* (*sim*). In contrast to *D. melanogaster*, midline expression of these genes is absent or severely diminished in *A. aegypti*; instead the genes are co-expressed laterally. This suggests that the *D. melanogaster* "midline GRN" has been redeployed from medial to lateral regions in *A. aegypti*. To characterize the responsible mechanisms, we identified relevant CRMs in both species. Analysis of these regulatory sequences in transgenic *D. melanogaster* suggests that the altered gene expression observed in *A. aegypti* is primarily a result of a *trans*-dependent redeployment of the GRN. Potentially, this stems from a *cis*-mediated change in the expression of *sim* and other unidentified upstream regulators. We are continuing to evaluate midline CRMs for both species using transgenic *D. melanogaster* along with reciprocal reporter gene assays in transgenic *A. aegypti*. To elucidate *trans*-acting mechanisms underlying the altered gene expression, we are ectopically expressing combinations of candidate transcription factors in the lateral *D. melanogaster* CNS with the goal of phenocopying the *A. aegypti* lateral expression pattern. Our results illustrate a novel mode of evolution, "repeal, replace, and redeploy", in which a conserved GRN functions at a new site while its original function is co-opted by a different GRN.

2262C Evaluation of serotonin-modifying toxicants using a standardized tracking and behavioral model in *C. elegans* *Courtney McClure*[1], Patrick Allard[1] 1) University of California, Los Angeles.

CPF is a commonly used organophosphate pesticide in the US despite evidence that it can cause life-long, persistent changes in the serotonergic system of mammals. Serotonin is responsible for modulating behavior and plays an important role in behavioral plasticity, memory, and learning. Here, we use the model system *C. elegans* as it shows a high degree of conservation of the serotonergic system with mammals including serotonin receptor, transporter, and synthesis and shows alterations in behavior in response to serotonin deficiency. We will evaluate this pesticide, CPF, using a self-designed and standardized behavioral model for serotonin-mediated behaviors, and monitor its effects on behaviors and alterations to the serotonergic system. We will also evaluate how this exposure can influence gene expression and epigenetic mechanisms transgenerationally. We hypothesize that CPF will alter serotonin-controlled behaviors and the serotonergic system, and that this will be seen transgenerationally and is influenced by changes in gene expression and epigenetic mechanisms. We have characterized the serotonin-controlled behaviors of enhanced slowing responses (ESR) via food deprivation, and basal slowing responses (BSR) under normal conditions for both wild type worms and mutant strains deficient in serotonin, dopamine, or both. *C. elegans* mutant strains chosen each contain a mutation in a particular part of the synthesis pathways of either serotonin and/or dopamine, enabling us to be able to do pathway specific genetic analysis of behavior. Wild type worms display a significantly slower locomotion rate in their ($P < 0.0001$) when compared with their BSR by measuring either center point speed (fold change= 1.9) or absolute peristaltic speed (fold change 1.5). Other new insights into the behavior of mutants such as *mod-5* (deficient in serotonin) and *cat-2* (deficient in dopamine) reveal that *mod-5* wavelength shows no difference when compared to wild type for the BSR, while *cat-2* shows significantly (ESR = 1.27 fold change, BSR= 2.6 fold change) higher wavelength in both ESR and BSR($P < 0.0001$). This in-depth individual behavioral analysis and subsequent dose response study using our behavioral model we have developed is the first of its kind and will address a significant gap of knowledge into the transgenerational effects of serotonin modifying agents on behavior.

2271C Developing a high content, whole organism behavioral screening platform for plant based compounds *Emily Fryer*[1], Sujay Guha[2], Lucero Rogel[2], Angela Xu[1], Theresa Logan-Garbisch[2,3], Iris Molhoff[1,4], Adam Nekimken[2], Ehsan Rezaei[2], Lauren A. O'Connell[4], Shaul Druckmann[5], Thomas R. Clandinin[5], Seung Y. Rhee[1], Miriam B. Goodman[2] 1) Plant Biology Dept, Carnegie Institution for Science, Stanford, CA; 2) Molecular and Cellular Physiology, Stanford University, Stanford, CA; 3) Neuroscience Graduate Program, Stanford University, Stanford, CA; 4) Biology, Stanford University, Stanford, CA; 5) Neurobiology, Stanford University, Stanford, CA.

Plants have evolved complex chemical strategies to influence animal behavior and humans have used plants for their medicinal properties for centuries. Well-known plant-derived compounds such as nicotine, cannabinoids, and opiates are some of the most potent chemical actuators of brain function. These compounds have also provided insight into the molecular basis of neural communication and starting points for treatments of nervous system disorders.

Many high-throughput compound screens have focused on biochemical and cell-based assays, followed by phenotypic assays at the organismal level. However, these widely applied methods limit the identification of new targets. Since nervous system disease biology is complex, targeted biochemical and cell-based assays may not be effective. To address these issues we initiated the Neuroplant Project which engages experts in neuroscience, plant biology, chemistry, and engineering. Our goal is to leverage medicinal plants and animal behavior to identify new chemical and molecular entry points for the treatment of neurological and psychiatric diseases. We are adapting a hybrid approach using intact animals and rapid phenotypic screens with the model organism, *Caenorhabditis elegans*. This nematode is especially well suited for compound screening due to its ease of handling and well characterized genome. Additionally, the coevolutionary relationship between terrestrial nematodes and plants underscores the importance of using *C. elegans* as our model.

Specifically, we tested plant-derived compounds for their ability to evoke chemotaxis (repulsion/attraction) in *C. elegans.* We selected compounds based on surveys of the scientific literature and documented ethnobotanical use. We coupled classical chemotaxis assays with automation and computer vision to increase the throughput. Our preliminary findings revealed both repulsion and attraction to several compounds and these data will be used in forward genetic screens to isolate the neurons that sense these compounds. We are also poised to identify ligands for the ~1280 chemosensory G-protein coupled receptors (GPCRs) that are part of the *C. elegans* genome, which will serve as a primer for understanding the molecular underpinnings of chemosensation. These are the first steps towards the development of a multi-phase, high-throughput drug discovery pipeline to screen compounds and complex mixtures of plant extracts and discover the molecular pathways and chemical tools to probe and treat neurological and psychiatric diseases.

Funding for this research is from the Wu Tsai Neurosciences Institute to SYR, TRC, SD, & MBG and an NRSA fellowship to AN.

2301C Role of the Drosophila small lateral ventral neurons in the regulation of behavioral responses to alcohol *Maria Ramirez*[1], Genesis Ayala-Santiago[1], Jose Agosto-Rivera[1], Alfredo Ghezzi[1] 1) Department of Biology, University of Puerto Rico, Rio Piedras Campus, San Juan, PR.

Alcohol consumption is known to disturb a variety of biological processes that affect normal physiological function. In the nervous system, alcohol is known to affect several molecular targets leading to an overall suppression of neuronal activity. In response, the organism produces a series of neuroadaptive changes that help restore neuronal homeostasis and that lead to the development of alcohol tolerance, dependence and ultimately addiction. These adaptations are also believed to be the root of a series of sleep disturbances, which often manifest during the development of alcoholism. As both, alcohol addiction and sleep regulation are under homeostatic control, we hypothesize that these processes share a common mechanism. Here, we use Drosophila melanogaster as a biological model to understand the molecular underpinnings of the effects of alcohol on the neuronal substrates that control sleep. We show that in Drosophila, a single acute alcohol exposure causes long-term sleep disruptions that resemble those described in mammals. These disturbances include an increase in total sleep duration, decrease sleep latency, and a significant reduction in morning anticipation. Furthermore, we show that the lateral ventral neurons (LNv), a small set of neurons known to control sleep/wake cycles through the secretion of the neuropeptide PDF, is an important regulator of the behavioral responses to alcohol. Silencing of the LNv neurons, either through a mutation in PDF or through a genetic block of synaptic release, significantly increases resistance to alcohol and prevents the development of tolerance. Our results suggest that sleep and alcohol tolerance share common regulatory mechanisms. We believe that the integration of genetic analyses with physiological modulation of neural activity within specific sleep circuits has tremendous potential to uncover the functionally relevant molecular targets whose action contributes to the deleterious effect of alcohol on sleep.

2303B Nociceptor sensitivity and plasticity in *Drosophila* larvae is regulated by translation initiation factors Kate Machen[1], Gita Gajjar[1], Haley McGuirt[1], *Andrew Bellemer*[1] 1) Appalachian State University, Boone, NC.

Nociception is the process of detecting noxious thermal, mechanical, or chemical stimuli by specialized sensory neurons known as nociceptors. Following tissue damage or inflammation, nociceptors may become sensitized, leading to increased pain perception and nociceptive behavior. Aberrant or prolonged nociceptor sensitization may underly many chronic pain conditions, highlighting the importance of understanding the mechanisms that underly the process of nociceptor sensitization. In *Drosophila* larvae, Class IV multidendritic neurons act as nociceptors that are activated by noxious thermal, mechanical, and UV stimuli in order to generate a nocifensive escape locomotion behavior. When larvae experience UV-induced tissue damage, they undergo nociceptor sensitization controlled by Eiger, Hedgehog, and Tachykinin signaling pathways that results in heightened nociceptive responses to noxious thermal stimuli. We show that nociceptor sensitivity under basal conditions and nociceptor sensitization is regulated by the function of the eukaryotic translation initiation factors (eIFs) of the eIF4F complex, the assembly of which is the rate-limiting step of translation initiation. Knockdown of eIF4A, eIF4G, and eIF4E subunits in the nociceptors results in decreased nociceptive responses to noxious thermal stimuli and a loss of nociceptive sensitization following UV-induced tissue damage. Loss of eIF4A specifically results in severe branching defects in Class IV neuron dendrites, suggesting that eIF4F subunits may have differing roles in nociceptor development and morphogenesis. These results suggest that regulation of gene expression during *Drosophila* nociceptor sensitization may be controlled at the level of translation initiation. Ongoing experiments will characterize the cellular mechanisms that translational regulators use to shape nociceptor function and also identify the molecular targets subject to translational regulation.

2307C A conserved mechanism of cooling detection drives both Drosophila warmth avoidance and Anopheles warmth attraction Chloe Greppi[1], Willem Laursen[1], Gonzalo Budelli[1], Elaine Chang[1], Abigail Daniels[1], Lena van Giesen[1], Andrea Smidler[2], Flaminia Catteruccia[2], *Paul Garrity*[1] 1) Brandeis University, Waltham, MA; 2) Harvard T. H. Chan School of Public Health, Boston, MA.

Mosquito-borne diseases kill over a million people annually. Blood-feeding requires detecting host-associated visual, chemical and thermal cues. Mosquitoes and fruit flies diverged ~250 Mya and have evolved distinct anatomies, receptor repertoires and behaviors (e.g., blood feeding). Work in *Drosophila* has substantially contributed to understanding how mosquitoes detect host-associated visual and chemical cues, indicating significant conservation in how these sensory stimuli are detected. However, the mechanisms that drive mosquito heat-seeking remain unknown. We are using *Drosophila* and mosquitoes to probe the basis of heat-sensing and heat-seeking.

Recent studies have shown that neither of the receptors activated by warming in *Drosophila*, TrpA1 or Gr28b, are required for heat-seeking in mosquitoes (Corfas and Vosshall, 2015). While this could mean different warmth-activated receptors are involved, an alternative is that heat seeking instead relies on cooling-activated receptors. We have explored the latter possibility using studies in *Drosophila* and the malaria mosquito *Anopheles gambiae*.

Working in *Drosophila*, we recently found that three Ionotropic Receptors, Ir21a and its co-receptors Ir25a and Ir93a, are critical for the detection of cooling (Budelli et al., Neuron, 2019). Importantly, these receptors are not only required for avoiding cool temperatures, they are also essential for avoiding excessively warm temperatures.

We are currently examining whether these functions are conserved between mosquitoes and flies. In *Drosophila*, we have generated "mosquito-ized" flies expressing mosquito IRs to interrogate their function and evaluate evolutionary similarities and distinctions. In *Anopheles*, we have

used molecular genetics, physiology, and behavior to probe how these and related IR receptors modulate heat-seeking. Our initial results indicate that we have identified a mosquito receptor responsible for detecting cooling, and that this receptor drives both heat-seeking and warmth-stimulated blood-feeding. The notion that mosquitoes locate warm-blooded hosts through a cooling-activated receptor suggests attraction to body heat may actually be driven by avoidance of cooler surroundings. The identification of a receptor for heat-seeking offers new potential targets for vector control strategies to help combat the spread of deadly diseases.

2357B In silico cholinergic pathway analysis indicates possible role for exogenous choline in modulating sensory processing in autism spectrum disorder *Audrey Olson*[1, 2], Fuquan Zhang[3], Hongbao Cao[1, 4], Ancha Baranova[1], Margaret Slavin[2] 1) George Mason University, College of Science, School of Systems Biology, Manassas, VA; 2) George Mason University, College of Health and Human Services, Department of Nutrition and Food Studies, Fairfax, VA; 3) Department of Psychiatry, The Affiliated Brain Hospital of Nanjing Medical University, Nanjing, China; 4) Department of Psychiatry, First Hospital/First Clinical Medical College of Shanxi Medical University, Taiyuan, Shanxi Province, China.

Background:
Sensory processing dysfunction is common in autism spectrum disorder. Sensory processing relies on sensory gating - ligand-activated, ion-channel mediated pathways built upon cholinergic signals. Acetylcholine plays roles in cognitive function, memory, learning, and sensory processing, including auditory signal pathways.
A majority of children on the autism spectrum do not consume adequate intake levels for choline, even as research highlights choline's criticality in neurodevelopment. Choline-deficient diets are associated with lower levels of brain acetylcholine in rodents, and with impaired sensory gating function in both rodents and humans. Therefore, investigation of the impact of dietary choline intake upon sensory processing in autism is warranted.

Methods:
Gene set enrichment analysis was conducted in order to identify Gene Ontology database pathways shared between an MSigDB-curated cholinergic pathway gene sets (345 genes total) and a set of 53 autism genes identified by meta-analysis of genome wide association studies (GWAS, meta-analysis by Zhang F et al., in press). Associated pathways were integrated graphically, using the Pathway Studio environment (Elsevier) to describe the potential influence of dietary choline deficiency on sensory processing pathways affected in autism.

Results:
Two Gene Ontology database pathways were found to be shared between the cholinergic set and the autism GWAS gene set ($p < 0.05$): those for ion transport regulation, and positive ion transport regulation. Further enrichment analysis each for a broader set of auditory genes and a prepulse inhibition gene set (related to auditory startle) from MSigDB revealed regulation of ion transport as the single shared Gene Ontology pathway between all four sets: the autism gene set, the cholinergic signaling gene set, the auditory gene set, and the prepulse inhibition set.

Using the same autism gene set, computational models built identified the cascade initiated by the binding of neurotransmitter gamma-aminobutyric acid (GABA) to the membrane protein GABBR1 as one pathway potentially impacting acetylcholine supply, because of its role in inhibiting eventual release of acetylcholinesterase (ACHE), an enzyme which degrades released acetylcholine in the interneuronal junction.

Conclusions:
Because acetylcholine has long been identified as a modulator of ion transport throughout the body, the findings highlight a potential use for dietary choline as a means to bolster acetylcholine supply, and therefore, ion transport related functionality across sensory domains. Future study of sensory processing associated with autism should include not only identified genetic components, but also associated sensory processing pathway components, including acetylcholine and its precursor, dietary choline**.**

2361C Autism-like behaviors are likely to be regulated by distinct genetic factors in the Mexican cavefish. *Michael Ito*[1], Kimberly Lactaoen[1], Masato Yoshizawa[1] 1) University of Hawaii.

Autism Spectrum Disorder (ASD) is widely distributed among the human population where 1 of 58 children is diagnosed with ASD in the US (2018). The proposed model, 'the burden of ASD risk alleles', in which a person who carries many common ASD risk alleles has a higher risk to express ASD, is widely accepted as a genetic mechanism of ASD. The most parsimonious expectation from this model is that many ASD risk alleles regulate multiple ASD associated behaviors (i.e. pleiotropic). However, it is largely unknown whether the majority of ASD risk genes are pleiotropic. The major challenge to address this possibility is that we only have a few proxy animal systems implementing the multigenic nature of ASD. One of such systems is the Mexican tetra, Astyanax mexicanus. The cave-dwelling morph of A. mexicanus displays a large part of associated symptoms of ASD: loss of socialness, repetitive behaviors, loss of social interaction, increased stress hormone, hyperactivity, adherence to particular stimuli, and loss of sleep. The cave morph also exhibits similar trends in overall gut microbiota diversity to ASD patients, with firmicute species depleted. We then hypothesize that multiple ASD-like traits are correlated in F_2 hybrids derived from a pair of surface and cavefish. Here, we used behavioral assays to quantify sleep, adherence, hyperactivity, sleeplessness, and repetitive turning in 8 individuals each from the surface and cave morphs, and 40 F_2 hybrids derived from a pair of surface and cave morphs. Contrary to our hypothesis, our results show that most of these five behaviors were not correlated in the F_2 hybrids, suggesting that majority of ASD risk alleles may regulate single or a few ASD-like traits, that is, non-pleiotropic. We also dissected the guts of F_2 hybrids and are testing whether gut microbiota (i.e. another emerging epigenetic component) play a role for regulating comorbid ASD-like behaviors in cavefish. We will then present the latest data of genetic relationships between ASD-like traits and gut microbiota compositions.

2365A Monoamine oxidase polymorphisms in Japanese and rhesus macaques (*Macaca fuscata* and *M. mulatta*): Implications for the evolution of macaque behavioral diversity Danielle N. Jones[1], Cody A. Ruiz[1], Anthony J. Tosi[1], Mary Ann Raghanti[1], Hiroyuki Tanaka[2], Yukiori Goto[2] 1) Kent State University, Kent, OH, USA; 2) Primate Research Institute, Kyoto University, Inuyama, Japan.

Monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B) are enzymes that degrade several monoamines of the central nervous system and have long been implicated in the modulation of social behavior. Macaque monkeys are a suitable model for investigating the role of functional monoamine oxidase polymorphisms in behavior modulation given the high amount of social diversity among the nearly two dozen species.

The present study reports allele frequencies for two polymorphisms, *MAOA-LPR* and *MBin2*, in samples of rhesus (*Macaca mulatta*) and Japanese (*M.fuscata*) macaques. Our results suggest that the two species may differ in high and low-activity *MAOA-LPR* allele frequencies. Specifically, 89% of the Japanese macaque alleles in our sample were the low-activity variant, whereas only 41% of the rhesus macaque alleles were of this sort. In our samples, the two species possessed similar allelic variation at the *MBin2* locus, with each possessing some species-specific alleles. We also tested for associations between *MAOA-LPR* genotype and plasma serotonin (5-HT) and dopamine (DA) concentrations in a subset of rhesus macaques, which revealed no association with genotype. Our findings point toward potential differences in the monoaminergic system of two closely related macaque species. Interpretation of our results are centered on implications for future investigations that aim to better understand the potential role of monoamine oxidase polymorphisms in the evolution of primate social styles.

2377A Initial swim bladder inflation in larval zebrafish is mediated by the mechanosensory lateral line *Alexandra Venuto*[1], Shannon Crowe[1], Timothy Erickson[1] 1) East Carolina University, Greenville, NC.

Larval zebrafish achieve neutral buoyancy between 3-4 days post-fertilization by gulping air from the water's surface to inflate their swim bladders. We define this behavior of swimming to the air-water interface as "surfacing." Currently, little is known about the sensory basis for this underappreciated behavior of larval fish. A good candidate is the mechanosensory lateral line, which is a hair cell-based sensory system that detects hydrodynamic information from sources such as water currents, predators, prey, stationary objects, and surface waves. However, the influence of the lateral line on the larval behaviors that mediate swim bladder inflation have not been examined.
To examine the connection between the lateral line and surfacing behaviors, we utilize a genetic mutant (*lhfpl5b*) where the lateral line is non-functional from birth but hearing and balance are normal. We observe that 45% of lateral line mutants over-inflate their swim bladder during initial inflation, lose neutral buoyancy, and subsequently die. Thus, we hypothesize that larval zebrafish use their lateral line to sense the water-air interface and regulate swim bladder inflation. To test this hypothesis, we address two central questions: 1) are lateral line defects in mutants responsible for swim bladder over-inflation? and 2) is this over-inflation caused by an excess of surfacing behaviors? To address the causal association between the lateral line and swim bladder inflation, we have *i)* mimicked the congenital loss of lateral line function using ototoxic treatments, and *ii)* performed a hair cell-specific transgenic rescue of the lateral line mutation. We have addressed the second question of whether excess intake of air is responsible for the over-inflation phenotype by: *i)* blocking access of larvae to the water-air interface, and *ii)* comparing the frequency and duration of surfacing behaviors between wild type and lateral line mutants. Additionally, we have evidence to suggest that phototaxis works together with lateral line input to mediate surfacing. In summary, we have discovered a novel sensory basis for achieving neutral buoyancy where larval zebrafish use their lateral line to sense the water-air interface and regulate swim bladder inflation.

Monday, April 27 12:00 PM - 3:00 PM

New Technology and Resources/Genomics and Systems Biology 2 - Poster Q&A 517A Enhancing enhancer studies in non-traditional insect models: a new suite of reporter vectors for diverse insect species *Kevin Deem*[1], Marc Halfon[2], Yoshinori Tomoyasu[1] 1) Miami University; 2) University at Buffalo-State University of New York.

Enhancers are central to the regulation of gene expression and to the structure of gene regulatory networks. In recent decades, functional analyses of enhancers have produced invaluable insights into the molecular genetic mechanisms underlying evolution, development, and disease. While methods have been developed to identify potential enhancers in a variety of species using sequence or epigenetic characteristics, assessing the function of potential enhancers is possible in only a handful of model organisms. Typically, enhancer function is assessed via one of two methods: loss-of-function genome editing, or enhancer-reporter assay. Enhancer-reporter assays utilize the expression of a reporter gene under the control of a core promoter and an upstream potential enhancer sequence. These assays provide the benefit of real-time, *in vivo* visualization of enhancer activity. A major setback has been the paucity of core promoters which function in distantly related organisms. Within the insects, this limitation has restricted the use of enhancer-reporter assays primarily to *Drosophila melanogaster*. To address this setback, our lab recently established a cross-species compatible reporter construct utilizing a Drosophila Synthetic Core Promoter (DSCP). This construct can report the activity of the same enhancer in homologous tissues in *Drosophila* and the red flour beetle, *Tribolium castaneum*. The ability of the DSCP to function with enhancers from lineages that diverged >300Mya suggests a great potential for use in a variety of insects. Further, as transgenic techniques in non-traditional insect models can be resource intensive, our system allows for prior screening of potential enhancers for activity in *Drosophila*, quickly and cheaply. Utilizing the DSCP, we have produced multiple new reporter constructs with different fluorescent proteins, both piggyBac transposon and PhiC31 site-directed insertion, as well as the Gal4-UAS binary expression system. We are also developing a piggyBac-inserted PhiC31 landing site, with a removable reporter, to screen genomes for insertion sites with minimal position effects. All constructs were tested for proper function using *Drosophila* as a proving ground. Select constructs were then functionally vetted in *Tribolium*. Our new series of enhancer-reporter constructs provide tools with applications ranging from pest management to molecular biology research, and will greatly enhance the study of enhancers in non-traditional insect models.

528C Development of a computer-guided robot for microinjection of embryos for the fruit fly, *Drosophila melanogaster* *Andrew Alegria*[1], Amey Joshi[1], Benjamin Auch[1], Daryl Gohl[1], Suhasa Kodandaramaiah [1] 1) University of Minnesota Twin-Cities, Minneapolis, MN.

Microinjection is commonly used to generate transgenic flies and perform gene editing and silencing. However, due to the labor-intensive nature of Drosophila microinjection, this process would benefit from automation. Here, we describe the development of a computer-guided robot that will automate microinjection into single isolated Drosophila embryos. The robot uses a camera to capture a high resolution image of an entire petri dish containing embryos. This image is then divided into smaller images and a machine learning algorithm detects single isolated embryos in each image. Morphological algorithms are used to detect key anatomical features and estimate injection points near the posterior of each embryo. To determine the exact injection depth for an embryo, we use computer vision algorithms to determine the focal plane of the microscope for an embryo. Other computer vision algorithms are then applied to detect the tip of the micropipette and calculate the 3-D spatial coordinates. At this point, transformation matrices are used to calculate the correct spatial coordinates for the XYZ stage to guide each embryo on the petri dish to the micropipette for injection. Once the micropipette penetrates the embryo, a pressure controller delivers femtoliters of DNA solution or other injection material to each embryo. This process is repeated for every detected embryo on the petri dish. Currently, the robot can successfully detect and microinject single isolated embryos on a petri dish. The injection procedure takes approximately 57 seconds per injection. An automated injection procedure

could reduce cost, increase injection throughput, and may ultimately allow for novel experimental approaches.

543C RefSeq Select: a curated set of representative transcripts for mouse protein-coding genes *Shashikant Pujar*[1], Alex Astashyn[1], Eric Cox[1], Olga Ermolaeva[1], Catherine Farrell[1], Tamara Goldfarb[1], John Jackson[1], Vinita Joardar[1], Vamsi Kodali[1], Kelly McGarvey[1], Mike Murphy[1], Lillian Riddick[1], Bhanu Rajput[1], Craig Wallin[1], David Webb[1], Terence Murphy[1] 1) National Center for Biotechnology Informaation, National Library of Medicine, NIH, Bethesda, MD.

Alternative splicing results in multiple transcript variants within a gene. About half of the genes in the RefSeq's mouse annotation have multiple transcript forms. While the full complement of transcripts may be of interest to carry out in-depth studies about a gene, several other analyses, such as comparative genomics and evolutionary studies, often require the use of one transcript per gene. Several genomic databases offer a representative transcript, however, they are often based on simplistic criteria such as transcript length or the oldest accession. At NCBI's (National Center for Biotechnology Information) RefSeq group, we have developed a method to identify a representative transcript or the 'RefSeq Select' based on functionally relevant properties of a gene, including conservation of the coding region and expression. The RefSeq Select pipeline leverages manual curation carried out by a group of expert curators. The Select transcript is usually well-supported by archived data and represents the biology of the gene. We initially released the RefSeq Select set for the human genome and have now expanded the set to include the mouse genome (GRCm38). The RefSeq Select transcripts can be accessed from multiple NCBI resources such as Gene, RefSeq, Genome Data Viewer and annotation files, and are available for bulk download from NCBI's FTP sites. In the long term, we hope to expand the RefSeq Select data further to include a set of key high-value taxa.

545B Phenotypic mouse allele sequence variant annotation at Mouse Genome Informatics *Laurens Wilming*[1], Meiyee Law[1], Cynthia Smith[1], Carol Bult[1] 1) The Jackson Laboratory.

The power of the mouse as a model for human disease can only be fully exploited if researchers are able to find suitable mouse models for their human disease of interest. Many human diseases are ultimately caused by simple genomic mutations (single or multiple nucleotide variations (SNVs, MNVs) small insertions or deletions (indels)). However, the large number of genetic variants uncovered from individual patients presents challenges in identifying the causal gene or genomic regions. Although the Mouse Genome Informatics (MGI) database provides gene and genotype connections to phenotype annotations, the sequence context of the genome variants for phenotypic alleles was not yet available.

To provide researchers with a searchable, structured dataset of mouse mutations for comparative analysis, we have started annotating mouse variants, concentrating on SNVs, MNVs and small indels. These variants, characterized by their genomic position and sequence changes, are associated with engineered and spontaneous phenotypic alleles in the MGI database (www.informatics.jax.org). Variant attributes include variant type (insertion, point mutation, etc.) and molecular consequence (frameshift, stop gain, etc.). Data is available in Human Genome Variation Society (HGVS) notation to provide transcript and protein contexts. Additional variants from large sequencing and mouse mutagenesis projects will be added to complement the manually curated data. By tying variant data and associated phenotype data to the genome, researchers will be able to search using human variants and find models with variants/mutations that result in the same amino acid change, have the same variant effect (missense, etc.), have the same functional impact (pathogenic, etc.), occur in the same protein domain(s), or have the same mode of inheritance (recessive, dominant, etc.), resulting in phenotypes similar to a patient.
We will present an update on this data, which will be accessible from MGI and the Alliance of Genome Resources (Alliance; www.alliancegenome.org) from March 2020.

549C Building high quality, chromosome-scale, *de novo* genome assemblies by scaffolding Next-Generation Sequencing assemblies with Bionano genome maps Andy Wing Chun Pang[1], Jian Wang[1], Ernest T Lam[1], Benjamin Clifford[1], Sven Bocklandt[1], Steffen Oeser[1], Thomas Anantharaman[1], Alex Hastie[1], Henry B Sadowski[1], Mark Oldakowski[1] 1) Bionano Genomics, San Diego, CA.

Except for a few model organisms, many biologically and economically important plants and animals still lack a reference-quality genome assembly that is crucial to the understanding of their biology. Their genomes are often complex and highly repetitive, making generation of high-quality assemblies almost impossible with next generation sequencing (NGS) alone and without access to long-range structural information. Bionano whole genome imaging provides a solution to reconstruct the full genomic architecture of large and complex genomes. Here, we present a direct enzymatic labeling approach which maintains the integrity of the DNA and enables us to create very contiguous Bionano genome maps which can then be used to scaffold NGS sequence assemblies to produce highly contiguous and structurally accurate hybrid assemblies that can span most repeat regions. This direct labeling method is compatible with a vast array of organisms. We validated our approach with the human NA12878 genome. Starting with NGS assemblies with N50 ranging from 0.18 to 0.9 Mbp, we produced hybrid assemblies with N50 from 70 to 80 Mbp. Chromosome-arm length scaffolds were assembled in 20 chromosomes, and alignments show that they were consistent with the hg19 reference. The hybrid assemblies incorporated 80-90% of total NGS sequences with over 99% scaffolding accuracy. We will also show equally impressive scaffolds for a variety of plants and animals. For a low cost and only several days from sample-to-scaffold, this new method promises to set a new standard for producing high-quality genome assemblies.

555C The development of CRISPR-Cas9 based genome-editing tools in entomopathogenic nematode *Steinernema carpocapsae* as a model organism to study microbial symbiosis *Mengyi Cao*[1], Paul Sternberg[1] 1) California Institute of Technology.

Entomopathogenic nematode ***Steinernema carpocapsae*** is parasitic to insects and mutualistic to bacterial symbionts *Xenorhabdus nematophila*, therefore is valuable to study naturally-occurring microbial symbiosis. As genetic tools in *Xenorhabdus* bacteria proved to be powerful to reveal the mechanisms underlying bacteria-host interactions, the lack of genetic tools on *Steinernema* nematodes hampered the study in the molecular pathways regulating the host side of the symbiotic conversation. Here we present our attempts to establish a CRISPR-Cas9 based approach to introduce mutations in *S. carpocapsae dpy-10,* a gene predicted to involve in nematode cuticle development. In our preliminary trials, we microinjected CRISPR-Cas9 mixture into *S. carpocapsae* adult female gonads (P0 generation) and successfully introduced modifications in the targeted gene by template-based homologous recombination or non-homologous end-joining. Using a single-worm genotyping method, we have detected the introduced mutations in both F1 and F2 progenies, confirming the genome-editing is heritable. One *dpy-10* mutant strain showed a minor defect in cuticle structure as expected. Our current efforts aim to develop CRISPR-Cas9 co-conversion markers in *S. carpocapsae* by introducing mutations that cause distinctive and heritable phenotypes. We will present our progress in genome-editing of *S. carpocapsae* targeting genes involved in

cuticle development or locomotion. Our CRISPR-Cas9 based tools will open a new revenue to study molecular pathways in host-microbes signaling particularly in naturally occurring parasitic and mutualistic symbiosis.

561C GeneLab: the NASA Systems Biology Platform for Space Omics Repository, Analysis and Visualization *Samrawit Gebre*[1], Sylvain Costes[1], Jonathan Galazka[1], Amanda Saravia-Butler[1] 1) NASA Ames Research Center, Moffett Field, CA.

The NASA GeneLab project capitalizes on multi-omic technologies to maximize the return on spaceflight experiments. To do this, GeneLab maintains a publicly accessible database (GLDS) that houses spaceflight and spaceflight relevant multi-omics data, and collaborates with NASA principal investigators and projects to generate additional omics data. GeneLab houses more than 220 transcriptomic, proteomic, metabolomic and epigenomic datasets from plant, animal and microbial experiments, with a growing number of these having been produced by the GeneLab sample processing lab. The GLDS contains rich metadata about each experiment and has recently integrated radiation dosimetery data from experiments flown on the Space Shuttle. GeneLab has also recently implemented an effort to present processed data in the GLDS in addition to the raw omics data. The processed data will enable interpretation of the data by a larger group of students, scientists and the general public. Standard pipelines for the transformation of raw data into visualizations were developed by four GeneLab Analysis Working Groups (animals, plants, microbes, multi-omics) comprised of over 120 scientists from NASA, industry, and academia. To explore the data, the GLDS provides users various tools for data analysis, collaborative workspace for file storage and sharing, and a visualization portal. The analysis platform built using the Galaxy toolshed provides access to a broad variety of users including those with limited bioinformatics experience and students to learn how to analyze spaceflight omics data. The visualization portal takes GeneLab one step closer to data democratization by removing all bioinformatics requisites to interpret transcriptomics data hosted in the repository. Discoveries made using GeneLab have begun and will continue to deepen our understanding of biology, advance the field of genomics, and help to discover cures for diseases, create better diagnostic tools, and ultimately allow astronauts to better withstand the rigors of long-duration spaceflight.

568A Annotation of Drosophila genomes by NCBI's RefSeq project *Terence Murphy*[1], Francoise Thibaud-Nissen[1], Valerie Schneider[1] 1) NCBI/NLM/NIH.

Advances in sequencing technology over the last decade have led to a cornucopia of Drosophila genome assemblies, with over 140 assemblies from 70 Drosophila species now available in public sequence archives. Many species have new, high-quality assemblies based on PacBio, Oxford Nanopore (ONT), or other technologies along with abundant RNA-seq datasets, generated by many researchers from around the world. To help maximize the utility of these genomes for the Drosophila research community, NCBI's Reference Sequence (RefSeq) project is now the primary annotation source for over 30 Drosophila species, allowing FlyBase to focus their resources on D. melanogaster. NCBI's automated annotation pipeline provides rapid, high-quality gene annotations across many taxa, with consistent processing that benefits comparative genomic studies. Annotations are available in NCBI's Gene resource, BLAST databases, and Genome Data Viewer (GDV). Gene and GDV also provide access to other genomic information including orthologs, RNA-seq expression data, and whole genome alignments to previous assembly versions or assemblies from different strains. This presentation will give an overview of these resources, including the scope and quality of annotation data currently available, and options for data access. Further information about NCBI's annotation resources and GDV is available at: https://www.ncbi.nlm.nih.gov/genome/annotation_euk/ and https://www.ncbi.nlm.nih.gov/genome/gdv/.

572B Genetic diversity and footprints of selection in wild house mice Raman Akinyanju Lawal[1], Beth Dumont[1] 1) The Jackson Laboratory.

A major goal of evolutionary genetics is to decipher the mechanisms of adaptation to diverse environments. Here, we used 154 whole-genome sequences from wild-caught house mice to perform genome-wide scans for selection. These samples span three core house mice subspecies (*Mus musculus domesticus, M. m. castaneus, M. m. musculus*) and the outgroup taxon, *M. spretus*, and include organisms inhabiting diverse ecological environments. We employ multiple established population genetic methods and conservative statistical thresholds to spotlight 71 loci in *M. m. domesticus*, 52 in *M. m. castaneus*, 48 in *M. m. musculus*, and 50 in *M. spretus* that are evolving non-neutrally. Included among these loci are well-established targets of positive selection among mammals, including olfactory receptors, genes involved in reproduction, and *Epas1*, which has been previously implicated in physiological adaptation to hypoxic environments. Several loci are also evolving via distinct evolutionary selection regimes in different subspecies, including *Hbb-bh2*, *Prl2c3, Cntnap2, Lrrc25,* and *Susd6*. Our analyses also underscore a key role for balancing selection in the maintenance of genetic diversity at several genes, including *Cwc22*, *Zswim2* and *Fam171b*. Taken together, our findings comprise a catalog of putative targets of positive and balancing selection in a powerful biomedical model system poised for facile discovery of the genetic and underlying physiological mechanisms of adaptation in the wild

581B High throughput screening for chemical inhibitors of mammalian adenylyl cyclases expressed in fission yeast *Charles Hoffman*[1], Jeremy Eberhard[1], Sheng Xiang Huang[1], Juliane Dessalines[1], Nicholas Ollila[1], Harrison Silva[1], Patricia Dranchak[2], James Inglese[2] 1) Boston College, Chestnut Hill, MA; 2) National Center for Advancing Translational Sciences, NIH, Rockville, MD.

The fission yeast *Schizosaccharomyces pombe* is an ideal host for high throughput screens (HTSs) to identify inhibitors of heterologously-expressed mammalian proteins involved in cyclic nucleotide metabolism. This is due to the following: 1) PKA is not essential in *S. pombe*, 2) PKA activity dramatically affects growth, mating and transcription of the *fbp1* gene, for which several reporters have been developed, 3) phenotypic screens in *S. pombe* are inexpensive and readily identify compounds that are both active against the target protein and are permeable to mammalian cells, 4) target identification is relatively straightforward using strains that express the fission yeast homolog of the target protein. Our previous HTSs using *S. pombe* strains expressing mammalian phosphodiesterases, identified compounds that are biologically active in mammalian cell culture and can be used as tool compounds or lead compounds for drug development. We have now successful deployed both GFP- and luciferase-based screens for mammalian adenylyl cyclase (AC) inhibitors using *S. pombe* strains that express mammalian ACs together with a mutationally-activated human GNAS G_s protein [1]. These screens were carried out in 1536-well microtiter dishes, allowing for quantitative HTSs in which compounds are tested at multiple concentrations. By recording GFP signals with an Acumen laser cytometer, we avoided the significant background generated by soluble fluorescent compounds. Successful screens of 100,000 to 125,000 compounds (the NCATS Genesis library) were completed with strains expressing human AC4, AC7, and AC9. This library is composed of collections of molecules with shared scaffolds to aid in the identification of lead compounds that are amenable to medicinal chemistry to develop more effective and drug-like molecules. Follow-up assays of cAMP production in response to compound treatment have identified two functional scaffolds that are likely to act as direct inhibitors of mammalian transmembrane ACs. Given the challenge of using biochemical approaches to inhibitor development for these integral membrane proteins, this work represents a significant break-

through in the discovery of AC inhibitors that are likely to be effective in mammalian cells.
[1] Getz, R.A. et al. Cell Signal, 2019. 60: p. 114-121.

604A 3D Imaging of Zebrafish Larvae Using the VAST BioImager *Yongwoon Kim*[1], Faiyaz Rahman[1], Yifei Wang[1], Mikalai Malinouski[1] 1) Union Biometrica.

3D tomographic visualizations have become a powerful approach in medical and scientific imaging. The mechanical stability of the microscope and the embryo are essential when acquiring tomographic projection datasets. We have developed software and methodology to make 3D reconstructions of live and fixed zebrafish embryos using the VAST BioImager. The VAST BioImager (Vertebrate Automated Screening Tool) is a modular, expandable platform for high throughput imaging and sorting of zebrafish larvae 2-7dpf. The system reliably and reproducibly detects, orients, and rotates the larvae to a user-defined field of view, eliminating manual manipulation. Identifying the center of rotation (COR) of the specimen is key for contrast volumetric reconstructions. We tested the efficiency of different approaches for COR detection.

The software was tested to perform tomographic reconstructions from different tissues. Here we will report volumetric reconstructions of craniofacial features, heart, and tumors and neuromast in developing zebrafish. Typical reconstructions were collected and processed in less than 5 minutes. 3D tomographic software allows to acquire and analyze morphological features at key stages of zebrafish embryonic development. In conjunction with the VAST system's high throughput positioning and orientation of zebrafish larvae, large numbers of 3D reconstructions of organs to whole fish are easily collected, enabling additional insight into morphology, structure, and phenotype.

631A Heterochromatin-dependent transcription of satellite DNAs in the Drosophila female germline *Xiaolu Wei*[1], Danna Eickbush[2], Iain Speece[2], Amanda Larracuente[2] 1) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY; 2) Department of Biology, University of Rochester, Rochester, NY.

Satellite DNAs (satDNAs) are tandemly repeated DNAs primarily found near centromeres, telomeres, and on sex chromosomes. They are abundant in eukaryotic genomes. Although typically thought of as junk DNAs, there is accumulating evidence that satDNAs play important roles in chromosome organization and maintaining genome stability. Abnormal satDNA activity is associated with chromosome missegregation, aging, and cancer. Despite its association with important phenotypes, we currently know little about satDNA maintenance at the chromatin level, or if satDNAs have specific functions. Previous studies have reported satDNA-derived transcripts. However, whether or not satDNA expression is regulated remains an open question. Using the Drosophila germline as a model system, we characterized the expression pattern and regulatory network of satDNAs using a combination of genomic, cytological, and molecular approaches. Our data revealed that the satDNAs are transcribed into long noncoding RNAs (lncRNAs) and then processed into piRNAs (PIWI interacting RNAs) in the germline. piRNAs are a subset of small RNAs that function to repress transposable elements (TEs) to maintain genome stability. We find that satDNA piRNA production is regulated by the same piRNA pathway as dual-strand piRNA clusters, involving Rhino and Moonshiner. The piRNA pathway is important for the establishment of heterochromatin at satDNA loci. Taken together, our findings suggest that satDNAs are regulated by piRNAs originating from their own genomic loci. This novel mechanism for satDNA regulation provides insight into general features important for understanding the roles of satDNAs in the germline.

636C Centromeric Determinants of Host Tolerance to Transposable Elements *Jyoti Lama*[1], Satyam Srivastav[2], Sadia Tasnim[3], Donald Hubbard[4], Erin Kelleher[1] 1) University of Houston, Houston, TX; 2) Cornell University, Ithaca, New York; 3) University of Texas Medical Branch Galveston, TX; 4) Texas A&M, College Station,TX.

Transposable elements (TE) are mobile genetic parasites whose unregulated activity in germline causes DNA damage, thereby disrupting gametogenesis. Hosts respond to this fitness cost either by evolving resistance, where transposition is regulated, or by evolving tolerance, where the germline is robust to the damaging effects of TE proliferation. Host resistance to TEs through small RNAs, particularly piRNAs is the focus of extensive research. However, little is known about host factors that could confer tolerance to TE activity.
We took advantage of Recombinant Inbred lines (RILs) that do not produce piRNAs targeting the *D. melanogaster P*-element to uncover genetic factors that determine tolerance to *P*-element transposition. Through Quantitative trait locus (QTL) analysis of 617 RILs, we identified a complex locus surrounding the 2ndchromosome centromere. Females harboring "tolerant" alleles are more likely to produce gametes despite unregulated *P*-element transposition. By comparing total RNA and small RNA pools between RILs harboring tolerant or sensitive alleles, we discovered that tolerant alleles are characterized by reduced expression of pericentromeric genes, and a corresponding increase in expression of piRNA clusters. Our observations suggest that tolerant alleles promote heterochromatin spreading, making the production of piRNAs regulating resident TEs more efficient in the dysgenic germline. Tolerance may therefore be determined by cryptic variation in genomic instability, which is revealed in the presence of acute genotoxic stress.

637A *bruno* and P-element transposition: positive regulator or cellular responder? *Lorissa Saiz*[1], Erin Kelleher[1] 1) University of Houston.

Transposable elements (TE) are obligate genetic parasites that guarantee transmission to offspring by replicating in the nuclei of germline cells. TE replication harms hosts by causing DNA damage and deleterious mutations that prevent the production of viable gametes. In response, hosts can employ two different strategies to minimize these effects: resistance and tolerance. Resistance mechanisms prevent TE replication, while tolerance mechanisms allow host cells to withstand the damaging effects of TE activity. However, the cellular mechanisms of tolerance remain largely unknown.
We recently identified *bruno* as a possible source of natural variation in tolerance of *D. melanogaster* females to *P*-element transposition. *bruno* loss of function alleles are strong suppressors of *P*-element-induced germline loss, but *bruno* has no known function in TE regulation. To conclusively determine whether *bruno* regulates *P*-element transposition, or represents a tolerance factor, we are comparing *P*-element expression and excision rates between *bruno* mutants and wild-type flies, as well as between natural tolerant and sensitive alleles of *bruno*. If *P*-element transposition is independent of *bruno*, then expression and excision should not differ between bruno genotypes. Alternatively, if expression or excision increase with *bruno* function, it will suggest that *bruno* influences germline loss by positively-regulating *P*-element activity. Our preliminary data suggest that *P*-element transcription does not differ between tolerant and sensitive alleles, consistent with *bruno's* proposed role in tolerance.

638B The Role of Bacterial Genotype in Persistence of the Microbiota of Drosophila melanogaster *Sarah Gottfredson*[1], John Chaston[1] 1) Brigham Young University.

The microbiome of *Drosophila melanogaster* can have significant effects on the host, and many of these have been studied. However, the reason why the bacterial species associate with and persist in *D. melanogaster* has not been studied in depth. Here we define persistence as how long a microbe associates with a host. The early assumption has been that the *D. melanogaster* gut microbiome is established solely through diet, but recent work suggests that other factors may be at play in the microbiome establishment. This experiment aims to study the correlation between bacterial genotype and persistence in the *D. melanogaster* microbiome. In this study, a metagenome wide association (MGWAS) was done using 40 different strains of bacteria to find distinct bacterial genes that are significantly correlated with persistence. To do this, each strain was mono-associated with twenty-four individual flies. The flies were reared for fourteen days, transferred onto new food three times a day for two days, homogenized, and plated. Using the significant genes found through the MGWAS, the same experiment protocol will be used to test mutants of these genes for their effect on persistence. These data will provide us with distinct genes that are necessary for effective bacterial persistence.

668B Exclusion of SINE Inverted Pairs from the Genome of the Dog (*Canis familiaris*) *Cassandra Ward*[1], Sara E. Kalla[3], Allison Seebald[2], Nathan B. Sutter[1] 1) La Sierra University; 2) Cornell University; 3) Baylor College of Medicine.

Retrotransposons make up about one third of mammal genomes. The genome of the domestic dog (*Canis familiaris)*, is no exception: there are 171,386 copies of SINEC_Cf in the reference genome. This particular type of sine is so young that many insertions have not yet gone to fixation making the dog a prime model for research on genome patterns, retrotransposon insertions, and disruption of gene expression. To discover polymorphic SINEC_Cfs, we collected a total of 279M next-gen sequence reads from 62 libraries enriched for flanks of the head end of SINEC_Cf. The libraries represent 59 distinct pure breeds. While most reads map to reference SINEC_Cfs that are presumably fixed in all dog chromosomes, approximately 8% of reads map to insertion loci not present in the reference genome, which we define as polymorphic. We found 81,747 such putatively polymorphic SINEs. These putative polymorphic SINEs and the fixated insertions found in the boxer reference genome we used to find and analyze SINE pairs. We define a SINE pair as two SINE insertions that are within a certain distance of each other. There are four different orientations for these pairs: head-to-head, tail-to-tail, head-to-tail, and tail-to-head and in addition we track the orientation of pairs relative to a gene transcript, if present. The head-to-tail and tail-to-head orientations are inverted SINE pairs. In other mammals, including humans (with Alu pairs), such inverted SINE pairs are observed at a much lower density than direct repeats and when present in transcripts have been shown to disrupt gene expression. The conclusion from such work is that these pairs are under negative selection. We looked for a similar loss of SINE pairs in the dog genome. Comparison of SINEC_Cf pairs in the reference genome shows that inverted pairs less than 100 base pairs apart are much less frequent than the pairs in the same orientation. This relation holds for pairs within introns as well as in nongenic sequences. We also found an orientation bias against inverted SINEC_Cfs as their pairwise alignment scores improved. We also looked at pairs in which one is a polymorphic SINE detected in our libraries, the majority of which will be SINEC_Cf. At short distances we find low proportions when polymorphic SINEs are paired with SINEC_Cf and SINEC_Cf2. No such drop occurs for pairs with LINE1 or MIR-type SINEs.

671B See More of Your Data with NCBI's Genome Data Viewer (GDV) *Sanjida Rangwala*[1], Ravinder Pannu[1], Anatoliy Kuznetsov[1] 1) National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health.

For three decades, NCBI has been the go-to source in the scientific research community for sequence and genome annotation. Over the years, NCBI has supported annotation of model and non-model organism genomes, including an expanding number of animals, plants, fungi, and other species across the tree of life. The Genome Data Viewer (GDV), NCBI's flagship genome browser, allows our users to view NCBI's RefSeq and Gene annotation for over 700 genome assemblies. For many genomes, including those for commonly-studied organisms, users can view genome sequence alongside gene annotation, NCBI-generated RNA-seq analyses, and data indexed in the SRA and GEO databases.

Users of NCBI genome browsers and sequence viewers can view their own custom data, including data hosted on a remote URL or organized in track hubs. Currently, users can stream data in BAM, bigWig, BigBed and tabix VCF formats. BLAST searches can also be run directly from within the browser, and the resulting alignments can be viewed in the context of gene annotation, known repetitive regions, and other genomic features. Putting data from multiple sources together in a visual format can help uncover relationships among different types of biological data, stimulating insights that can inform further scientific investigation and discovery.

GDV can also serve as a natural entry point for retrieving NCBI sequence and annotation for a genomic feature or region of interest. Here, we'll highlight recent updates to getting downloads from the genome browser, including the ability to export gene annotation in GFF3, BED, and human-readable tabular format. We'll demonstrate how researchers can combine NCBI-provided and external datasets within the GDV browser to create customized visual representations of their research stories for viewing, analysis, and further dissemination.

698B The influence of a natural diet and microbiota community on the metabolic phenotype of *Drosophila melanogaster* *Andrey Bombin*[1], Owen Cunneely[1], Sergei Bombin[1], Kira Eickman[1], Abigail Ruesy[1], Abigail Myers[1], Caroline Hart[1], Mengting Su[1], Ryan O'Rourke[1], Rachael Cowan[1], Laura Reed[1] 1) The University of Alabama.

Obesity is an increasing worldwide epidemic and contributes to physical and mental health losses. The development of obesity is caused by multiple factors including genotype, hormonal misregulation, psychological stress, and gut microbiota. Our project investigates the influence of a microbiota community, acquired from the environment and horizontal transfer, on traits related to obesity; such traits include weight gain, fat storage, and blood (hemolymph) sugar. The study applied the novel approach of raising *D. melanogaster* from ten, wild-derived, genetic lines (DGRP) on naturally fermented peaches, thereby preserving genuine microbial conditions. In order to control for the effect produced only by live microorganisms, we used autoclaved rotten peach diet as a control for the natural diets. Our results showed that the microbial composition of lab and peach food types differed in beta diversity, as well as, in abundance of the major microbial taxa. Consumption of the natural diet led to decreased weight, protein and glucose levels but higher triglyceride concentrations. Larvae raised on the autoclaved fermented peach diet, thus deprived of original environmental microbiota, had lower survival and developmental rates, lower dry weight, and elevated levels of protein, triglycerides, and sugars. Thus, our results suggested that a live microbial community played an active role in shaping metabolic phenotype. In addition, we found that the abundance of several major microbial taxa produced a significant effect on larval metabolic phenotypes. Surprisingly, the strength of association with a particular microbial taxa could vary with the diet and even show a specific diet by taxa interactive effect. Our results also indicated that inheritance of parental microbiota partially rescued the phenotype induced by a lack of the natural microbial community in the food. In addition the presence of parental microbiota further increased the development rate and weight gain on the natural diet, even in presence of original environmental microbes

suggesting beneficial additive effects of both. Among the experimental groups we also observed a significant interactive effect between parental microbiota, diet, and genotype in shaping larval metabolic profile.

702C Wolbachia effects on Drosophila microbiome and metabolic phenotypes vary during seasonal adaptation *Lucas Henry*[1,2], Julien Ayroles[1,2] 1) Princeton University, Dept. of Ecology and Evolutionary Biology; 2) Princeton University, Lewis-Sigler Institute for Integrative Genomics.

Wolbachia, a maternally transmitted alpha-proteobacterium, is a master manipulator of invertebrate biology, affecting numerous host traits from sex ratios to fecundity to pathogen blocking. Because of pathogen blocking, Wolbachia has emerged as powerful tool to combat vector-borne diseases. However, abiotic stressors in natural environments frequently diminish the efficacy of Wolbachia control measures. The microbiome may play an important role in enabling hosts to buffer environmental stressors, but the interaction between Wolbachia and the microbiome is poorly understood in natural environments. To test how Wolbachia infection influences the microbiome, we performed a field mesocosm experiment using replicated, outbred D. melanogaster populations in Princeton, NJ. We started each mesocosm with ~2500 flies (N=4 for Wolbachia infected, N=4 for uninfected) in July 2019 until the first frost in early November 2019. Through weekly sampling, we identified shifts in microbiome composition during seasonal adaptation in Drosophila. Providencia bacteria were most prevalent at the start of the experiment, shifting to Acetobacter and Commensalibacter dominance mid-season, but Providencia increased in relative abundance again in late October. Wolbachia infection reduced bacterial diversity but did not strongly facilitate or exclude bacteria species compared to uninfected flies. For metabolic phenotypes, we measured starvation resistance and triglycerides at 4 timepoints (roughly every 2 weeks) from early September to late October in age matched flies sampled from the mesocosms. Wolbachia infection generally did not affect metabolism—except for one timepoint in mid-October. In mid-October, Wolbachia infected flies, especially males, were significantly less starvation resistant than uninfected flies. Taken together, our data suggest that Wolbachia effects on fly metabolism and microbiome are context dependent in natural mesocosms, frequently with no or only modest effects. These results highlight how complex interactions among microbes and the environment shape host adaptive responses to seasonal environments. Future work could apply these results to improve Wolbachia control measures through eco-evolutionary dynamics.

Tuesday, April 28 12:00 PM - 3:00 PM

Disease Models and Aging - Poster Q&A 1938C Exposure to a Potential Environmental Contributor of Parkinson's Disease: *S. venezuelae* alters lifespan and mitochondrial phenotypes in *C. elegans* *Jennifer Thies*[1], Hanna Kim[1], Guy Caldwell[1], Kim Caldwell[1] 1) University of Alabama.

Parkinson's Disease (PD) is characterized by the loss of dopaminergic (DA) neurons and the formation of proteinaceous inclusions that contain the α-synuclein (α-syn) protein in the midbrain. Overexpression of human α-syn in the eight DA neurons of *C. elegans* causes neurodegeneration in an age- and dose-dependent manner. Only 5-10% of PD cases have a direct genetic origin; however, exposure to herbicides, pesticides, and a rural lifestyle are all potential risk factors. A soil bacterium, *Streptomyces venezuelae* (*S. ven*), produces a secondary metabolite that causes age- and dose-dependent DA neurodegeneration in *C. elegans* following acute or chronic exposure. It also exacerbates α-syn-induced DA neurodegeneration. Initial studies from our lab determined that the *S. ven* metabolite increased oxidative stress and reactive oxygen species and that the metabolite worked through the insulin-signaling associated transcription factor *daf-16* to activate *sod-3*. We also found that *S. ven* toxicity increased mitochondrial fragmentation. To determine whether the metabolite impaired the longevity of worms, we subjected wild-type worms to lifespan assays. We discovered that *S. ven* metabolite exposure has a hormetic effect on lifespan whereby higher concentrations decrease lifespan and lower concentrations extend lifespan. Notably, mutants in *daf-16* displayed no significant differences in lifespan at either concentration of *S. ven*, suggesting the hormetic response is *daf-16* dependent. We additionally investigated the impact of *S.ven* metabolite on lifespan in *C. elegans* mitochondrial fission and fusion mutants, *drp-1* and *fzo-1*, respectively, because *drp-1*, but not *fzo-1*, is upregulated in response to *S. ven* exposure. We found exposure to the higher concentration of *S.ven* increased lifespan in a *drp-1* mutant background suggesting that this cellular pathway might be important for combating toxicants following chronic exposure. Continued investigation of the concentration-dependent effects of *S. ven* through additional genetic analyses and transcriptional profiling might illuminate mechanisms of neurodegeneration. Taken together, these studies indicate a novel environmental toxicant might be a contributor underlying susceptibility to PD via an intersection of lifespan and mitochondrial dynamics

1949B Protective role of the *C. elegans* DBL-1/TGF-β signaling pathway in innate immune defenses against a panel of Gram-negative and Gram-positive bacteria *Bhoomi Madhu*[1], Laura Hanson[1], Tina Gumienny[1] 1) Texas Woman's University, Denton, TX.

The innate immune response coordinates several molecular activities, including a cell-cell signaling pathway called Transforming Growth Factor-β (TGF-β), conserved in species from the simplest animals to humans. In *C. elegans*, the DBL-1/TGF-β pathway is required for an effective innate immune response to fight some fungal and bacterial challenges. *C. elegans* exhibit different levels of protection from immune challenges: 1) avoidance of pathogens, 2) physical and mechanical protection, and 3) induction of antimicrobial genes upon infection. To determine if DBL-1 is specifically required to mount an effective innate immune response against a broad range of bacteria, we challenged normal and *dbl-1(nk3)* mutant nematodes with a selected panel of bacteria that are opportunistic human pathogens. This panel comprises Gram-positive *Bacillus megaterium*, *Enterococcus faecalis*, and *Staphylococcus epidermidis*; and Gram-negative *Enterobacter cloacae*, *Klebsiella oxytoca*, and *Serratia marcescens*. We compared avoidance behavior, nematode survival, bacterial colonization, and pharyngeal pumping of wild-type and *dbl-1(nk3)* animals on our bacterial panel. Loss of DBL-1 function has a strong, specific effect on some of these *C. elegans* innate immunity-associated traits. To identify the innate immune genes regulated by DBL-1 upon exposure to our test bacterial challenges, we performed RNA sequencing of wild-type and *dbl-1(nk3)* populations exposed to *S. marcescens* and *E. faecalis* for 48 hours from the L4 stage. Preliminary results indicate that classes of genes encoding innate immune response peptides and proteins, including C-type lectins, aspartyl proteases, saposins, lipases, and lysozymes, are regulated by DBL-1 upon infection by *S. marcescens* and *E. faecalis*. These genes include many that are commonly regulated in response to these two pathogens, but others are specific to a response to one pathogen. Future work will use transcriptional reporters and real-time PCR to determine if DBL-1 regulates select target genes upon exposure to the other test Gram-positive and Gram-negative bacteria. This work will expand our knowledge about how the DBL-1/TGF-β pathway protects animals from a variety of immune challenges.

1993A An *in vivo* small molecule screen to identify therapeutics for NGLY1 deficiency *Kevin Hope*[1], Randall Peterson[2], Clement Chow[1] 1) University of Utah, Human Genetics; 2) University of Utah, College of Pharmacy.

Rare diseases affect more than 30 million Americans, however, treatment options are limited or non-existent in approximately 90% of cases. NGLY1

deficiency, a rare disease with no effective treatment, is caused by loss-of-function mutations in the *N-glycanase 1* (*NGLY1*) gene and is characterized by global developmental delay, hypotonia, alacrima, and seizures. NGLY1 is a deglycosylase that removes GlcNAc sugar chains from glycoprotein substrates, and is thought to play a role in endoplasmic reticulum associated degradation (ERAD) by deglycosylating misfolded glycoproteins prior to degradation by the proteasome. Recent work demonstrated that the transcription factor NRF1 is translated into the ER membrane, glycosylated, retrotranslocated to the cytoplasm, and immediately degraded. Under proteasome stress NRF1 is deglycosylated by NGLY1, allowing it to be cleaved and subsequently act as a transcription factor to upregulate proteasome genes. Therapeutic discovery approaches targeting the known interaction between NGLY1 and NRF1 have yielded limited results, and effective treatments are still lacking. In this study we took an unbiased, drug repurposing approach to limit the time and cost associated with drug development. To identify FDA approved drugs amenable for repurposing in NGLY1 deficiency, we conducted an *in vivo*, whole organism screen of the Prestwick Chemical Library (1520 compounds, 99% FDA/EMA approved) for molecules that rescue lethality in NGLY1 deficient flies. *In vivo* screens account for complex interactions between tissue types that are lost in other screening approaches to facilitate the identification of compounds that are orally available, metabolically stable, and low in toxicity. Additionally, this screen focused on FDA approved compounds because these drugs have known safety profiles, are biologically active, and limit the time and cost to bring a treatment into the NGLY1 deficiency population. The results from this ongoing screen will be presented. This project demonstrates the utility of *Drosophila* in rare disease modeling and drug development, and similar strategies can be taken to address other rare diseases.

2015B Selective vulnerability of dopaminergic neurons revealed by genome wide analysis *Jacinta Davis*[1], Claire Da Silva Santos[1], Linette Zavala[1], Nicholas Gans[1], Daniel Patracuolla[1], Daniel Babcock [1] 1) Lehigh University, Bethlehem, PA .

The hallmark of Parkinson's Disease (PD) is the loss of dopaminergic (DA) neurons in the brain. However, little is known about why DA neurons are selectively vulnerable to PD. To identify genes that are associated with DA neuron loss, we screened through over 200 wild-caught populations of *Drosophila melanogaster* as part of the Drosophila Genetic Reference Panel (DGRP). Here we identify the top associated genes containing SNPs that render DA neurons vulnerable. We tested these genes further by using mutant analysis and tissue-specific knockdown for functional validation. We found that this loss of DA neurons caused progressive locomotor dysfunction in mutants and gene knockdown analysis as well. We also investigate *sestrin,* one of the most significant candidates from our screen. As Sestrin is a known regulator of TOR signaling, we examine the mechanism by which the loss of Sestrin results in DA neuron loss. Further analysis of these genes should help to identify the factors that render DA neurons selectively vulnerable in conditions such as PD.

2022C Examining Mechanisms of Nuclear Pore Complex Disruption in Neurodegeneration *Kirstin Maulding*[1], Sandeep Dubey[1], Kai Ruan[1], Thomas Lloyd[1] 1) Johns Hopkins University.

Disruption of the nuclear pore complex and of nucleocytoplasmic transport have been increasingly implicated in neurodegenerative disease pathogenesis, including in ALS, Alzheimer's disease, and Huntington's disease. In addition, cell cycle re-entry has been linked with many of the same disorders. We propose that neuronal cell cycle re-entry may drive nuclear pore disruption, as it is well known that dividing cells must disassemble their nuclear pore. We aim to examine the link between these two processes, as well as signaling pathways upstream of cell cycle re-entry and potential interactions with fly neurodegenerative models such as our C9orf72 ALS model. To do this we will assess the ability of cell cycle re-entry to cause nuclear pore disruption, and of nuclear pore disruption to cause neurodegeneration via use of Gal-4/UAS driven overexpression and RNAi constructs against cyclin/CDKs and nucleoporins. We will also assess the ability of these manipulations to modify phenotypes in our ALS models. Our results suggest that Wg signaling is upstream of cell cycle re-entry, as upregulation of Wg signaling using the Gal-4/UAS system leads to altered expression of Nup214 and Nup98 as well as defects in nucleocytoplasmic transport of a shuttle GFP construct. In addition, Wg signaling is upregulated in our ALS model. These studies have the potential to establish a novel link between these processes in a broad array of neurodegenerative diseases, which may suggest a novel therapeutic target.

2026A Genetic and chemical modulation of the immune deficiency pathway influences the immune response and neurodegeneration in a *Drosophila* model of Machado-Joseph Disease *John Warrick*[1], Ethan Fenton[1], Lizzie Godscall[1], Grace Ward[1] 1) Univ Richmond.

Machado-Joseph Disease (SCA-3), a member of the polyglutamine family of human neurodegenerative diseases, causes neurodegeneration through poorly understood mechanisms. In human neurodegenerative diseases, it has been suggested that inflammation may contribute to neurodegeneration. To test this hypothesis, we have modulated the expression of Relish, a nuclear transcription factor and *Drosophila* homolog of NF-kB, which regulates the immune response through the immune deficiency pathway. Increased expression of activated Relish increases degeneration. Preliminary data suggest that up and down regulation of Dredd, a caspase which activates Relish, also influences neurodegeneration. Curcumin, a biologically active polyphenolic compound, known antioxidant, and an anti-inflammatory molecule in vertebrates was shown to slow degeneration in preliminary data. In vertebrates it prevents NF-κB from entering the nucleus and preventing an inflammatory response. However, curcumin did not prevent Relish from entering the nucleus in our model. These data suggest that neurodegeneration in MJD may be influenced by reducing inflammation or by increasing antioxidants.

2057B Toll signaling in the *Drosophila* larval fat body shifts programs of anabolic lipid metabolism and dysregulates nutrient storage *Brittany Martínez*[1], Rosalie Hoyle[1], Scott Yeudall[1], Norbert Leitinger[1], Michelle Bland[1] 1) University of Virginia, Department of Pharmacology.

Activation of the innate immune Toll signaling pathway leads to synthesis and secretion of antimicrobial peptides that kill invading pathogens. During an immune response, the *Drosophila* fat body secretes AMPs in quantities that can reach concentrations of hundreds of micromolar in hemolymph. The fat body is also a critical metabolic organ that stores dietary nutrients as glycogen and triglycerides. How these two processes – mounting an immune response and regulation of energy metabolism – are coordinated within the fat body is poorly understood. To address this question, we chronically activated Toll signaling in the fat body by expressing a constitutively-active Toll receptor, Toll^{10b}, and assessed lipid metabolism. Expression of Toll^{10b} in the fat body leads to a tissue-autonomous decrease in triglyceride levels. Key fatty acid synthesis enzymes, such as *acetyl co-A carboxylase*, *ATP citrate lyase*, and *fatty acid synthase*, exhibit normal expression in animals with active Toll signaling. However, we observe 50% decreases in fat body transcript levels of both *lipin*, a phosphatidic acid phosphatase, and *midway,* the homolog of diacylglycerol acyltransferase, both of which carry out triglyceride synthesis. In contrast to effects on triglyceride storage, Toll signaling in fat body increases levels of fat body phosphatidylethanolamine and phosphatidylcholine. These increases are mirrored by 2.5-fold increases in transcript levels of key phospholipid synthesis enzymes, such as *easily shocked,* an ethanolamine kinase homolog, and *Pcyt1*, a phosphocholine cytidylyltransferase, in Toll^{10b}-expressing fat bodies. Overall, chronic Toll pathway activity leads to an anabolic shift from triglyceride storage to phospholipid synthesis. A potential role for

increased phospholipids may be to support endoplasmic reticulum (ER) function to sustain AMP biosynthesis required to combat infection. Indeed, transcript levels of ER resident proteins are increased in fat bodies expressing Toll^{10b}, suggesting that Toll signaling drives ER expansion. Transmission electron microscopy analysis of fat bodies with chronic Toll signaling shows extensively dilated ER that takes up 40% more volume within the cell compared with control fat bodies. Conversely, there is 40% less volume of organelle-free cytoplasm in Toll^{10b}-expressing fat bodies compared with controls. Together, these results point to a switch from triglyceride storage to phospholipid biogenesis during chronic Toll activation that may allow the fruit fly to expand ER machinery to make sizeable quantities of AMPs for fighting off invading pathogens. Better understanding of how this anabolic switch in lipid metabolism is induced, as well as the long-term consequences of reduced triglyceride storage at the expense of phospholipid synthesis, should yield insight into metabolic diseases that stem from chronic inflammation.

2083A A genetic screen to identify the roles of human genes in *Drosophila* *Lily Paculis*[1], Ashley Avila[1], Susan Iherjirika[1], Dongyu Jia[1] 1) Georgia Southern University.

The tissue formation in an organism is strictly regulated by orchestrated gene expression. Alteration of gene expression can often lead to abnormal tissues, which sometimes develop into cancers. In recent years, many human genes have been identified to play a variety of roles in the tissue formation and progression of cancers. Due to its cheaper and quicker genetic manipulations and conserved genome, *Drosophila* has been developed as various human disease models. In our lab, we conducted a simple screen to identify the roles of hundreds of human genes in *Drosophila* by observing phenotypic changes of the eye morphology. Virgin female GMR-GAL4 flies were crossed with males containing human genes obtained from Bloomington *Drosophila* Stock Center. Offspring produced from the crosses with variable eye phenotypes were observed using both a light microscope and a SEM microscope. We found many genes altered the eye morphology, including ones that cause loss of eye bristles, extra eye bristles, ommatidia apoptosis, rough eye, reduction of eye size, and disorganization of ommatidia. All the finding suggest the human genes can be involved in altered tissue development and formation of the fly eyes. This screening also allowed us to identify which human genes will be of interest to experiment and analyze further with the fly model. Using this screen, we demonstrate how hundreds of human genes can be rapidly tested and evaluated for their interference with the gene expression in flies through morphological examination.

2086A *De novo* Mutations in *TOMM70*, a central receptor of the mitochondrial import translocase, Causes Developmental Delay and Neurological Phenotypes *Debdeep Dutta*[1,2], Lauren C. Briere[3], Paul C. Marcogliese[1,2], Oguz Kanca[1,2], Melissa A. Walker[4], Frances A. High[3], Adeline Vanderver[5,6], Cansu Kücükköse[7], Nora Vögtle[7], Joel Krier[8], Nikkola Carmichael[8], Christine Callahan[9], Ryan J. Taft[5,10], Cas Simons[5,11], Guy Helman[5,11], Michael Wangler[1,2,12], Shinya Yamamoto[1,2,12,13], David A. Sweetser[3], Hugo J. Bellen[1,2,12,13,14] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX 77030, USA; 3) Division of Medical Genetics and Metabolism, Department of Pediatrics, Massachusetts General Hospital for Children, Harvard Medical School, Boston, MA 02114, USA; 4) Department of Neurology, Massachusetts General Hospital, Boston, MA 02114, USA; 5) Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA; 6) Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA; 7) Institute of Biochemistry and Molecular Biology, University of Freiburg, 70104 Freiburg, Germany; 8) Brigham Genomic Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA; 9) Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA; 10) Illumina, Inc., San Diego, CA 92121, USA; 11) Murdoch Children's Research Institute, The Royal Children's Hospital, Parkville, Victoria 3052, Australia; 12) Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, USA; 13) Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA; 14) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA.

More than 1000 mitochondrial proteins are imported into mitochondria. The Translocase of Outer Mitochondrial Membrane (TOMM) complex is the entry gate for virtually all mitochondrial proteins and essential to build the mitochondrial proteome. TOMM70 as a central receptor assists mainly in the import of proteins with internal mitochondrial targeting signals. To date, there have been no reports of disease related to any of the TOMM proteins. Here, we identified two individuals with a childhood onset neurological disorder that displays behavioral symptoms, physical signs and medical imaging evidence of ongoing neurodegenerative brain disease that had no explanation prompting their evaluation and genomic sequencing in the Undiagnosed Diseases Network or at Children's Hospital of Philadelphia. Both patients were ultimately found to have de novo (i.e. mutations in their germline not identified in their biological parents) missense changes in highly conserved residues of the human *TOMM70* gene (p.Thr607Ile and p.Ile554Phe) raising the possibility that they have a novel syndrome related to *TOMM70* mutations.
To functionally assess *TOMM70* variants, we used a humanization strategy in the fruit-fly (*Drosophila melanogaster*). *TOMM70* is highly conserved in fruit-flies, and fly *Tom70* is expressed in the larval and adult brain of fruit-fly. We replaced the entire *Tom70* ORF with a Kozak-MiniGAL4 transgene using CRISPR. These animals die as pupae, but the lethality is rescued by the MiniGAL4 driven expression of human reference UAS-*TOMM70* cDNA. However, both patient variants lead to significantly less rescue indicating that they are loss-of-function alleles. *RNAi*-mediated knock-down of *Tom70* in the developing eye causes smoothened regions and necrotic spots. Electroretinograms (ERGs) of flies with *RNAi*-mediated knock-down also revealed a severe synaptic transmission defect. Co-expression of human reference *TOMM70* in the fly *Tom70 RNAi* background, partially rescues the eye morphology defect and fully rescues the ERG defects. However, the patient variants fail to rescue these defects, further supporting the notion that these variants are indeed loss-of-function alleles.
Altogether, our data indicates that loss-of-function mutations in *TOMM70* results in variable white matter disease and neurological phenotypes in patients. Hence, *Drosophila* can be used as a valuable model to study the human disease(s) associated with the TOMM complex.

2097C Exposure to Environmental Triggers Results in Disease Signs in Rats Carrying a Human *ATG16L1* Crohn's Disease Susceptibility Variant *Kari Chesney*[1,2], Marcia Hart[4], Elizabeth Bryda[1,2,3] 1) Comparative Medicine Program, University of Missouri; Columbia, MO, United States; 2) Area Pathobiology, University of Missouri; Columbia, MO, United States; 3) Rat Resource and Research Center, University of Missouri; Columbia, MO, United States; 4) IDEXX BioAnalytics, Columbia, MO, United States.

Crohn's disease (CD) is one of two chronic inflammatory bowel diseases (IBD) that affect the lining of the gastrointestinal system. Several environmental factors, either through acute insult or chronic exposure, contribute in large part to the multifactorial etiology of this disease. Using CRISPR-Cas9 genome editing technology, our laboratory generated a rat strain carrying a human variant for CD in the *ATG16L1* autophagy gene (F344-*Atg16l1^{em8}* – hereby referred to as *em8*). To determine whether CD onset could be triggered in this rat strain, we performed two studies, one acute and one chronic, in which we exposed heterozygous (HET) *em8* rats and their wild type (WT) litter mates to known triggers of CD. For the acute study, rats were orally gavaged with 10 mg/kg of the NSAID diclofenac once per day for 7 days to simulate a short-term course of high-dose

NSAID administration in humans. We found that HET *em8* rats displayed overt signs of poor health as well as gross and histologic signs of NSAID toxicity and CD-like lesions at necropsy, with females showing more severe signs of disease than males. For the chronic study, rats were either orally gavaged with diclofenac at 1.25 mg/kg once per day on a 10 day on, 7 day off cycle for 12 weeks or provided *ad libitum* Western diet formulated rodent chow as their sole source of feed for 12 weeks. We found that HET *em8* rats displayed changes in the gut microbiota composition when compared to WT litter mates. They also showed mild CD-like histologic signs in ileal and colonic tissues, with HET rats on the Western diet showing the most severe CD-like histologic lesions. Given that this rat strain carries the identical genetic susceptibility variant found in human CD patients and exhibits disease upon exposure to known environmental triggers of human disease, our findings support future use of this model to understand the underlying mechanism of *ATG16L1* and autophagy in CD.

2099B *High-throughput neuroanatomical screen uncovers 198 genes involved in mouse brain morphogenesis* *Valerie Vancollie*[1], Stephan Collins[2,3,4,5,6], Anna Mikhaleva[7], Katarina Vrcelj[8], Christel Wagner[2,3,4], Nestor Demeure[2,3,4], Helen Whitley[2,3,4,5], Meghna Kannan[2,3,4,5], Rebecca Balz[7], Lauren Anthony[1], Andrew Edwards[8,9], Hervé Moine[2,3,4,5], Jacqueline White[1], David Adams[1], Alexandre Reymond[7], Caleb Webber[10,11], Binnaz Yalcin[2,3,4,5,7], Christopher Lelliott[1] 1) Wellcome Sanger Institute; 2) Institut de Génétique et de Biologie Moléculaire et Cellulaire; 3) Centre National de la Recherche Scientifique; 4) Institut National de la Santé et de la Recherche Médicale; 5) Université de Strasbourg; 6) Inserm UMR1231 GAD, University of Bourgogne Franche-Comté; 7) Center for Integrative Genomics, University of Lausanne; 8) Woodland View Hospital, NHS Ayrshire and Arran; 9) Wellcome Centre for Human Genetics; 10) Department of Physiology, Anatomy and Genetics, University of Oxford; 11) UK Dementia Research Institute, University of Cardiff.

The Sanger Institute's Mouse Pipelines aim to make a significant impact on our understanding of gene function and their role in disease by generating, characterising & archiving knockout mouse lines. The mice are phenotyped using a battery of tests and at the end tissue samples are sent to collaborators that perform specialized screens for physical and functional abnormalities.

Here we report on the findings of a mouse brain histopathology screen which looked at the morphology of the brain, with the aim of highlighting genes that play a role in neural development and higher-order cognition in humans. Brains from 3 male mice per line were collected and fixed into blocks, to allow specific predetermined sections to be collected (either sagittal or coronal). These were double-stained (Luxol Fast Blue & Cresyl violet) before being scanned at cell-level resolution and quantatively analyzed on 118 neuroanatomical parameters.

A total of 1,566 alleles (1,446 unique genes) were examined and of these, 198 genes were found to affect areas of brain architecture implicated in brain connectivity. While 17% of these genes (e.g.: *Mcph1, Kptn, Cenpj*…) are known loci for cognitive dysfunction in the relevant human orthologues, 83% of them were previously unknown to be involved in brain morphology. Network analysis showed that these neuroanatomical phenotypes affected a diverse range of pathways and structures including the cell cycle, synapses and the cytoskeleton. The resulting phenotypes ranged from abnormal brain size (both micro- and macrocephaly), hydrocephaly, agenesis of the corpus callosum and more.

That a subset of the genes found are known to cause a variety of brain and cognitive disorders in humans is a good indication that the other 83% are prime candidates for further study of their biological and clinical relevance. They therefore compose a catalogue informing future research, both in mice and in humans.

2126B *Saccharomyces cerevisiae* var. *'boulardii'* infections: from diagnosis to in-host microevolution *Alexandra Imre*[1,2], Hanna Rácz[1,3], Péter Oláh[4,5], Zsuzsa Antunovics[6], Ilona Dóczi[7], László Majoros[8], Renátó Kovács[8,9], Ksenija Lopandic[10], Zsigmond Benkő[1], István Pócsi[1], Walter Pfliegler[1] 1) Department of Molecular Biotechnology and Microbiology, University of Debrecen, Debrecen, Hungary; 2) Kálmán Laki Doctoral School of Biomedical and Clinical Sciences, University of Debrecen, Debrecen, Hungary; 3) Doctoral School of Nutrition and Food Sciences, University of Debrecen, Debrecen, Hungary; 4) Department of Dermatology, Venereology and Oncodermatology, University of Pécs, Pécs, Hungary; 5) Department of Dermatology, University Hospital of Düsseldorf, Düsseldorf, Germany; 6) Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary; 7) Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary; 8) Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary; 9) Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary; 10) Institute of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria.

The use of probiotics is continuously on the rise due to conceived positive health effects and increased microbiome awareness, while their risk assesment still lags behind. An increasing number of infections originating from probiotic use is reported worldwide, with the majority of such cases caused by the yeast *Saccharomyces 'boulardii'*, a subtype of *S. cerevisiae*. Its frequent use poses a significant health risk for severely ill or infant patients, and for those under prolonged hospitalization. Following the genetic assessment of >60 clinical yeast isolates collected during the course of three years in Hungarian hospitals, we proposed a multiplex PCR protocol combining interdelta fingerprinting and microsatellite typing, to enable a quick and reliable identification of *S. 'boulardii'*. With this method we detected frequent probiotic origin among infectious *Saccharomyces* in all anatomical niches. The method can be applied for the identification of yeast infection sources, helping decisions on probiotic use. Subsequently, our isolate collection has been subjected to whole-genome sequencing and comparison with previously published probiotic-derived human isolates and various probiotic supplements. This revealed high uniformity among probiotic and related clinical yeasts and a low level of genome structure variations compared to other pathogenic *Saccharomyces*.
To facilitate probiotic strain improvement and risk assessment, we investigated the genetic basis of phenotypic adaptations in clinical isolates of the probiotic yeast and conducted *in vivo* microevolutionary experiments in mice. We performed stress-phenotyping of subclones retrieved from mice in order to reveal the traits under selection during pathogenic lifestyle. Our data suggests that the clinical isolates and subclones derived from the experimental infection show adaptations to higher osmotic stress and altered cell wall composition compared to the commercial probiotics, leading to increased survival in our mammalian model. Based on these results we explored the potential application of genetic engineering tools to create probiotic yeasts unable to adapt to the host environment outside the gut. Such a strain would be unable to cause systemic infections, in contrast to currently marketed products with questionable safety.

2144B High-throughput yeast screening reveals a new dimension of intracellular pathogenesis. Malene Urbanus[1], Harley Mount[1], Dylan Valleau[1], Eleanor Latomanski[4], Frederick Roth[1,3], Hayley Newton[4], Alexei Savchenko[2], *Alexander Ensminger*[1] 1) University of Toronto, Toronto, ON Canada; 2) University of Calgary, Calgary, AB Canada; 3) Lunenfeld-Tanenbaum Research Institute, Mt. Sinai Hospital, Toronto, ON Canada; 4) University of Melbourne, Peter Doherty Institute for Infection and Immunity, Melbourne, VIC Australia.

A central pillar of molecular pathogenesis is that bacteria inject proteins (effectors) into the host cell in order to modulate host proteins. An extreme example of this comes from *Legionella pneumophila*, an intracellular bacterial pathogen that injects over 300 proteins into the host cell during infection. Using yeast as a genetically tractable proxy for the eukaryotic host, we recently uncovered a novel class of effectors in *Legionella pneumophila*: "metaeffectors" (or "effectors of effectors") that break the rules by targeting other effectors rather than host proteins. This is a dimension of microbial biology that was not widely considered prior to our work and may be one explanation for the large size of *L. pneumophila*'s effector arsenal. Having first established metaeffector-discovery methodologies *in L. pneumophila* (300+ effectors), we have recently extended our search to other bacterial pathogens (Coxiella, Chlamydia, Yersinia) with promising results.

We will present our data resulting from two systems-level approaches to identifying metaeffectors and other effector-effector interactions. In the first ever comprehensive screen for metaeffectors, we used yeast to screen all 112,000 possible pairwise genetic interactions between a library of 330x330 *L. pneumophila* effectors. We identified all previously described instances of effector-effector antagonism as well as nine previously unknown metaeffectors. In a second approach, we have modified a high-throughput protein-protein interaction assay (BFG-Y2H) to directly measure all possible pairwise physical interactions between effectors. These studies have already informed several detailed structure-function studies of previously uncharacterized effectors, revealing new activities against the host and a diversity of effector regulatory mechanisms. These discovery pipelines are now primed to extend this work to a variety of other pathogens.

Our data suggest that metaeffector activity is a common feature of bacterial pathogenesis that has been hiding in plain sight. Backed by the awesome power of yeast genetics, we are beginning to shine a light on this new dimension of bacterial pathogenesis.

2145C Patient derived xenografts in zebrafish embryos (Zevatars) demonstrate differential drug responses to pancreatic cancer chemotherapeutics *Ceylan Metin*[1], Shaila Mudambi[1], Kamden Gray[1], Seray Er[1], Saumya Kasliwal[1], Michael Pishvaian[1], Stephan Byers[1], Eric Glasgow[1] 1) Georgetown University.

Cancer is currently the second leading cause of death in the world. Given that cancer is a highly individualized disease, it is very difficult to accurately predict the best chemotherapeutic treatment for individual patients. Ex vivo models such as mouse PDX and organoids are being developed to determine patient tumor responses before treatment in the clinic. Although promising, these models pose significant disadvantages including long growth times that introduce tumor genetic changes and high costs. To overcome these issues, we have developed a zebrafish xenograft assay (Zevatars) to evaluate the efficacy of chemotherapies on patient biopsies in a model that recapitulates the original tumor as closely as possible. Our preliminary focus is pancreatic cancer, the most lethal solid malignancy. Despite some recent progress in treatment, the median overall survival still remains less than one year. There is an urgent need to improve the efficacy of pancreatic cancer treatments survival of these patients. We have used fragments of primary pancreatic ductal adenocarcinoma (PDAC) or liver metastases from patient tumor biopsies for our assay. These samples are cut into 50µm pieces, fluorescently labelled, and implanted into hundreds of 2-day-post-fertilization (dpf) stage embryos. The implanted embryos are then treated with maximum tolerated doses of gemcitabine+abraxane and folfirinox (current standard of care drugs) and are imaged immediately after treatment. The embryos are re-imaged 2-5 days post-implantation to evaluate tumor growth, cell migration and metastases. Chemotherapy tumor response is assessed by change in tumor area and presence of metastasis using ImageJ software. Due to the transparency of the Zevatars, we can easily visualize rapid vascularization of the tumors and detect metastases at distal sites within five days. Currently, we have implanted over 1000 embryos with pancreatic cancer and liver metastases patient biopsy samples and have demonstrated that our assay is capable of determining differential drug responses, and is effective in doing so with either fresh or cryopreserved tissue. The Zevatar assay is ideal for large-scale chemotherapy screening and is a rapid, cost-effective method to positively impact prescription of effective chemotherapies for individual cancer patients.

Tuesday, April 28 12:00 PM - 3:30 PM

Gene Regulation/Disease Models and Aging 1- Poster Q&A 760A A Neuronal Atlas of RNA-Binding Protein Expression and Localization at Single-Cell Resolution *John Laver*[1], Eviatar Yemini[2], Oliver Hobert[2], Mihail Sarov[3], John Calarco[1] 1) Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada; 2) Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University, New York, NY, USA; 3) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

The nervous system is composed of many different types of neurons, each unique with regard to their functions and morphologies. The specification, development, and function of neurons and neuron subtypes require precise post-transcriptional control of gene expression, through regulation of mRNA splicing, localization, translation, and stability. These processes are regulated by *trans*-acting factors, such as RNA-binding proteins (RBPs) and small RNAs, hundreds of which are encoded in eukaryotic genomes. To gain a systems-level understanding of post-transcriptional regulation in the nervous system, we aim to define a comprehensive atlas of neuronal RBP expression in a whole organism, the nematode *Caenorhabditis elegans*, with single-cell resolution. To assess neuron-specific RBP expression and subcellular localization, we are using a microscopy-based approach, visualizing RBPs in *C. elegans* by expressing GFP-tagged RBP transgenes driven by endogenous regulatory elements. An initial survey of 40 RBPs has revealed a diversity of expression patterns: one-third of the RBPs exhibit expression restricted to particular subsets of neurons, and at the subcellular level, approximately equal proportions of the RBPs localize to either the cytoplasm, the nucleus, or both, with some RBPs displaying different subcellular localization patterns in different neurons. We have now begun to define the expression of each RBP with single-neuron precision, using NeuroPAL, a multi-colour neuronal identification strain in which individual neurons are marked with up to four different fluorescent proteins. A complete understanding of where and when each RBP is expressed in the nervous system will yield important insights into the RBP regulatory networks controlling neuronal development and function.

764B Genome-wide patterns of histone H2A ubiquitylation and effects on *C. elegans* developmental timing Daniel Fusca[1], Kailynn MacGillivray[1], Reta Aram[1], Arneet L. Saltzman[1] 1) Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada.

Histone H2A ubiquitylation is a post-translational modification associated with the regulation of gene expression and development. In *Drosophila* and mammals, Polycomb Repressive Complex 1 (PRC1) is responsible for H2A ubiquitylation (H2AK119ub) and is believed to work cooperatively with PRC2-mediated histone H3 lysine 27 trimethylation (H3K27me3) to repress gene expression. However, the distribution of H2A ubiquitylation across the genome and its role in developmental gene regulation are not fully understood. Our ChIP-seq profiling in *C. elegans* embryos revealed

unexpected patterns of H2AK119ub (H2Aub) enrichment. Many H2Aub peaks did not colocalize with H3K27me3 and were instead associated with H3K4me1 and H3K27ac, histone modifications associated with enhancer chromatin states. We also found that H2Aub was enriched at promoter regions of highly expressed genes and that a significant proportion (146/1929) of H2Aub-associated genes had functional annotations related to nervous system development. These findings are consistent with roles for the putative *C. elegans* PRC1 subunit homologues mig-32 and spat-3 in neuronal migration and axon guidance (Pierce *et al*. 2018 PNAS; Karakuzu *et al*. 2009 Development). We also found that mutations of *spat-3* or *mig-32*, which greatly reduce H2Aub levels, led to delayed and variable developmental timing. Using RNA-seq, we also identified 99 genes that were significantly differentially expressed in both mig-32 and spat-3 mutants, 36% of which were associated with promoter-proximal H2Aub enrichment. Our results reveal the relationship between histone H2AK119ub and chromatin states across the *C. elegans* genome and suggest that H2A ubiquitylation plays a role in the regulation, and potential activation, of developmentally important genes.

765C An RNAi screen to identify factors that enhance microRNA activity after dauer in *C. elegans* *Himal Roka (Pun)*[1], Xantha Karp[1] 1) Central Michigan University, Mount Pleasant, MI.

Stem cells have the ability to self-renew and differentiate into specialized cell types. Quiescence, a temporary cell cycle arrest, is an important feature of many mammalian adult stem cells. Adult stem cells maintain multipotency during quiescence, and loss of quiescence is linked to loss of stem cell maintenance. *C. elegans* larvae that develop in adverse conditions also exhibit cellular and developmental quiescence if they enter the dauer stage, which occurs after the second larval molt. Hypodermal seam cells are self-renewing and multipotent during larval development, and serve as our stem cell model. Seam cell fate is regulated by the heterochronic gene network. Transcription factors and RNA binding proteins promote early cell fates, and are downregulated by microRNAs in order to allow progression to later fates. MicroRNAs and proteins form the microRNA induced silencing complex (miRISC) that silences target genes post-transcriptionally. The miRISC complex includes core protein components such as Argonautes (ALG-1 and ALG-2) and GW182 proteins (AIN-1 and AIN-2). Loss of either heterochronic microRNAs, *alg-1,* or *ain-1* causes a "retarded" phenotype, where cell fates are reiterated and adult cell fate is delayed. Often, these mutants burst through the vulva, a phenotype that is readily observable in the dissecting microscope. Interestingly, some of these mutants develop normally after dauer. Indeed, the retarded *alg-1(0)* or *ain-1(0)* mutant phenotypes are completely suppressed in post-dauer adults, suggesting that *alg-2* or *ain-2* are sufficient to mediate microRNA activity after dauer, but not during continuous development. These data, together with other genetic data, suggest that microRNA activity is enhanced by unknown factors in post-dauer animals. In order to find genes involved in enhancing microRNA activity after dauer, we are performing an RNAi screen for genes that are required for post-dauer suppression of *alg-1(0)* retarded phenotypes. As a secondary screen, we are testing hits for their ability to suppress retarded phenotypes in *ain-1(0)* mutants. We are focusing our screen on conserved RNA-binding proteins and kinases as factors likely to impact miRISC activity.

769A The RAB-6.2 GTPase is a novel regulator of small RNAs and Ras in *C. elegans* *Sarah Gagnon*[1,2], Kimberley Gauthier[1,2], Christian Rocheleau[1,2,3] 1) Department of Cell Biology, McGill University, Montreal, Canada; 2) Research Institute of the McGill University Health Center, Montreal, Canada; 3) Department of Medicine, McGill University, Canada.

Epidermal growth factor receptor (EGFR)/Ras signaling promotes cell growth and can drive tumorigenesis when overactive. *C. elegans* vulva development can model this pathway as signaling from EGFR induces the vulval cell fate in vulva precursor cells (VPCs). As such, signaling defects translate into over- or under-induced vulval phenotypes. The RAB-6.2 GTPase regulates retrograde trafficking from the trans-Golgi network, but is otherwise poorly characterized. While *rab-6.2(-)* mutants do not show vulval defects, loss of RAB-6.2 in signaling-deficient backgrounds rescues proper vulva development, indicating restored EGFR/Ras signaling and posing RAB-6.2 as a negative regulator of this pathway. GFP-tagged EGFR localization is unchanged in the VPCs of *rab-6.2(-)* mutants, indicating that while RAB-6.2 is most well known to regulate trafficking, its regulation of EGFR/Ras does not impinge on EGFR transport. VPS-52 is a RAB-6.2 effector that promotes the activity of the *let-7* family of microRNAs (miRNAs), known to repress Ras in *C. elegans* and mammals. We thus hypothesize that RAB-6.2 negatively regulates EGFR/Ras through VPS-52 and the *let-7* family of miRNAs. We have evidence that RAB-6.2 regulates small RNAs at large, as *rab-6.2(-)* mutants show enhanced transgene silencing, a process mediated by small RNAs in *C. elegans*. Moreover, *rab-6.2(-)* mutants are hypersensitive to RNA interference, further indicating dysregulation of small RNAs. More directly in line with a RAB-6.2/VPS-52/*let-7* family of miRNAs regulatory axis, deletion of *mir-48* (a *let-7* family member) rescues vulva development in signaling-deficient backgrounds. Also, while miRNA levels are unchanged in *rab-6.2(-)* mutants, the punctate localization of a GFP-tagged ALG-1 Argonaute, a component of miRNA-induced silencing complexes (miRISCs), is lost in *rab-6.2(-)* VPCs. As punctate miRISCs are thought to be sites of active silencing, this supports decreased miRNA activity in *rab-6.2(-)* mutants and suggests that RAB-6.2 may regulate miRNAs through the recruitment of miRISC components. To further investigate miRNA regulation by RAB-6.2 and determine whether this underlies its role in EGFR/Ras signaling, we will test for altered expression of *let-7* family targets and increased signal from a Ras reporter in *rab-6.2(-)* mutants. Overall, our results pose RAB-6.2 as a regulator of small RNAs and Ras and expand our understanding of the functions of RAB GTPases and the pathways regulating small RNAs and gene expression. As these elements are implicated in numerous diseases, our research also provides the groundwork to investigate the therapeutic potential of these newly defined regulatory pathways.

794B Identification and characterization of potential enhancers of Robo2 in the *Drosophila* embryonic nervous system *Muna Abdal-Rhida*[1] 1) University of Arkansas.

Neurons, the longest cells in the human and animal bodies, are grown during nervous system development in pathways to connect the both sides of the body. These pathways are tightly controlled by specific genes which decide whether or not axons to cross the midline to the other side of the animal body. The *Drosophila* Robo2 axon guidance receptor is a member of the evolutionarily conserved Roundabout (Robo) protein family that is involved in this process. The various roles of Robo2 depend both on distinct functional domains within the receptor protein, and on the dynamic transcription of *robo2* in various subsets of cells throughout embryogenesis. Thus, understanding how Robo2 regulates distinct guidance outcomes depends in part on understanding how its expression is regulated during embryogenesis.
To determine how *robo2* transcription is regulated in distinct subsets of cells during embryogenesis, we screened a series of 17 transgenic lines in which GAL4 expression is placed under the control of putative regulatory regions derived from DNA sequences in and around the *robo2* gene. We crossed each line to a *UAS-GFP* reporter and characterized GFP expression in the embryonic ventral nerve cord during stages when axon guidance is occurring. We identified two non-overlapping DNA regulatory regions located within *robo2* that can activate transcription in distinct subsets of *robo2*-expressing lateral longitudinal neurons in the embryonic ventral nerve cord, where Robo2 acts to promote formation of longitudinal axon pathways. We also identified two additional regions that can activate transcription in midline cells including midline glia, where Robo2 acts non-au-

tonomously to promote midline crossing of commissural neurons during early stages of axon guidance.
Using these identified regulatory regions, we have built transgenic constructs which allow us to restore *robo2* expression to defined subsets of neurons to test for rescue of *robo2*-dependent axon guidance phenotypes in the embryonic CNS and to distinguish cell-autonomous vs non-autonomous roles of *robo2* in specifying distinct axon guidance outcomes. Our results suggest that *robo2's* dynamic expression pattern is specified by multiple distinct regulatory regions, and that its expression in specific subsets reflects a combination of genetically separable regulatory sequences.

829A The NR5A nuclear receptor Ftz-f1 promotes follicle maturation and ovulation via bHLH/PAS transcription factor Single-minded Elizabeth Knapp[1], *Jianjun Sun*[1] 1) University of Connecticut, Storrs, CT.

The NR5A family of nuclear receptors are highly conserved and function within the somatic follicle cells of the ovary to regulate folliculogenesis and ovulation in mammals; however, their roles in *Drosophila* ovaries are largely unknown. Here, we discover that Ftz-f1, one of the NR5A nuclear receptors in *Drosophila*, is transiently induced in follicle cells in late stages of oogenesis via ecdysteroid signaling. Genetic disruption of Ftz-f1 expression prevents follicle cell differentiation and transition into final maturation stage, which lead to anovulation. In addition, we demonstrate that the bHLH/PAS transcription factor Single-minded (Sim) acts downstream of Ftz-f1 to promote follicle cell differentiation/maturation and that Ftz-f1's role in regulating Sim expression and follicle cell differentiation can be replaced by its mouse homolog steroidogenic factor 1 (mSF-1). Our work provides new insight into the regulation of follicle maturation in *Drosophila* and elucidates the conserved role of NR5A nuclear receptors in regulating folliculogenesis and ovulation.

867C Natural variation in R-loop formation in *Drosophila melanogaster* *Timothy Stanek*[1], Christopher Ellison[1] 1) Rutgers University.

R-loops are three-stranded nucleotide structures consisting of a DNA:RNA hybrid and a displaced ssDNA non-template strand. Originally viewed as a byproduct of transcription, R-loops are now recognized as important regulators of gene expression and genomic stability. R-loops are primarily associated with active Pol II transcription but have also been found at tRNA genes, mitochondrial genes, and transposable elements. Persistent dysregulation of R-loop maintenance can result in replication stress, DNA double-strand breaks, and chromosomal rearrangements that contribute to diseases such as neurological disorders and cancer. Although R-loops are conserved across cell types in mammals, little is known about natural variation in R-loop formation between individuals and throughout development. Using DNA:RNA immunoprecipitation followed by high-throughput sequencing (DRIP-seq), we have mapped the R-loop profiles of two *D. melanogaster* individuals from the Drosophila Genetic Reference Panel (DGRP) at both embryonic and adult stages. Our current work assesses *in vivo* the underlying sequence and architectural determinants of R-loop formation and variation with respect to development and sex.

876C Nucleolar Dominance, a Locus-Level Regulation of Ribosomal DNA Expression, in *D. melanogaster* Females *Duojia Li*[1,2], Natalie Warsinger-Pepe[1,3], Yukiko Yamashita[1,4,5] 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI; 3) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 4) Cell and Developmental Biology, University of Michigan, Ann Arbor, MI; 5) Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI.

Ribosomal DNA (rDNA) codes for the catalytic RNA components of ribosomes, and is organized in tandem repeats of 100s-1000s copies in eukaryotic genomes. In *Drosophila*, rDNA loci are on the X and Y chromosomes where each locus contains ~200-250 rDNA copies. Across eukaryotes, a large-scale regulation of rDNA expression called nucleolar dominance, where individual rDNA locus is entirely silenced or activated, operates to regulate the dosage of rRNA. Nucleolar dominance was originally found to occur in interspecies hybrids and has been shown to occur within a species. Particularly in male *D. melanogaster*, the Y rDNA locus is preferentially transcribed while the entire X rDNA locus is silenced. This preferential expression/silencing, however, does not occur between two X rDNA loci in female larval brains. Previous studies were unable to comprehensively characterize female nucleolar dominance in other tissues and developmental stages due to technical limitations and the high sequence homology between rDNA repeats. Here we identify sequence variation between two X rDNA loci of *D. melanogaster* strains from geographically distinct regions and utilize these sequence differences with fluorescent *in situ* hybridization to characterize nucleolar dominance in females. We expand on previous studies and show that nucleolar dominance does not occur in X/X females in multiple tissues and throughout development. By comparing X/X/Y females vs X/X females, we further found that nucleolar dominance is not dictated by cellular sex since the Y rDNA can dominate over the X rDNA in female tissues. Utilizing compound X:Y chromosomes, we further found that the short arm of the Y chromosome (with the Y rDNA) is important for nucleolar dominance. Preliminary data suggests that the long arm of the Y chromosome is not important for nucleolar dominance and that heterochromatin components on the X chromosome may be important for female nucleolar dominance. This study begins to unravel what chromosomal regions dictate the patterns that we see in both female and male nucleolar dominance and will help us understand how this large-scale preferential silencing/expression of particular rDNA loci occurs.

919A Tbx2 mediates dorsal patterning and germ layer suppression through inhibition of BMP/GDF and Activin/Nodal signaling *Shoshana Reich*[1], Peter Kayastha[2], Sushma Teegala[2], Daniel Weinstein[1,2] 1) PhD Program in Biology, The Graduate Center, CUNY; 2) Department of Biology, Queens College, CUNY.

Members of the T-box family of DNA-binding proteins play a prominent role in the differentiation of the three primary germ layers. VegT, Brachyury, and Eomesodermin function as transcriptional activators and, in addition to directly activating the transcription of endoderm- and mesoderm-specific genes, serve as regulators of growth factor signaling during induction of these germ layers. In contrast, the T-box gene, *tbx2*, is expressed in the embryonic ectoderm, where Tbx2 functions as a transcriptional repressor and inhibits mesendodermal differentiation by the TGF-beta ligand Activin. Tbx2 misexpression also promotes dorsal ectodermal fate via inhibition of the BMP branch of the TGF-beta signaling network. Here, we report a physical association between Tbx2 and both Smad1 and Smad2, mediators of BMP and Activin/Nodal signaling, respectively. We perform structure/function analysis of Tbx2 to elucidate the roles of both Tbx2-Smad interaction and Tbx2 DNA-binding in germ layer suppression. Our studies demonstrate that Tbx2 associates with intracellular mediators of the Activin/Nodal and BMP/GDF pathways. We identify a novel repressor domain within Tbx2, and have determined that Tbx2 DNA-binding activity is required for repression of TGF-beta signaling. Finally, our data also point to overlapping yet distinct mechanisms for Tbx2-mediated repression of Activin/Nodal and BMP/GDF signaling.

951C Role of mediator subunit Med8 in ethanol tolerance of yeast Ali Nabhani[1], William Chollett[1], Jackson Valencia[1], *William Park*[1] 1) Texas A&M University.

By transforming med8Δ0 BY4741 with a series of Med8 mutants on centromeric plasmids, we found that interactions between the mediator head module subunits Med8 and Med18 and the middle module subunit 31 play a key role in ethanol tolerance. Cells were still able to grow on YPD + 6% ethanol at 30°C after deletion of the N-terminal region of Med8, which tethers Med18 and Med20 to the rest of the mediator complex. Further deletion of a region between the Med18/20 tether and the CTD binding region of Med8 blocked growth on YPD + 6% ethanol. However, it had only modest effects on growth on 2% glucose or 2% galactose without ethanol or on YPD containing 2 M glycerol, 1 M KCl or 0.05% MMS. Replacing this region with unrelated sequences in constructs lacking the Med18/20 tether also resulted in a loss of ethanol tolerance, but had little effect on growth on YPD. To further define the sequences involved, we mutated the amino acids in this region which are conserved among species that produce large amounts of ethanol under aerobic conditions, replaced nine amino acid blocks with an unrelated sequence, and also used proline mutagenesis to disrupt the conserved helical structure. Surprisingly, despite the importance of $Med8_{139-172}$ in constructs lacking the Med18/20 tether, replacing it with unrelated sequences did not have a significant effect on growth on YPD + 6% ethanol in full length Med8 constructs - which contain the Med18/20 tether However, in full length Med8 constructs, this region again became critical for ethanol tolerance when assayed in med31Δ0 cells

961A A putative condensin loading factor that controls yeast chromosome III architecture *Manikarna Dinda*[1], Ryan D Fine[1], Mingguang Li[1,2], Jeffrey S Smith[1] 1) Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, VA; 2) Department of Laboratory Medicine, Jilin Medical University, China.

Condensin plays an important and evolutionarily conserved role in mitotic chromosome condensation, three-dimensional genome organization and regulation of gene expression. Condensin is a multi-subunit complex that has natural affinity for the promoters of highly transcribed genes, and also associates with specific transcription factors. However, a general mechanism for functional loading of the complex onto chromatin remains elusive. Our lab previously demonstrated that in haploid *MATa* yeast cells, condensin and Sir2 (a histone deacetylase) both associate with the recombination enhancer (RE), a cis-acting element on chromosome III that directs donor preference of mating-type switching. Here, Sir2 locally regulates transcription of a small gene called *RDT1*, while condensin contributes to the 3-dimensional organization of chromosome III, as well as donor preference, indicating that the RDT1 promoter region acts as a locus control region (Li et al., 2019). We have now further characterized the mechanism of condensin recruitment to the *RDT1* promoter LCR, and uncovered a critical role for a non-meiotic version of the monopolin complex, known as cohibin (Lrs4 and Csm1 subunits), analogous to its known role in recruiting condensin to the rDNA locus. To test if cohibin functions more generally in condensin loading, or as a condensin accessory factor, we have performed ChIP-seq for genome-wide condensin binding sites in WT and *lrs4Δ* strains. Numerous Brn1 peaks (including *RDT1* and the rDNA) were eliminated or significantly reduced by *lrs4Δ*, consistent with the condensin loader hypothesis. Micro-C XL was then used to characterize the general effects of defective condensin recruitment on genomic conformation in a *lrs4Δ* mutant, or when the Brn1 condensin subunit was depleted using an auxin-inducible degron system. Alterations were observed on multiple chromosomes, though the most severe changes occurred on chromosome III, which had significant negative effects on the efficiency of mating-type switching, as well as donor preference. We therefore hypothesize that cohibin (Lrs4/Csm1) is indeed a condensin loader at the *RDT1* promoter region, where it establishes chromosome III conformation, potentially through a loop extrusion mechanism. Evidence for direct recruitment of condensin by the cohibin complex at other genomic sites and any possible role for cohibin in chromosome condensation are currently under investigation.

1940B PI3K/Akt signaling influences dopamine function in *C. elegans* *Meretta Hanson*[1], Stephanie Fretham[1] 1) Luther College.

Phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway integrates external and internal cues such as insulin and insulin-like growth factors, nutrients, energy levels, and oxidative stress to regulate cellular functions necessary for growth, plasticity, and stress responses. PI3K/Akt signaling is altered in many different neurological conditions though its role in these diseases is not clear. In order to better understand the how disruptions in PI3K/Akt signaling affect neuronal function this study examined dopamine in wild type and mutant *Caenorhabditis elegans* (*C. elegans*). Wild-type (N2) and strains with mutations causing increased and decreased PI3K/Akt activity (*daf-18* and *age-1*) were cultured on standard nematode growth media. Dopamine-dependent basal slowing behavior and tap habituation was reduced relative to control in young adult *daf-18* mutants and enhanced in *age-1* mutants. Furthermore, HPLC quantification of dopamine demonstrated reduced dopamine levels in *daf-18* but not *age-1* mutants. Together, these findings demonstrate an inverse relationship between PI3K/Akt signaling activity and dopamine function and suggest that pathway activity may play a role in dopamine production or dopaminergic neuron survival.

2042B Fs(2)Ket rescues hTDP-43^{M337V}-mediated toxicity in Drosophila *Deepak Chhangani*[1], Lorena de Mena[1], Edward HoLostalo[1], Pedro Fernandez-Funez[1], Diego Rincon-Limas[1] 1) Department of Neurology, University of Florida, Gainesville, FL.

Tar DNA binding Protein-43 (TDP-43) is a major DNA/RNA binding protein involved in multiple cellular processes including transcriptional regulation, mRNA splicing and stress granules formation. Mutations in TDP-43, such as TDP-43^{M337V} cause Amyotrophic Lateral Sclerosis (ALS). Toxic TDP-43 aggregates significantly contribute to Frontotemporal Dementia and Alzheimer's disease also. Despite of its role in several devastating diseases, toxic properties of TDP-43 are less understood, and hence, lesser is known about modifiers of its toxic effects. Here, we performed the first ever genetic screen of over six thousand next generation RNAi lines against a Drosophila model expressing human TDP-43^{M337V}. We found ~200 genetic modifiers of TDP-43 toxicity in our model using fly eye phenotype as screening platform. We discovered nuclear transport related genes enriching as second largest ontology group among all the modifiers. Most of the modifiers from nuclear transport category were suppressors, but interestingly, an RNAi against importin Fs(2)Ket enhanced the phenotype. We further validated our results by using an overexpression line of Fs(2)Ket, and established it as a strong suppressor. We also proved that overexpression of Fs(2)Ket suppresses phospho-TDP-43 staining, and reduces toxicity in fly CNS, which leads to partially restoring the lifespan and relative locomotor activity. We are currently working on additional nuclear transport-related modifiers to unravel the pathway more comprehensively. We anticipate that modifying or altering the nuclear transport pathways in neuronal cells can suppress toxic effects caused by mutant TDP-43. Our results provide evidences suggesting nuclear import as modifier of TDP-43^{M337V} toxicity *in vivo*. Testing Fs(2)Ket orthologs, along with other nuclear transport related proteins in higher animal models, may lead to development of potential therapeutic approaches against such neurodegenerative conditions. This work was supported by NIH grant AG059871 to DERL.

2093B Differential gene and protein expression from the aging heart of Diversity Outbred mice *Isabela Gerdes Gyuricza*[1], Kwangbom Choi[1], Duy Pham[1], Andrew Deighan[1], Gary Churchill[1] 1) The Jackson Laboratory, JAX, Bar Harbor, ME.

The Diversity Outbred (DO) mice are a source of genetic variability and a robust tool to study human phenotypes. In humans, about one quarter of differences in life expectancy are explained by genetic factors, and cardiovascular health appears to be the key for longevity. Based on that, this

work aimed to investigate the molecular pathways involved in the aging heart of DO mice. To address that we used transcriptomics and proteomics data from mice on three different ages: 6, 12 and 18 months, and performed the differential expression followed by enrichment analysis. We obtained 2287 differentially expressed genes involved in 4 main pathways. The pathways showed reduced expression of genes associated with muscle contraction, fatty acid metabolism and cardiomyocytes survival with aging. Concurrently, we observed higher expression of genes associated with inflammation, cellular proliferation and fibrosis. For the differential protein expression analysis, we found 1240 proteins and 3 enriched pathways, including oxidative phosphorylation, intracellular protein transport and positive regulation of cellular proliferation. We observed reduced expression of *Actn3* with age, which is associated with skeletal muscle metabolism and oxidative phosphorylation induction but has not been described in the heart. We also found increased expression for subunits of complexes II, III and IV of mitochondria respiratory chain, as well as proteins associated with mitochondria membrane transport (translocase of the inner mitochondrial membrane complexes). These findings show the predominance of mitochondrial metabolism in the differential protein expression analysis, suggesting this might be the primary cause of changes in the heart with age, whereas the pathways found in the gene expression analysis seem to be an adaptive effect.

2125A Understanding how nuclear genetic variation in a population can affect mutant, *ND2* gene phenotypes associated with complex-I mitochondrial diseases using *Drosophila melanogaster* *Valeria Aizen*[1], Ben Harrison[1], Daniel Promislow[1] 1) University of Washington, Department of Pathology.

Mitochondrial diseases are frequently characterized by mitochondrial myopathy, encephalomyopathy, lactic acidosis, and stroke-like symptoms. Leading causes of mitochondrial diseases are mutations affecting the assembly of complex-I, reducing its ability to couple electron transfer to proton pumping, ATP production, and efficient energy production. The *ND2* (mitochondrially encoded NADH dehydrogenase 2) gene encodes the production of the ND2 protein, a subunit of complex-I. In *Drosophila melanogaster*, the $ND2^{del1}$ mutation is associated with signs of progressive neurodegeneration and paralysis, similar to the the effects of mitochondrial Complex I mutations in humans. These behaviors are known as 'bang sensitivity', characterized by sudden paralysis after *Drosophila* are vigorously shaken in a test-tube (bang assay). The objective of our research was to understand how nuclear genetic variation present in a population can exacerbate or ameliorate the effects of the *ND2* gene mutation. To model genetic variation in a large population, we used the Drosophila Genetic Reference Panel (DGRP). These consist of 200 fully sequenced, inbred lines derived from a genetically diverse wild population. Preliminary experiments using 164 DGRP lines crossed to ND2 demonstrated significant variation in average recovery times for flies across lines (Kruskal Wallis: $\chi^2 = 2357.8$, df= 166, $P < 2.2x10^{-16}$). These results also demonstrated no background effect of DGRP alone on bang sensitivity. To test for reproducibility and account for possible confounding factors, we crossed males from 20 DGRP lines exhibiting maximal and minimal recovery times to ND2 females who were previously backcrossed to w1118 (wild type) flies all within the same batch. Progeny were collected and at 21 days, subjected to bang assays, and their recovery times recorded. Our results demonstrated significant variation across differing genotypes (Kruskal Wallis: $\chi^2 = 266.85$, df = 21, P < 2.2e-16). Furthermore, results from the two assays were correlated (Pearson's $r = 0.69$, df = 20, $P < 0.0004$), demonstrating significant reproducibility in median recovery time across genotypes. Collectively, these results point to significant epistatic interactions between nuclear and mitochondrial alleles for a mitochondrially encoded mutation associated with neurodegeneration. Previous studies have demonstrated links between neurodegenerative disease susceptibility, variable NAD^+ concentrations, and complex-I dysfunction. Furthermore, our unpublished research has identified correlations between the *Drosophila* metabolome and age-related disease phenotypes. As such, future research will test the hypothesis that variation among genotypes is associated with variable NAD and other metabolite profiles.

2141B Anti-prion systems in *Saccharomyces cerevisiae* cure most prion variants as they arise *Reed Wickner*[1], Moonil Son[1], Songsong Wu[1], Herman Edskes[1] 1) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

[PSI+] and [URE3] are yeast prions composed of amyloid filaments of Sup35p and Ure2p, respectively. These amyloids have an in-register, folded, parallel beta-sheet architecture, but each prion has a large number of variants consisting of the same protein but in amyloids of distinct conformation. Each prion variant is rather stably propagating, and may differ in pathogenicity, intensity of the prion phenotype, stability of propagation, ability to cross inter-species or intra-species barriers, or sensitivity to overproduction or deficiency of various chaperones and other cellular components. We have shown that there are at least five normal anti-prion systems, meaning systems that eliminate prions as they arise, without artificial over-expression or deficiency of any cellular components.

Btn2p and Cur1p cure ~90% of variants of [URE3] prions that arise in their absence. Btn2p cures by collecting Ure2p amyloid at one place in the cell, increasing the probability of prion loss on cell division. Hsp104 has a disaggregation activity necessary for prion propagation, and a second activity producing asymmetric segregation of amyloid filaments. This latter activity cures most [PSI] prions as they arise. The Upf1,2,3 proteins responsible for nonsense-mediated decay normally form a complex with Sup35p on ribosomes, and thereby block the propagation of most [PSI+] variants that arise in the absence of any one of the Upf proteins. Siw1p is a pyrophosphatase specific for 5-pyrophospho-inositol polyphosphates, and by limiting the levels of such IP-PPs, prevents the propagation of some [PSI+] variants that need a higher level of these compounds.

We are currently screening for more anti-prion components, examining the effects of multiple defects of anti-prion systems and seeking the mechanism of the effects of inositol polyphosphates on the [PSI+] prion.

Tuesday, April 28 12:00 PM - 3:30 PM

Gene Regulation - Poster Q&A 627C Improved enhancer discovery in *Drosophila* and other insects *hasiba asma*[1], Chad M. Jaenke[2], Michael L. Weinstein[2], Thomas M. Williams [2], Marc S. Halfon[1,3,4] 1) University at Buffalo - State University of New York ; 2) University of Dayton; 3) NYS Center of Excellence in Bioinformatics and Life Sciences; 4) Roswell Park Comprehensive Cancer Center.

Enhancer (or "*cis*-regulatory module," CRM) identification is critical for understanding transcriptional regulation. We previously developed a computational method, SCRMshaw, that discovers CRMs with a high rate of true positive predictions. SCRMshaw uses the wealth of known *D. melanogaster* CRMs as training data to facilitate CRM discovery in not just *Drosophila* but in diverse holometabolous insects including mosquitoes, beetles, and bees. We present here three approaches for increasing SCRMshaw's effectiveness.
pCRMeval: We developed a pipeline, *pCRMeval*, that provides a general *in silico* evaluation framework to be applied to SCRMshaw or any other CRM prediction method. *pCRMeval* compares prediction results with the existing extensive corpus of validated *Drosophila* CRMs to calculate recovery of true CRMs and estimate the specificity of a given method. *pCRMeval* can also assess the performance of a specific training set in terms of

both sensitivity and specificity.
WCC score: Despite SCRMshaw's aggregate strong performance, it is difficult to discriminate between true and false positives at the level of individual predictions, absent empirical testing. We are developing a method to assign each predicted CRM a "Weighted Comparative Confidence" (WCC) score. This score compares predictions from groups of closely-related species to determine whether CRMs are predicted in a common genomic location (assessed by proximity to conserved sequence blocks). The greater the number of genomes with a common prediction, the higher the WCC score for that prediction, weighted by the evolutionary divergence between the considered species. Incorporating the WCC score will give a confidence measure not just to each predicted enhancer, but also to each training set (providing a means of evaluation in addition to *pCRMeval*).
Iterative searching: In order to identify CRMs within a gene regulatory network (GRN) for a rapidly evolving *Drosophila* pigmentation trait, we used SCRMshaw on a small training set of 7 CRMs. Empirical testing of 18 predictions revealed 10 new CRMs with pigmentation GRN-like regulatory activity. These new CRMs were combined with the original 7 and the 2.5-fold expanded training set used for a new round of SCRMshaw prediction. Notably, the top prediction results from this set did not include the sequences shown to be false-positives by empirical testing, but did contain all the previous true positives. This suggests that iterative approaches can serve to augment weak training sets to improve true-positive:false-positive ratios.

748A Gene expression analysis and temporal ablation of AMA-1/PolII show that transcriptional regulation supports survival deep into starvation *Amy Webster*[1], Rojin Chitrakar[1], Ryan Baugh[1] 1) Duke University.

C. elegans larvae hatch in the L1 stage, and in the absence of food they remain developmentally arrested as L1s (L1 arrest). L1 arrest is reversible, and worms continue development upon feeding. However, in the complete absence of food, worms can survive L1 arrest for several weeks. L1 arrest was originally described as an "ageless" state, because duration of L1 arrest was not found to subsequently affect adult lifespan [1]. However, worms late in L1 arrest exhibit signs of aging [2], and they incur fitness costs upon recovery [3]. The transcriptional response to starvation is rapid and appears to approach a steady state within one day [4]. However, gene expression has not been analyzed genome-wide late into developmental arrest. We used mRNA-seq to determine the gene expression dynamics of L1 larvae in a time course spanning both early and late L1 arrest. While the initial response to starvation is rapid, we found that many genes increase in expression late in L1 arrest, suggesting substantial transcription or, alternatively, widespread transcript decay with a minority of transcripts remaining stable. To distinguish between these possibilities, we used the auxin-inducible degron (AID) system to generate a strain that allows us to temporally and spatially control the stability of the largest RNA polymerase II subunit, AMA-1. We found that transcription of mRNA is required in the soma, but not the germline, for survival late in L1 arrest, consistent with active transcription. We are performing additional mRNA-seq experiments using the AID strains to disentangle the effects of transcript stability and *de novo* transcription early and late in starvation in the soma and germline. These results will allow us to identify classes of genes that are specifically transcribed late in starvation to support survival, in conjunction with genes that may exhibit exceptional stability over time and in different tissues.

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753C Exploring the *xol-1*-independent role of SEX-1 in dosage compensation *Eshna Jash*[1], Gyorgyi Csankovszki[1] 1) University of Michigan - Ann Arbor.

The composition, function and the mechanisms of action of condensin complexes are conserved across all eukaryotes. In addition to their better known roles in mitosis, condensin complexes have major roles in interphase gene regulation that are not very well understood. We study the condensin-like C. elegans dosage compensation complex as a model to dissect the mechanisms of gene regulation used by condensin complexes. In *C. elegans,* transcription from the two X chromosomes of the hermaphrodite are reduced by half to match the output from that of the XO male. This dosage compensation is established by the condensin-like *C. elegans* dosage compensation complex (DCC). The DCC consists of Condensin I^{DC}, a *C. elegans* specific condensin complex that differs from the canonical Condensin I by one subunit. The DCC uses multiple mechanisms to repress the X chromosome, not all of which have been characterized. We present evidence for a novel pathway that relies on the activity of a transcription factor, SEX-1.
SEX-1 is a nuclear hormone receptor (NHR). The known role of *sex-1* is as an X signal element that represses the expression of *xol-1*, which is responsible for turning off dosage compensation in males. When *xol-1* is mutated, male-specific lethality is observed due to the ectopic activity of the DCC. Mutations in *sex-1* in this background cause disruptions in DCC-mediated dosage compensation which are exacerbated when combined with mutations in known DCC-regulating genes. These results suggest that *sex-1* has a unique role in dosage compensation. We evaluate the degree of disruption by quantifying male-specific rescue of *xol-1* mutants. Since this disruption of dosage compensation is observed in a *xol-1* null background, this role of *sex-1* is independent of its *xol-1* regulatory function. Furthermore, homologs of *sex-1* in other model organisms have been shown to interact with condensin complexes indirectly through a co-repressor.
As an NHR, SEX-1 is capable of directly binding to gene promoters in order to control their expression. Based on this, we will use localization, gene expression and imaging techniques to characterize the *xol-1*-independent role of SEX-1 in dosage compensation. Characterizing this SEX-1-mediated pathway will provide us with insight about mechanisms employed by the DCC to repress the X chromosome.

778A *REDfly*: The regulatory element database for *Drosophila* Marc S. Halfon[1,2,3], Soile V. E. Keränen[4], Angel Villahoz-Baleta[1], Andrew E. Bruno[1], Steven M. Gallo[1] 1) University at Buffalo-SUNY; 2) NYS Center of Excellence in Bioinformatics and Life Sciences; 3) Roswell Park Comprehensive Cancer Center; 4) independent consultant.

The REDfly database provides a comprehensive curation of experimentally-validated *Drosophila cis*-regulatory modules (CRMs, "enhancers") and transcription factor binding sites (TFBSs). The database seeks to include all functionally tested sequences, both with and without observable regulatory activity, so that all experimental data are available for exploration. These data have numerous uses ranging from detailing the regulatory structure of a single locus, to large-scale studies of the regulatory genome, to providing training and/or validation data for machine-learning

analyses of gene regulation. A key REDfly feature is extensive expression pattern annotation for each CRM's activity using the *Drosophila* anatomy ontology, which allows for detailed searching of the data at varying levels of granularity. We introduced this year an expanded data model that also captures information on temporal aspects of gene regulation, regulation in response to environmental and other non-developmental cues, sexually dimorphic gene regulation, and non-endogenous (ectopic) aspects of reporter gene expression. The growth in regulatory sequence deletion experiments made possible using CRISPR/Cas9 technology has led to an increase in sequences tested for regulatory necessity, as compared to the sufficiency studies provided by traditional reporter gene experiments. A new "CRM_segment" data category now better handles data from such experiments. The past year has seen a substantial increase in database contents such that REDfly currently covers over 1000 publications and contains more than 31,000 records of reporter constructs regulating over 1530 genes, including over 24,000 "minimal" CRMs from transgenic in vivo reporter assays and over 10,000 from cell-culture assays, as well as over 2500 TFBSs. Priorities for the coming year include continued curation, addition of non-fly insect species, and improved search and download features. REDfly is freely accessible at http://redfly.ccr.buffalo.edu and can be followed on Twitter at @REDfly_database.

784A Sequence independent self-assembly of germ granule mRNAs into homotypic clusters *Tatjana Trcek*[1,2], Tyler E. Douglas[2], Markus Grosch[2], Yandong Yin[3], Whitby V.I. Eagle[4], Elizabeth R. Gavis[4], Hari Shroff[5], Eli Rothenberg[3], Ruth Lehmann[2] 1) Department of Biology, Johns Hopkins University; 2) HHMI, Skirball Institute of Biomolecular Medicine, Department of Cell Biology, NYU School of Medicine; 3) Department of Biochemistry and Pharmacology, NYU School of Medicine; 4) Department of Molecular Biology, Princeton University; 5) Section on High Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, NIH.

mRNAs enriched in membraneless condensates provide functional compartmentalization within cells. How mRNAs sort and organize within these condensates is poorly understood. Here, we use genetics and quantitative super-resolution imaging and report on a self-sorting mechanism by which multiple mRNAs derived from the same gene assemble into homotypic mRNA clusters. In *Drosophila* germ granules, clusters formed by mRNAs originating from different genes are de-mixed from each other within the same granule and located at distinct positions within the homogeneously-distributed protein environment of the granule. The specificity for self-sorting is not dependent on any particular RNA sequence and the intermolecular RNA:RNA interactions among clustered mRNAs are transient. We propose that the ability of mRNAs to self-sort into homotypic assemblies is their inherent property that is augmented under conditions that increase local mRNA concentration, such as upon enrichment in RNA-protein condensates, a process that appears conserved in diverse cellular contexts and organisms. This work was supported by the Intramural Research Programs of the US National Institute of Biomedical Imaging and Bioengineering, by the National Institute of Health (NIH) grant R01 GM067758 awarded to E.R.G and by the NICHD K99HD088675 grant awarded to TT. W.E. was supported by NIH training grant T32 GM007388. RL is an HHMI investigator.

786C Initiation and Maintenance Mechanisms of the Drosophila Histone Locus Body *Greg Kimmerer*[1], Gwyn Puckett[1], Leila Rieder[1] 1) Emory University.

In order to facilitate important cellular processes, certain nuclear regulatory factors concentrate into structures known as nuclear bodies. For the function of many of these bodies, formation and maintenance at a precise genomic location is crucial, but precisely how this is achieved remains unclear. The Histone Locus Body (HLB), a nuclear body that concentrates factors to regulate histone mRNA biogenesis, forms early in development. While the mechanisms behind HLB initiation are becoming clear, initiation is not the only requirement; the body must also be maintained throughout development. The only DNA element necessary and sufficient for HLB initiation in Drosophila is the bidirectional H3-H4 promoter. It is unknown whether this same DNA element directs HLB maintenance. To address this question, we have engineered Drosophila that allow us to delete the promoter cis elements after HLB initiation and assay the impact on HLB maintenance.

787A Very short fragments containing just the core promoter are sufficient to drive testis-specific gene expression in *Drosophila*. *Helen White-Cooper*[1], Athena Zitti[1], Ben Gambrill[1], Cerian Thomas[1], Charis Garside[1], Daniela Dominguez[1], Darius Pease[1], Edward Foster[1], Ffion Boxall[1], Ioannna Kontou[1], Jenni Hughes-Davies[1], Jessica Brocklebank[1], Kate Hurlow[1], Matthew de Guzman[1], Molly Hall[1], Nadiyah Mughal[1], Ryan Jones[1], Steph Jones[1], Tabitha Paul[1], Tyler Davies[1], Will Beaumont[1], Glen Sweeney[1] 1) Cardiff University.

Differentiation of sperm from morphologically unremarkable cells into highly specialised free-living, motile cells requires the co-ordinated action of numerous gene products. Many genes essential for spermatogenesis are not used elsewhere in the animal, or are expressed elsewhere, but using a different transcription start site. Spermatogenesis is thus a good system for elucidating the principles of tissue specific gene expression.
Several published transcriptional reporter constructs support a hypothesis that *Drosophila* testis-specific promoters are short (100bp or less in some cases), and that distal enhancers are not required. However, an unbiased approach has not been used to test the generality of this finding, and very few genes in total have been tested.
Most, but not all, testis-specific transcripts in *Drosophila* depend on the activity of a pair of testis-specific protein complexes - TMAC and tTFIID. TMAC comprises 8 subunits, including four DNA binding proteins. tTFIID is a variant of the general transcription factor complex TFIID. How TMAC and tTFIID recognise and activate target promoters is still unknown.
An "Advanced Research Methods" module class of undergraduate students included 10 working lab days. Each student was assigned a testis-specifically expressed gene and designed and generated a set of LacZ promoter-reporter constructs containing proximal promoter, TSS, and 5'UTR fragments of varying length (long ~ 400bp, mid ~200bp and short ~65-125bp). Generation of transgenic *Drosophila* was outsourced, and then expression of the reporter in testes was assayed. We tested 19 TMAC-dependent and 5 TMAC-independent genes.
14/19 short fragments and 16/17 long or mid fragments were able to drive reporter expression. Several extremely short DNA fragments, for example 64bp flanking the TSS of *CG14891*; 70bp from *CG17196* and 72bp from *Rpn12R* generated robust expression of LacZ in testes. Expression of both *CG14891* and *CG17196* is TMAC dependent, while *Rpn12R* expression is TMAC-independent.
Our data provides compelling evidence that the core promoter typically contains all the sequence information needed to direct testis-specific gene expression in *Drosophila*, and that more distal regulatory regions are not usually required. Surprisingly this was found both for TMAC-dependent and -independent genes. We demonstrate how a group undergraduate project with very significant time constraints can generate data that addresses real research questions.

788B The tudor-domain protein Tdrd5l identifies a novel germline granule and is important for post-transcriptional regulation of maternal RNAs during oogenesis *Caitlin Pozmanter*[1], Sydney Kelly[1], Harrison Curnutte [1], Mark Van Doren [1] 1) Johns Hopkins University.

Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development including post-transcriptional gene regulation. Recent work in our lab identified *tudor5-like (tdrd5l)*, which promotes male germline identity in germline stem cells (GSCs) in the testis, but is repressed by the RNA binding protein Sex lethal (SXL) in female GSCs. Interesting, Tdrd5l is also expressed in the differentiating germline in both sexes, indicating that it may also act to control germline differentiation in males and females.
We found that Tdrd5l localizes to the periphery of a novel germline RNA granule in both sexes. In males, Tdrd5l granules are observed adjacent to foci of Decapping protein 1 (Dcp1), suggesting that Tdrd5l granules are docking with processing bodies and could play a role in post-transcriptional gene regulation. Consistent with this, we observe genetic interactions between *tdrd5l* and *dcp1,* as well as between *tdrd5l* and the RNA deadenylase *twin* of the CCR4-NOT complex. Interestingly, in egg chambers of the ovary, Tdrd5l granules are found in nurse cells, and a single large granule is also observed at the anterior of the oocyte directly adjacent to the four ring canals. This suggests that *tdrd5l* may be important for post-transcriptional regulation of maternal RNAs being transported into the oocyte. Gurken (Grk) is normally only observed at the dorsal-anterior corner of later stage oocytes where it specifies dorsal identity. However, in *tdrd5l* mutants we observe ectopic expression of Gurken (Grk) protein in nurse cells. Additionally eggs laid by *tdrd5l* mutants have a decreased hatch rate and dorsal appendage defects consistent with *grk* misregulation. Lastly, we observed ectopic Oskar protein in the center of the oocyte in *tdrd5l* mutants rather than its normal posterior localization. This could indicate either that Grk protein also failed to signal to form posterior follicle cell identity, or that *oskar* is an additional target for regulation by *tdrd5l*. Taken together, our data suggest that the Tdrd5l granule is essential for regulating repression of maternal RNAs during transport from nurse cell to oocyte, and possibly for their correct localization within the oocyte.

789C The histone modifier KDM5 links cell cycle regulation with endocrine control of development in *Drosophila* Coralie Drelon[1], *Michael Rogers*[1], Helen Belalcazar[1], Julie Secombe[1] 1) Albert Einstein College of Medicine, Bronx, NY.

Across species, steroid hormones function as key signaling molecules that coordinate the cellular processes required for development. In *Drosophila*, the larval prothoracic gland integrates nutritional status with developmental signals to regulate growth and maturation through the secretion of the steroid hormone ecdysone. While the nutritional signals and cellular pathways that regulate prothoracic gland function are relatively well studied, the transcriptional regulators that orchestrate the activity of this tissue remain less characterized. Here we show that lysine demethylase 5 (KDM5, aka Lid), a histone modifier linked to human developmental disorders, is essential for prothoracic gland function. Although KDM5 is ubiquitously expressed throughout development, we have demonstrated that restoring KDM5 expression specifically within the prothoracic gland of *kdm5* null mutant animals is sufficient to rescue both the larval developmental delay and pupal lethality caused by loss of KDM5. Our studies show that KDM5 functions by promoting the endoreplication of prothoracic gland cells, a process that increases ploidy and is rate-limiting for the expression of ecdysone biosynthetic genes. Molecularly, we show that KDM5 activates the expression of the receptor tyrosine kinase *torso*, which then promotes polyploidization and growth through activation of the MAPK signaling pathway. Taken together, our studies provide key insights into the biological processes regulated by KDM5 and expand our understanding of the transcriptional regulators that coordinate animal development. We are currently carrying out genome-wide experiments to define the extent of the transcriptional defects in *kdm5* null mutant prothoracic gland cells. We are also further characterizing the temporal requirements of KDM5 to determine whether its prothoracic gland function is limited to larval development or whether it also plays key roles during pupation.

800B The selector gene *midline* controls ventral leg pattern by both inhibiting Dpp signaling and specifying ventral fate *Lindsay Phillips*[1], Pia Svendsen[1], Jae-Ryeon Ryu[1], William Brook[1] 1) University of Calgary.

The patterning of Drosophila limbs is controlled in part by the regional expression of selector genes, which are transcription factors that mediate binary patterning choices. The T-box transcription factor gene *midline (mid)* (fly Tbx20) acts as a selector gene, causing cells to adopt a ventral pattern instead of dorsal. The ventral signal Wg (fly Wnt) specifies ventral fate by activating *mid* in all ventral cells. Dorsal fate is controlled by Dpp (fly BMP). Our work shows that *mid* specifies ventral fate via at least two pathways. In the first pathway, *mid* inhibits dorsalization by blocking Dpp signaling. Ectopic dorsal fate induced by tkv^{QD}, an activated Dpp receptor, is inhibited by simultaneous expression of *mid*. As well, *mid* mutant cells have increased levels of phosphorylated Mothers-Against-Decapentaplegic (pMad), a readout of the level of Dpp signaling. Expression of *mid* reduces the levels of pMad accumulation. These results are consistent for the Dpp-target gene reporter dad-lacZ which displays increased expression in *mid* mutant cells and decreased expression when ectopic *mid* is present. Taken together, these results imply that the role of mid in dorsal inhibition is downstream of the Dpp receptor. We suspect that mid is interacting with genes involved in Mad phosphorylation, activation, or nuclear transportation and our research is currently investigating these possibilities. In the second pathway, *mid* directly promotes ventral fate. Genetic mosaics that lack mid and are blocked for Dpp signaling are not rescued to ventral fate in all but one ventral structure. Thus, mid also specifies ventral fate independent of Dpp signaling. Specification of ventral fate by *mid* requires a known repressing domain (eh1) and putative activating domains (TD1/2). Mid is a direct transcriptional repressor of several genes expressed in the ventral domain and a *mid* mutant in the eh1 domain is compromised in ventral fate specification in gain-of-function assays and rescue experiments. Although we have not identified genes activated by Mid, mutants in the *mid* TD1/2 domains are also compromised in ventral fate specification. We propose that mid specifies ventral fate though (1) inhibition of Dpp signaling and (2) coordinating the regulation of genes in the ventral leg.

803B Regulatory crosstalk between ecdysone-induced transcription factors confers temporal specificity to chromatin-state & gene expression during metamorphosis *Spencer Nystrom*[1,2,3,4], Daniel McKay[2,3,4] 1) Curriculum in Genetics and Molecular Biology; 2) Department of Biology; 3) Department of Genetics; 4) Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill.

Organismal development requires proper temporal and spatial coordination of gene expression by transcription factors (TFs). Binding of TFs *in vivo* is strongly influenced by packaging of DNA into chromatin; accessible binding sites are permissive for TF binding, whereas inaccessible sites are a barrier to TF binding. Despite the central role of chromatin accessibility in regulating dynamic gene expression during development, the underlying mechanisms remain unclear.

Our work has identified the ecdysone pathway as playing a central role in control of chromatin accessibility over time. Pulses of ecdysone initiate transitions between developmental stages by inducing expression of a cascade of temporal-specific TFs (tTFs) that activate & repress target genes. Prior work revealed thousands of changes in chromatin accessibility in the developing wing during the larval to pupal transition. Many of these changes fail to occur in mutants of the ecdysone-induced tTF *E93*. Interestingly, E93 appears to perform two opposing functions. In the absence of E93, late-acting target enhancers fail to open & activate and early-acting target enhancers fail to close & deactivate. How E93 performs these two

opposing actions is unclear. We identified DNA binding motifs for the early-acting tTF, Broad (Br), at sites that are opened by E93 later in development, suggesting the potential for regulatory crosstalk between early and late-acting tTFs.

To identify whether Br, like E93, is required for regulating temporal changes in accessibility, we performed FAIRE-seq in *brRNAi* wings during late larval and early pupal stages. We identify hundreds of late-opening sites that precociously open in *brRNAi* wings, many of which are bound and opened by E93 later in pupal stages. Therefore, we hypothesize that Br actively prevents TF access to chromatin at late-acting enhancers to provide temporal specificity to target gene expression.

In support of these findings, RNA-seq in *brRNAi* wings identifies hundreds of late-acting genes that are precociously expressed during larval stages. To examine these effects further, we profiled Br binding in wings using CUT&RUN. We identify hundreds of binding sites that correspond to dynamic chromatin sites.

Finally, we find that E93 is both necessary & sufficient to repress *br* transcription during wing development, supporting a model wherein E93 acts to both repress *br* expression and antagonize Br repressive activity at target enhancers to drive developmental forward.

812B Translational regulation of *cycB* in the *Drosophila* male germline *Catherine Baker*[1], Margaret Fuller[1] 1) Stanford University School of Medicine.

Tissue-specific regulation of the cell cycle is critical for proper development and homeostasis. Such regulation can be mediated by the function of cell-type-specific proteins to control the expression and/or activity of the core cell cycle machinery. The *Drosophila* male germline contains both mitotic cells (spermatogonia) and meiotic cells (spermatocytes), and the regulation of cell division in these two cell types is dramatically different. Spermatogonia divide regularly and efficiently; spermatocytes, in contrast, undergo a meiotic G2 prophase that lasts 3.5 days, and the concurrent delay of the meiotic divisions is mediated by fine-tuned control of the temporal expression of core cell cycle components. One such cell cycle factor is Cyclin B (CycB). CycB protein expression is high in mitotic spermatogonia, and then low in immature spermatocytes. CycB protein levels spike again just before spermatocytes enter the meiotic divisions. Published work from our lab has shown that the RNA-binding protein Rbp4 and its co-factor Fest repress *cycB* translation, mediated by sequences in the 130nt *cycB* spermatocyte 3'UTR (Baker, Gim, & Fuller 2015). Fest has no recognizable protein domains but is conserved in protostomes. Subsequent work has revealed that a novel protein, Lutin (Lut, formerly CG1690), is also required for *cycB* repression in early spermatocytes. Lut binds Fest independent of RNA, and co-precipitates with Rbp4 in the presence of Fest. In addition, we have found that testis-specific isoforms (the product of spermatocyte-specific transcription and splicing) of the RNA regulator Syp are required for activation of *cycB* translation in mature spermatocytes. Loss of function of *syp* in the testis causes germ cells to advance to the late spermatocyte stage, but these germ cells fail to translate *cycB* RNA and arrest prior to meiotic division. Syp, like Rbp4, binds the 130nt *cycB* spermatocyte 3'UTR in biotin pulldown experiments. Curiously, Syp binds to Fest independent of RNA (and can co-precipitate with Rbp4 in the presence of Fest). Further experiments should reveal whether Lut and Syp can co-precipitate in the presence of Fest, or whether their binding to Fest is mutually exclusive. Experiments exploiting a synchronized differentiation time-course technique (Kim et al 2017) are underway to determine whether any of the major interactions (Fest-Rbp4, Fest-Lut, and Fest-Syp) change as spermatocytes mature to allow *cycB* translation to switch from off to on.

819C Regulation of Gonad Morphogenesis and Gametogenesis by the BTB Protein Ribbon *Jennifer Jemc*[1], Shannon McDonnell[1], Pauline Sulit[1], Adriana Soriano[1], Danielle Talbot[1], Manuel Alvarez[1], Usama Khan[1], Sana Moqeet[1] 1) Loyola University Chicago.

Cell adhesion and cell-cell signaling are critical for the establishment and maintenance of organ structure and function. In the case of the gonad, defects in organ formation or gametogenesis can result in sterility. The *Drosophila* gonad has proven an excellent model for identifying genetic mechanisms underlying organogenesis. The gonad is formed when the somatic gonadal cells and germ cells migrate and coalesce during embryogenesis. Subsequent development results in the establishment of the germline stem cell niche and stem cell populations that will be maintained throughout the lifetime of the organism. In previous studies, the BTB transcription factor Ribbon (Rib) was identified as a gene required for embryonic gonad formation. Further study has revealed that Rib continues to be expressed during larval gonad development and in the adult ovaries and testes. These results suggest that Rib may regulate significant morphological changes that occur in the larval gonad, as well as gametogenesis in the adult. We have found that overexpression of *rib* in somatic cells throughout development causes significant defects in ovary and testis development. In females overexpressing *rib*, niche structures fail to form, resulting in ovaries with a blob-like appearance and a failure to produce eggs. In males overexpressing *rib*, niche structures form, but testes appear truncated and sperm do not progress through meiosis. The effect of clonal loss of *rib* are being examined in males and females. In order to specifically examine the role of Rib in gametogenesis, *rib* overexpression was limited to adult tissues. When *rib* is overexpressed in somatic cells of the adult ovary, defects in oogenesis occur and a reduction in the number of follicle cells surrounding the egg chamber is observed. *rib* overexpression in somatic cells in the adult testis results in a failure of cells to transition to meiosis and abnormalities in somatic cell gene expression. Given that oogenesis in *rib* overexpression ovaries arrests at a key transition regulated by the Notch signaling pathway, and Notch and Rib have been found to regulate a common target gene, we are currently examining the relationship of Rib to the Notch signaling pathway in the ovary and testis.

825C OrganSizeRegulationInRpMutantsInDrosophila *Walter Otu*[1], Nicholas Baker [2], Amit Kumar[2] 1) University of Texas Rio Grande Valley, Brownsville, TX; 2) Albert Einstein Einstein College of Medicine, Bronx, NY.

Ribosomal proteins (Rp) function as components of ribosomes which are essential for the synthesis of proteins. Given how important ribosomes are for translation, organisms with homozygous mutations for Rp die, while heterozygous mutants present with abnormal disease in humans as can be seen in Diamond- Blackfan anemia and Drosophila with reduced size in bristles and an extended growth period (the minute phenotype). In the case of Drosophila, previous results in the lab suggests that Xrp1 are able to regulate this phenotype through a developmental delay that contributes to the extended growth period compensating for the missing copy of the Rp gene. The Hippo signaling pathway is a kinase cascade that plays a role in organ size control in animals by regulating the activity of a transcription co-activator yorkie (yki) that promotes organ growth. During the course of this research wing size was used as a measure for the genetic interactions of the manipulated genes. The question that we explored was whether Xrp1 regulates organ size in Rp mutants through the regulation of the hippo pathway. Our hypothesis is that there will be an increase of yki activity in Rp (ribosomal protein) mutants to maintain organ size. Crosses were set for the desired genotypes, wings from the obtained flies were dissected and measurements were taken. From the taken measurements we found evidence that RpS3 heterozygous flies display mild but significant increase

in wing size and reduction in aspect ratio, a phenotype dependent on Xrp1. Moreover, additional genetic interaction data is presented with the members of the Hippo pathway. From results we were able to support our hypothesis in the case of yki but not in wts, suggesting that these measurements should be repeated with a greater number of flies to show the extent of this control.

905B A role for *MED15* in the domestication of wine yeast *David Cooper*[1], Yishuo Jiang[1], Jan Fassler[1] 1) Department of Biology, University of Iowa, Iowa City, IA.

The propensity for *Saccharomyces cerevisiae* to ferment sugars into ethanol and CO_2 has long been used to produce a wide range of foods and drinks and more recently used in a variety of industrial applications. In the pursuit of superior wine, winemakers have domesticated yeast for fermentation capacity and optimized production of desirable aromas and flavors. The yeast strain chosen for wine fermentation is critical because not all strains can tolerate the stresses of fermentation equally nor ferment as effectively. One possible mechanism by which wine yeast have adapted to fermentation stresses is through changes in expression of fermentation and stress response genes.
Polyglutamine (poly-Q) tract containing proteins are enriched and conserved in eukaryotic transcriptional regulators suggesting a poorly understood functional and/or adaptive role for poly-Q tracts in regulating gene expression. *MED15* is a glutamine-rich general transcriptional regulator and RNA Pol II Mediator subunit with both positive and negative roles in expression of metabolic and stress response genes. In our previous computational analyses we found that the three longest poly-Q tracts of Med15 display considerable across-strain length variation (10-30 CAG or CAA repeats), and that there is a strong association between beer and wine yeast domestication phenotypes and polyglutamine tract lengths in Med15. We hypothesize that these variations impact Med15 structure, activity and/or its affinity for its interaction partners, thus resulting in the altered expression of Med15 target genes.
We find that strains lacking *MED15* exhibit reduced fermentation and fermentation stress responses. To study the impact of Med15 poly-Q variation on stress response and domestication phenotypes we transplanted *MED15* alleles from wine yeast strains, differing from the lab allele primarily in the lengths of three polyglutamine tracts, into a lab strain lacking the *MED15* locus. We found that certain wine yeast alleles improved fermentation capacity and response to acetic acid and ethanol stresses in the lab strain. Expression analysis of Med15-dependent metabolic and stress response genes revealed that wine yeast *MED15* alleles change the expression of fermentation genes in the lab strain relative to the native *MED15*. These data are consistent with a role for polyglutamine tract length variability in the domestication of wine yeast by modulating the activity of a general transcriptional regulator.

907A Allele-specific expression in reciprocal crosses of Virginia Tech high-growth and low-growth selection lines Fernando Lopes Pinto[1], Göran Andersson[1], Leif Andersson[3,1], Elizabeth Gilbert[2], Christa Ferst Honaker[2], Paul Siegel[2], *Dirk Jan de Koning*[1] 1) Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Virginia Tech, Blacksburg, Virginia; 3) Uppsala University, Uppsala, Sweden .

The Virginia Tech high-growth and low-growth selection lines of White Plymouth Rock chickens have been developed by more than 50 generations of divergent selection for body weight at 56 days of age. These lines have been extensively used for QTL mapping in order to reveal the genetic basis for the considerable selection response. To further clarify the mechanisms for how QTLs control phenotypic differences, we measured allelic RNA expression ratios to detect differential expression between alleles derived from the two lines.

By performing whole transcriptome sequencing, we set out to detect allelic imbalance in the RNA expression of 6 F1 progeny from reciprocal crosses between generation 54 parents from the high and low parental lines. Using RNA samples extracted from liver, hypothalamus and pectoralis major, we generated circa 250 M (100 bp) RNA sequencing reads per F1 individual. This experimental design also allows us to detect allele-specific expression.

Initially, the sequencing reads were filtered following the GATK Best Practices workflow for RNAseq. The downstream pipeline included applying a Bayesian approach for estimating allele-specific expression from RNA-Seq data with diploid genomes, followed by a generalized linear model for decomposing cis-regulatory, parent-of-origin, and maternal effects on allele-specific gene expression.
Our results show that allelic imbalance exists, especially in the liver, and to a lesser degree in the chest muscle and the hypothalamus. Also, we found evidence for parent of origin effect in liver and muscle.

923B Deciphering the transcriptional regulation of flocculation via CWI pathway in *Saccharomyces cerevisiae* SANTHOSH KUMAR SARIKI[1], RAMESH KUMAWAT[1], Dr. RAGHUVIR TOMAR[1] 1) Laboratory of Chromatin Biology, Department of Biological Sciences, Indian Institute of Science Education and Research Bhopal, India..

Flocculation is an essential characteristic of yeast cells required for survival under adverse conditions. The multicellular structure (flocs) of yeast provides a suitable microenvironment to enhance the chances of survival during stress conditions. Although the signaling events triggering flocculation have been studied earlier, the molecular mechanism remained elusive. In present study, we used flocculating sen1 mutants to identify the mechanism of flocculation. Based on the abnormal cell surface morphology and constitutive phosphorylation of Slt2 in flocculating Sen1 mutant cells, we assumed that flocculation is regulated by the Cell Wall Integrity (CWI) pathway. Up-regulation of *FLO* genes in wild type cells was observed upon activation of the CWI pathway either by chemical treatment or by deleting Slt2 phosphatase (Msg5). By using Slt2 mutants our study reveals that active state of Slt2 is indispensable for flocculation. The flocculation was reduced after the deletion of *SLT2* or *RLM1*. Further, we revealed overlapping binding sites for Rlm1 and Tup1 at the promoters of almost all the *FLO* genes. Finally, we show higher Rlm1 and lower Tup1 occupancy at the promoters of *FLO1* and *FLO5* in flocculating cells. Altogether we demonstrate that CWI MAPK (Slt2) pathway uses a non-catalytic mechanism to activate the transcription of *FLO* genes.

928A Translational control of fatty acid synthesis controls nuclear morphology in the cell cycle *Nairita Maitra*[1], Heidi Blank[1], Clara Kjerfve[1], Vytas Bankaitis[1], Michael Polymenis[1] 1) Texas A & M University.

Cells maintain a tight balance between growth and division to ensure that with each generation cells do not become progressively larger or smaller. A critical requirement to achieve this balance is the regulated synthesis of proteins and lipids during every cell cycle. Bulk protein synthesis is necessary to maintain the cell's mass, and lipids are required for membrane biogenesis, and as key signaling components. However, how lipid homeostasis is maintained during the cell cycle is poorly understood. Fatty acids are the building blocks of lipids. A genome-wide study performed in budding yeast in our lab identified the translational efficiency of mRNAs encoding two fatty acid synthesis enzymes (ACC1 and FAS) to be under periodic

control, peaking late into the cell cycle. ACC1 catalyzes the carboxylation of acetyl CoA to malonyl CoA. Malonyl CoA serves as the substrate for FAS (FAS1 and FAS2) to synthesize 14-16 carbon fatty acids. The translational efficiency of ACC1 is controlled by an upstream open reading frame (uORF) present 341 nucleotides upstream of the ACC1 start codon. FAS1 has two uORFs located respectively at 141 and 279 nucleotides upstream of the FAS1 start codon. We propose that these uORFs control the translational efficiency of FAS1. We found that de-repressing the translation of ACC1 nd FAS1 increases lipogenesis and accelerates nuclear division. Moreover, mutating the uORFs of ACC1 and FAS1 suppresses the bigger cell size, abnormal nuclear morphology, and the G2/M delay of sec14-1 mutants. Sec14p is a phosphatidylinositol transfer protein, which regulates membrane trafficking. Taken together, our results suggest that translational control of lipid homeostasis during cell division links cell growth to nuclear envelope remodeling in mitosis.

Tuesday, April 28 12:00 PM - 3:30 PM

Gene Regulation/Disease Models and Aging 2 - Poster Q&A 763A Chromodomain proteins CEC-3 and CEC-6 affect chromatin and small RNA pathways and protect germline immortality *Victor Lao*[1], Reta Aram[1], Elizabeth Yan[1], Arneet Saltzman[1] 1) University of Toronto.

Proper regulation of histone modifications and small RNA pathways play an important role in the protection of germline immortality. Two *C. elegans* chromodomain containing proteins, CEC-3 and CEC-6, recognize H3K9 and H3K27 methylation *in vitro* and *cec-3Δ; cec-6Δ* animals show progressive sterility or a 'mortal germline' phenotype. To understand how CEC-3 and CEC-6 contribute to germline immortality, we used several established phenotypic and transgenic reporter assays for gene silencing. We found that CEC-6 but not CEC-3 is required for silencing of a repetitive transgene in the germline, and that this silencing can be rescued by maternal CEC-6. Unexpectedly, both CEC-3 and CEC-6 also contribute to silencing of a repetitive transgene expressed in the soma (*rol-6*). Dysfunctional RNA interference (RNAi) pathways are linked to both germline desilencing and somatic silencing of repetitive transgenes, so we investigated the role of CEC-3 and CEC-6 in RNAi. Whereas CEC-3 and CEC-6 are dispensible for exogenous RNAi and the maintenance of piRNA-initiated silencing, we find that *cec-3Δ; cec-6Δ* animals have reduced germline and enhanced nuclear RNAi responses. Together, our results suggest a role for these chromodomain proteins in the interplay between endogenous small RNA and chromatin silencing pathways. We are currently investigating if CEC-3 and CEC-6 affect subcellular localization of the nuclear RNAi Argonaute NRDE-3 or expression of an endogenous siRNA reporter, as well as conducting a directed RNAi screen to determine the genetic requirements of CEC-3/6-mediated transgene silencing.

835A Integration of posterior positional cues patterning the follicular epithelium of the Drosophila ovary *Baptiste Rafanel*[1], Scott De Vito[1], Kelvin Ip[1], Mariana Fregoso Lomas[1], Laura Nilson[1] 1) Department of Biology, McGill University, Montreal, QC, CA.

In multicellular organisms, specification of cellular fate requires genes to be expressed at the right place and time. This process is regulated by intercellular signals. However, only a small number of signaling pathways specify a wide range of cellular fates, implying that the same signaling pathway can generate multiple outcomes.

The follicular epithelium of the Drosophila ovary provides a model in which localized activation of the epidermal growth factor receptor (EGFR) leads to different outcomes depending on the timing and location of signaling. Early in oogenesis, posteriorly localized EGFR activity induces expression of the T-box transcription factors Midline (Mid) and H15. In subsequent stages, EGFR activity translocates to the dorsal anterior where the homeodomain transcription factor Mirror (Mirr) is induced. This diversity of EGFR output allows the same localized signal to define both the AP and DV axes. Which of these two EGFR signaling outcomes is chosen is mediated by opposing gradients of JAK/STAT and BMP activity. At the posterior, JAK/STAT signaling activates *mid* and prevents *mirr* expression. At the anterior, BMP signaling activates *mirr* and prevents *mid* expression. In addition, Mid and Mirr mutually repress each other. We propose that this regulatory network generates a bistable switch ensuring proper patterning of this tissue. However, how these signals are integrated and interpreted by the cells remains unknown.

To address this question, we focus on how the signals of this network are integrated to regulate transcription of *mid*. We have characterized two genomic regions recapitulating expression of Mid, asking whether each captures the same signals regulating *mid*. While both are sensitive to loss of the EGFR effector *pointed* (*pnt*), only one is sensitive to loss of the JAK/STAT effector *Stat92E*. However, disruption of putative STAT92E binding sites does not affect the ability of this region to drive reporter expression, suggesting that inputs from JAK/STAT are not captured through direct binding of STAT92E to these sequences. In addition, we find that Pnt is regulated by the JAK/STAT pathway in the posterior follicle cells, consistent with previous data of Pnt reporters, leading to the hypothesis that JAK/STAT indirectly regulates *mid* through regulation of Pnt levels. We propose that inputs from EGFR and JAK/STAT signaling are being integrated at the level of Pnt regulation to induce *mid* expression and posterior fate.

843C Protein acetylation as a metabolic switch for *de novo* lipogenesis in *Drosophila* development *Ting Miao*[1], Jinoh Kim[1], Hua Bai[1] 1) Iowa State University.

Lipogenesis is tightly regulated during animal growth and development. Despite the well-established transcriptional regulation of lipogenic genes (e.g., fatty acid synthase, FASN), it remains largely unclear how post-translational modifications (PTMs) of these lipogenic enzymes modulate lipogenesis and contribute to the regulation of metabolic homeostasis. With a FLAG-tag knock-in fly line generated using CRISPR-Cas9 system, we observed an intriguing dynamics of the acetylation of endogenous *Drosophila* FASN1 protein, the rate-limiting enzyme in *de novo* lipogenesis, during larval development. The acetylation of FASN1 proteins varies with developmental stages and peaks at 96h after egg laying (AEL), which is positively correlated to the FASN1 enzymatic activity and fast larval growth. Mass-spectrometry analysis identified two evolutionarily conserved lysine residues that are acetylated in both fly and human FASN proteins. One of the lysine residues, K813, is located nearby the active site of the malonyl/acetyltransferase (MAT) domain. Acetylation of K813 is predicted to enlarge the binding pocket of MAT domain to allow fast substrate loading. Indeed, lysine to arginine substitution mutants (K813R, acetylation deficiency mutants) show decreased lipogenesis, reduced body weight, and delayed pupariation. Lastly, we identified a deacetylase, Sirt1, as the key negative regulator of FASN1 acetylation and *de novo* lipogenesis. Our epistasis analysis suggests that the repression of Sirt1-mediated deacetylation at 3rd instar stage might be responsible for the increased FASN1 acetylation and *de novo* lipogenesis in fast growing *Drosophila* larvae. Taken together, our results reveal a novel role of lysine acetylation in modulating pocket conformation of MAT domain of FASN1 protein and promotes its catalytic activity by enhancing substrate loading. Since acetyl-CoA is the key metabolite linking lipogenesis and acetylation, our findings further indicate that lysine acetylation may act as a fine tune mechanism to modulate enzymatic activity in responding to fluctuated metabolite availability during animal growth and development.

845B Investigating repeat-induced silencing in *Drosophila melanogaster* *Elena Gracheva*[1], Tingting Gu[2], Sukruth Shashikumar[1], Gary Huang[1], Sophia Bieser[1], Matthias Walther[4], Gunter Reuter[3], Sarah CR Elgin[1] 1) Washington University in St Louis; 2) Nanjing Agricultural University , China; 3) Martin-Luther-Universität Halle-Wittenberg, Germany; 4) Community school "J. F. Walkhoff "Gröbzig, Germany.

We investigated the role that repetitious DNA sequences play in the formation and spreading of heterochromatin by creating transgenic constructs containing a repeat fragment (transposable element *1360*, $lacO_{256}$ tandem repeats, or a GAA_{310} triplet repeat) upstream of an *hsp70-white* reporter. These constructs were placed in a euchromatic region in a site within an actively transcribed gene, *nesd*, but close to a block of repetitious DNA packaged as heterochromatin. The resulting PEV eye phenotypes suggest that ectopic heterochromatin formation has occurred in all three cases. However, the PEV phenotype is much stronger in $lacO_{256}$*-hsp70-white* and in GAA_{310}*-hsp70-white* than in *1360-hsp70-white* transgenic flies. Excision of the repeat fragment reverses the PEV phenotype to a full red eye in all cases, demonstrating that the stochastic silencing is dependent on the presence of repetitious elements. In all cases the PEV reporters are sensitive to HP1a depletion, confirming that the silencing is due to heterochromatin formation. Tests for sensitivity to mutations in *piwi* and *AGO3* indicate that the *1360-hsp70-white* reporter is dependent on the RNAi mechanism. Silencing in the $lacO_{256}$ repeat-containing case shows little dependence on H3K9 histone methyl transferases; dependence on *Su(var)2-1* for silencing, and loss of silencing on over-expression of *Gcn5* suggest reliance on the histone deacetylation process. $lacO_{256}$*-hsp70-white* transgenic flies show a surprising loss of silencing at 18°C. The GAA repeat-containing reporters are sensitive to both HMTs and HDAC pathway mutations. Overall, our results indicate distinct mechanisms for targeting and maintenance of local heterochromatin domains induced by various types of repeats.

861C A PRC2 sampling model for the initiation of Polycomb silencing in the *Drosophila* germline *Steven DeLuca*[1], Allan Spradling[1] 1) Carnegie Institute.

Polycomb silencing antagonizes transcription and underlies a molecular memory of past gene expression critical for animal development. While many of the proteins required for silencing have been heavily characterized, how these proteins are regulated to control where and when silencing is initiated during development is incompletely understood. We developed a reporter-based method to detect Polycomb silenced loci throughout the genome in single cells, and use the *Drosophila* germline to model the initiation of Polycomb silencing during differentiation. Nurse cells, which occupy a germline-derived dead-end lineage, induce Polycomb silencing not only at traditional Polycomb domains, but also at all transcriptionally inactive loci. Two Polycomb proteins, Pcl and Scm, are developmentally regulated to initiate silencing upon nurse cell differentiation. In nurse cell progenitors, high Pcl levels inhibit silencing by limiting the mobility of the Polycomb methylase, PRC2. In differentiated nurse cells, Pcl levels drop, generating two pools of PRC2- a highly mobile, Pcl-free PRC2, which samples and silences generally inactive chromatin and residual Pcl-PRC2, which is concentrated by Scm to enhance silencing on Polycomb domains. We propose that Polycomb silencing is generally regulated by the rate that PRC2 samples chromatin, which is developmentally controlled by PRC2 interacting proteins that modify both PRC2 mobility and local concentration.

863B Disruption of promoter-enhancer communication leads to *engrailed* mutants *Anna Horacek*[1], Victoria Blake[1], Judith Kassis[1] 1) National Institute of Child Health and Human Development, NIH, Bethesda, MD.

In Drosophila, the *invected* (*inv*) and *engrailed* (*en*) genes exist within a co-regulated complex and are expressed throughout development. Distinct enhancers drive co-expression of *inv/en* in stripes, the head, gut, and CNS of the embryo, as wells as in the imaginal discs of the larvae. Although the *inv/en* promoters are separated by ~54 kb, their expression is regulated by the same enhancers distributed across a 70 kb region, suggesting that enhancers can activate multiple promoters over long distances. Previous studies have identified a 2 kb regulatory DNA fragment upstream of the *en* promotor, that may serve as a promoter tethering element by facilitating interactions between the *en* promoter and distant enhancers. Interestingly, the deletion of 530 bp in the 2 kb fragment reduces En expression in the posterior compartment of imaginal discs, suggesting it's importance for interactions with the imaginal disc enhancers. We have generated a transgenic line containing the 2 kb regulatory fragment fused to a *lacZ* reporter gene inserted near the *en* promoter. When coupled with a *wild-type* chromosome, transgenic organisms expressed ß-Galactosidase and En only in the posterior compartment of the wing imaginal discs, consistent with appropriate enhancer communication. However, in the absence of a *wild-type* chromosome, transgenic organisms exhibited a marked reduction in En expression in addition to a de-repression of β-Galactosidase in the anterior compartment of the wing imaginal discs, suggesting the endogenous enhancers have been hijacked by the regulatory fragment within the transgene. Evidence of a reduction in En expression is also observed in the pharate adults produced by this genotype, which lack thoraxes and exhibit impaired wing development. Taken together, our data suggest that a specific regulatory fragment may serve as a promoter tethering element and is required to facilitate interactions between the *en* promoter and imaginal disc enhancers at discrete developmental stages.

878B Translational components driving heart morphogenesis in *Drosophila*: implications for Congenital Heart Disease *Analyne Schroeder*[1], Tanja Nielsen[1], Georg Vogler[1], Paul Grossfeld[2], Alexandre Colas[1], Rolf Bodmer[1] 1) Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA; 2) Department of Pediatrics, UCSD School of Medicine, La Jolla, CA, USA.

Deviations in the cardiogenic program can lead to malformations of the heart resulting in a range of Congenital Heart Disease (CHD) features in humans. It is becoming clear that the etiology of these deviations are complex and oligogenic, likely involving multi-cellular contributions. Therefore, the use of more simplistic, well-established cardiac models, such as the *Drosophila* heart and human *iPSC*-derived multipotent cardiac progenitor cells (MCPs), can help uncover genetic underpinnings and interactions, leading to identification and understanding of mechanisms that lead to cardiac pathogenesis.

In our search for novel genetic players driving CHD, we identified the large ribosomal subunit *RPL13* within a deleted Copy Number Variant segment from a CHD patient, that reduced proliferation and differentiation of MCPs into cardiomyocytes upon knockdown (KD), while increasing fibroblasts. In the fly, heart specific *RpL13* KD in the embryo was sufficient to produce a 'no heart' phenotype in adults, and remarkably KD at larval to adult stages had no longer an effect on heart development. Interestingly, late-stage embryonic cardioblasts are present and appear normal overall, suggesting that *RpL13* KD pre-programs the embryonic heart for histolysis at later developmental stages. In contrast, despite KD of the small ribosomal subunit *RpS15A* also leading to altered proliferation and differentiation of MCPs, KD caused a partial fly heart phenotype, retaining the posterior half. Furthermore, *RpS15A* KD is required throughout development to obtain the adult phenotype, not just in the embryo.

We have also examined translational chaperones Nascent polypeptide Associated Complex-alpha (*NAC*α) and Signal Recognition Particle (*SRP*) subunits. Interestingly, we identified a genetic interaction between *NAC*α and the *hox* gene *abd-B* specifically during metamorphosis implicating a car-

diac specific role for *NACα* in heart development. We have also uncovered a range of interesting cardiac relevant phenotypes with spatial specificity produced at distinct temporal stages during development following KD of individual *SRP* subunits.

In summary, despite the seemingly generic function of these translational components, we uncovered effects that were spatially and temporally specific leading to varied adult fly heart phenotypes. Translational genes should therefore be further explored as targeted drivers and regulators of heart development and pathogenesis that may have implications for CHD.

948C Systematically validated, genome-scale inference of quantitative regulatory networks and condition-specific TF activities *Cynthia Ma*[1], Michael Brent[1] 1) Washington University in St Louis.

Cells process and respond to information from their environments, in part, by changing the activity levels of transcription factors – the degree to which each TF exerts its regulatory potential on its target genes. To understand the cell's information processing, we must know which TFs change activity in response to specific extracullular stimuli or intracellular conditions. However, changes in TF activity (TFA) are difficult to measure directly because they have diverse molecular implementations, including changes in protein abundance, localization, and post-translational modifications. An alternative is to infer changes in TFA from changes in the expression levels of their target genes. This idea has been pursued in previous work, including Network Component Analysis (Liao et al 2003), RegulonProfiler (Boorsma et al 2009), and ISMARA (Balwierz et al 2014), but until now, systematic, genome-scale evaluation of such methods has not been possible. We present the first such evaluation. We find that TFA inference really does work, but only if the input network identifying the targets of each TF is of high quality. We compare multiple methods of constructing such networks, including from chromatin immunoprecipitation (ChIP) binding data, binding specificity models, or differential expression after direct perturbation of TFs. We find that perturbation response data are both necessary and sufficient for good performance, whereas the other sources are neither.

Our objective evaluation is made possible by the availability of two large data sets of gene expression after direct perturbation of TFA in yeast. One is based on knockouts of ~1,400 genes (Kemmeren et al 2014). The other is a new, unpublished data set in which gene expression is measured a few minutes after induction of each TF. We use one data set for learning a quantitative regulatory network and the other for testing TFA inference using that network. Two tests involve guessing which TF was perturbed in each expression profile, and whether the TF was activated or repressed. The third is to identify known examples of proteins regulating the activities of TFs. The fourth examines correlation between a TF's inferred activity and its observed mRNA. Further analysis using a new, unpublished data set of TF binding obtained with the transposon calling cards method also found that the inferred strength with which a TF regulates each of its targets correlates positively with its measured binding strength at each target. With these data sets and metrics, we are able to determine, with high confidence, what works and what doesn't in TFA inference. We also provide a validated, quantitative regulatory network for yeast.

957C The Paf1 transcription elongation complex interacts directly with the N-terminus of Rad6 to facilitate H2B ubiquitylation in *Saccaromyces cerevisiae* *Brendan McShane*[1], Nicole Horan[1], Jason True[2], Amber Mosley[2], Karen Arndt[1] 1) University of Pittsburgh, Pittsburgh PA; 2) Indiana University School of Medicine, Indianapolis IN.

The Polymerase Associated Factor 1 complex, Paf1C, couples chromatin changes to transcription elongation and is important for the deposition of transcription-associated histone modifications genome-wide. Our previous studies in *S. cerevisiae* revealed a direct interaction between the Histone Modification Domain (HMD) of Paf1C subunit Rtf1 and the ubiquitin-conjugating enzyme Rad6. The HMD is both necessary and sufficient to stimulate mono-ubiquitylation of H2B lysine 123 (H2Bub), a conserved modification that is required for several downstream modifications, including H3 K4 and H3 K79 di- and tri-methylation. While the region within the Rtf1 HMD involved in its interaction with Rad6 had been identified in our previous work, the region of Rad6 that contacts the HMD was unknown. Using an *in vitro* crosslinking-mass spectrometry approach exploiting an unnatural photoreactive amino acid, we identified the N-terminal helix of Rad6 as the likely site of interaction with the HMD. Using alanine-scanning mutagenesis, we verified the functional importance of this helix for H2Bub *in vivo* and identified several residues necessary for the function of Rad6 in this process. Interestingly, while several *rad6* mutations in the putative HMD-interacting site reduce H2Bub *in vivo*, they do not disrupt significantly other Rad6-dependent pathways, including DNA damage repair. Because this region within Rad6 has been suggested to interact with several enzymes involved in ubiquitylation, including the ubiquitin activating enzyme and the ubiquitin ligase Bre1 associated with H2Bub, we purified recombinant Rad6 mutant proteins and assayed them with an *in vitro* ubiquitylation reaction. These analyses have allowed us to dissect the importance of specific residues in the N-terminal helix of Rad6 in catalyzing the ubiquitylation of H2B. Collectively, our results provide mechanistic insight into the Paf1C-stimulated deposition of a histone modification with strong connections to human cancers.

968B DNA methylation is required for regional gene expression signatures in the zebrafish intestine *Gilberto Padilla Mercado*[1], Colin R. Lickwar[1], John F. Rawls[1] 1) Department of Molecular Genetics and Microbiology, Duke Microbiome Center, Duke University School of Medicine, Durham, NC 27710.

The intestinal tract is organized into distinct regions along the cephalocaudal axis with specific roles in the digestion and absorption of dietary nutrients. These distinct intestinal regions are associated with corresponding distinct regional gene expression programs that are conserved between zebrafish and mammals. However, the regulatory mechanisms underlying these gene expression programs are not fully understood. DNA methylation is an important general mechanism of epigenetic regulation of gene expression, and recently has been found to play a role in maintaining regional identity in human intestinal organoids. Additionally, genetic defects in DNA methylation maintenance in zebrafish disrupts barrier function and induces inflammation in the intestine. Similar DNA methylation mutants also show an increase of type I interferon responses due to derepression of endogenous retroviral elements. Utilizing a combination of zebrafish transgenic lines labelling distinct regions of the intestine, I show that regional identity requires DNA methylation maintenance *in vivo*. I will also describe our plans to test the hypothesis that antiviral innate immune pathways play a unique role in maintaining regional gene expression patterns involved in nutritional physiology and inflammation in the intestine.

971B Identifying the link between non-coding regulatory RNAs and phenotypic severity in a zebrafish model of *gmppb* dystroglycanopathy *Grace Smith*[1], Lily Charpentier[1], Erin Bailey[2], Mary Astumain[2,3], Michelle Goody[3], Clarissa Henry[2,3], Benjamin King[1,2] 1) Department of Molecular and Biomedical Sciences, University of Maine, Orono, ME; 2) Graduate School of Biomedical Science and Engineering, University of Maine; 3) School of Biology and Ecology, University of Maine, Orono, ME.

Muscular Dystrophy (MD) is characterized by varying severity and time of onset by individuals afflicted with the same forms of MD, a phenomenon that is not well understood. MD affects 250,000 individuals in the United States and is characterized by mutations in subunits of the dystroglycan complex. gmppb encodes an enzyme that glycosylates dystroglycan, making it functionally active; thus, mutations in gmppb cause dystroglycanopathic MD. The zebrafish (Danio rerio) is a powerful vertebrate model for musculoskeletal development and disease. Like human patients, gmppb mutant zebrafish present both mild and severe phenotypes. In order to understand the molecular mechanisms involved, we performed high-throughput RNA Sequencing (RNA-Seq) to measure gene expression at 4 and 7 days post-fertilization (dpf) in mild and severe gmppb mutants and sibling controls. We hypothesize that variable phenotypes in gmppb mutants are due to differences in gene regulation; therefore, we identified differentially expressed long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), both potent genetic regulators. In the severe mutants, 87 miRNAs were differentially expressed ($p<0.05$) whereas in the mild mutants, 57 miRNAs were differentially expressed at either time point. Using an established lncRNA annotation workflow, our lab identified approximately a dozen differentially expressed putative novel lncRNAs. In addition, we characterized the nearby protein-coding genes, highlighting lncRNAs with nearby "MD-relevant" genes - those involved in muscle organization, cell adhesion, mitochondrial function, the immune system, and transcriptional regulation. We linked gene expression of MD-relevant genes with miRNA expression by identifying differentially expressed miRNAs with gene targets (indicated by TargetScanFish) that were differentially expressed in the opposite direction, since most miRNAs regulate mRNAs by inducing degradation. Additionally, we generated network attack maps of the differentially expressed miRNAs in each set of the samples (siblings, mild mutants, and severe mutants) that allow for identification of miRNAs that contribute towards network stability. By integrating these analyses together, we constructed a genetic regulatory map that included miRNAs, novel lncRNAs, and MD-relevant genes. This work contributes toward our understanding of the molecular mechanisms of Dystroglycanopathy, highlighting genes that may serve as potential therapeutic targets.

1960A Transcription factor FOXO maintains neuromuscular junction homeostasis with aging in *Drosophila* *Allison Birnbaum*[1], Sean McLaughlin[1], Hua Bai[1] 1) Iowa State University.

The transcription factor FOXO is a known regulator of lifespan extension and tissue homeostasis. It has been linked to homeostatic maintenance of neuronal processes across many species, and has been shown to influence axonal trafficking and synaptic plasticity at the neuromuscular junction (NMJ). However, the role of FOXO on aging at the neuromuscular junction has yet to be evaluated. To identify the role of dFOXO (hereafter FOXO) on NMJ homeostasis, we profiled the abdominal ventral longitudinal muscles of adult Drosophila FOXO-null mutants and found they exhibit enlarged boutons and short branches that are morphologically similar to those of aged wild-type flies. FOXO has been shown to effect synaptic vesicle recycling, and when we knocked down FOXO specifically in the motor neuron we observed an abnormal accumulation of late endosomes in young flies. Endocytic pathways are also known to change with aging, and we found there is also an increase in Rab7-marked late endosomes associated with the axon in aged wild-type flies. Overexpression of wild-type FOXO in the motor neuron was able to delay the accumulation of Rab7 with aging as well as senescent NMJ morphology, suggesting FOXO is a positive regulator of neuronal homeostasis during aging. We used FOXO ChIP-seq and RNA-seq data to identify potential pathways downstream of FOXO activity in neuronal tissue, and performed a genetic screen of candidate genes in a motor neuron-specific FOXO-RNAi background to rescue the induced rab7 accumulation. Through this screening method we have identified a pathway through which we expect FOXO regulates endocytic homeostasis at the neuromuscular junction.

1968C Characterization of Genes Influencing the Age-specific Changes in Phagocytosis of *Drosophila melanogaster*, Using an In Vivo Phagocytosis Assay *Shonda Campbell*[1], Murielle Abissi[1], Jeff Leips[1] 1) University of Maryland Baltimore County.

The innate immune response is an evolutionarily conserved process that is essential for host survival in almost all multicellular organisms. As we age, immune functions begin to decline, or immunosenesces, reducing one's ability to fight infections, posing a serious risk to human health. The way that age affects the immune response can vary greatly among individuals and populations, and this variation has a significant heritable component. The genes responsible for this variation are not known. Age-related changes influence aspects of phagocytosis that are important for maintaining an adequate immune response; however, little is known about the genetic basis of cellular based immunosenescence. The goal of this project is to identify and characterize genes that regulate age-specific immune responses, with special focus on phagocytosis, to better understand the mechanisms that give rise to immunosenescence. This will be done using the model organism *Drosophila melanogaster*, using a newly developed phagocytosis assay to test to if previously identified candidate genes regulate changes in phagocytosis with age. This information could lead to targets for therapeutic treatments, and improve or restore immune function in elderly people.

Tuesday, April 28 12:00 PM - 3:30 PM

Gene Regulation/Disease Models and Aging 3 - Poster Q&A 844A Analyzing the Function of PcG Bound DNA Fragments Outside of H3K27me3 Domains *Joshua Price*[1], Lesley Brown[1], Judith Kassis[1] 1) NIH/NICHD. Bethesda, Maryland..

Polycomb group (PcG) proteins are repressors of developmental genes that act through diverse regions of non-coding DNA called Polycomb response elements (PREs). In *Drosophila*, PREs consist of multiple sequence motifs that interact with DNA-binding PcG recruiter proteins such as Spps, Cg, and Pho. These proteins act to recruit other PcG proteins which function as multi-protein complexes called Polycomb repressive complexes (PRCs). Predominantly, PRC1 and PRC2 coregulate gene silencing by compacting chromatin and creating H3K27me3 domains, a diagnostic mark of PRE regulated genes. Previous genome-wide chromatin immunoprecipitation (ChIP) studies on PcG recruiter binding showed the majority of PcG targets at active regions outside of H3K27me3 domains, apparent across multiple developmental stages and tissue types. To investigate the function of PcG proteins bound to these active regions, we are determining the region's abilities to act as PREs. We selected twenty DNA fragments outside of H3K27me3 domains with high PcG binding from a previous bioinformatics analysis of ChIP-seq data for Spps, Cg, and Pho. We are currently determining PRE function through analysis of each fragment's ability to repress a transgene and the outcomes will be presented.

869B Splicing factor Scaf6/CHERP regulates muscle and nervous system development in *Drosophila* *Shao-Yen Kao*[1], Keshika Ravichandran Ravichandran[1], Vanessa Todorow[1], Rippei Hayashi[3], Maria Spletter[1,2] 1) Biomedical Center, Department of Physiological Chemistry, Ludwig-Maximilians-Universität München, Munich, Germany; 2) Center for Integrated Protein Science Munich (CIPSM) at the Department of Chemistry, Ludwig-Maximilians-Universität München, Munich, Germany; 3) Institute of Molecular Biotechnology, Australian Academy of Sciences, Vienna, Austria.

Alternative splicing promotes transcriptome and proteome diversity by generating multiple mRNA transcripts from a single gene. Alternative

splicing is suggested to play an important role in muscle and neuronal development as alternatively spliced isoforms can have distinct functions in transcription, Ca^{2+} signaling or cytoskeletal organization. Proper regulation of splicing is important and mis-regulation of RNA splicing results in diseases such as myotonic dystrophy, dilated cardiomyopathy or spinal motor atrophy. The pathological mechanisms underlying these diseases are still debated, and more generally <10% of possible splicing regulators have been studied in muscle. While looking for novel splicing factors in *Drosophila* muscle, we identified the U2-associated protein Scaf6/DmCHERP. In vertebrate cell culture, CHERP has been shown to suppress cryptic splicing and regulate colorectal cancer tumorigenesis. We find that Scaf6 is expressed and plays an essential functional role in *Drosophila* muscle, as muscle-specific RNAi as well as whole-animal mutants are flightless. We show that the flight defect arises developmentally, as the flight muscle fibers are detached and atrophic from 48 h after puparium formation. Furthermore, loss-of-function of Scaf6 in muscle disrupts spontaneous muscle contraction during adult myogenesis, suggesting a possible role in muscle maturation that may be distinct from Scaf6's role in promoting progenitor cell proliferation. Interestingly, we find that loss of Scaf6 has distinct phenotypes in muscle and neurons, as neuronal-specific RNAi results in climbing, grooming, eclosion and motor neuron axon morphology defects. Our mRNA-Seq data demonstrates a function for Scaf6 in alternative splicing and the suppression of cryptic splicing specifically in muscle. Our results thus demonstrate a function for Scaf6 in multiple *Drosophila* tissues, highlighting the importance of alternative splicing during myogenesis and suggesting pleotropic functions for vertebrate CHERP.

872B A Dual-Activity Topoisomerase Complex Interacts with piRNA Machinery to Promote Transposon Silencing and Germ Cell Function *Seung Kyu LEE*[1], Weiping Shen[1], Yutong Xue[1], Shuaikun Su[1], Keji Zhao[2], Yongqing Zhang[1], Alexei Sharov[1], Weidong Wang[1] 1) National Institute on Aging / NIH; 2) National Heart, Lung, and Blood Institute / NIH.

Topoisomerase 3 beta (Top3b) is the only dual-activity topoisomerase in animals that can change topology for both DNA and RNA. Current evidence suggests that Top3b can facilitate transcription on DNA and translation of mRNAs. Top3b forms a complex with Tudor domain containing 3 (TDRD3), which interacts with the Fragile X Mental Retardation Protein (FMRP) to regulate mRNA translation in neurons. *Top3b* mutation in human has been linked to schizophrenia, autism, and cognitive impairment, whereas Top3b inactivation in mice results reduced lifespan and abnormal neurodevelopment. However, the mechanism of how Top3b maintains normal life-span and mental health remains largely unclear.
We have recently shown that the Top3b-TDRD3 complex interacts with the siRNA machinery to facilitate heterochromatin formation and transcriptional silencing of genes and transposable elements (TEs). Because mobilization of TEs can cause aging and defective neuronal function, one mechanism by which Top3b functions in aging and neurons could be through its interaction with siRNA machinery. In addition to siRNAs, PIWI-interacting RNAs (piRNAs) is the other major class of small RNAs that are germline specific and suppresses TEs. Here, we present evidence that Top3b-TDRD3 complex interacts with piRNA machinery to promote germline development and silencing of TEs. First, Top3b and TDRD3 form stable complexes with the piRNA machinery, including PIWI, in gonads of both mouse and *Drosophila*. Second, mutation of either *Top3b* or *TDRD3* results in reduced fertility with defective oogenesis and embryogenesis, as well as de-silencing of multiple transposons in gonads. Third, *Top3b* and piRNA machinery genetically interact to suppress expression of TEs. Notably, *Top3b* and *Tdrd3* mutants exhibit altered piRNA biogenesis and transcription of piRNA clusters. Together, our data reveal a novel role of Top3b-TDRD3 complex—interacting with the piRNA machinery to promote silencing of TEs and germ cell function.

888C Known Versus Predicted: RefSeq Functional Elements as a Reference Set of High-Confidence Non-Genic Elements in Mouse *Catherine Farrell*[1], Terence Murphy[1] 1) National Center for Biotechnology Information (NCBI), National Library of Medicine, National Institutes of Health.

The mouse genome contains many non-genic elements that function in diverse biological processes, such as gene regulation, chromosome organization, recombination or replication. Large-scale epigenomic mapping projects can predict the locations of gene regulatory elements, but those data are difficult to interpret in the absence of specialized research knowledge, are not generally visible in traditional genome annotation, have not been reconciled with experimental data in the literature, and do not always show function when tested experimentally. NCBI has therefore introduced a higher-confidence and more accessible dataset, RefSeq Functional Elements (www.ncbi.nlm.nih.gov/refseq/functionalelements/), which are annotated on the mouse genome alongside conventional genes. The dataset, which comprises richly annotated RefSeq records and descriptive records in the Gene database (www.ncbi.nlm.nih.gov/gene/), includes known enhancers, silencers, and other non-genic regions with known function. Data are derived from experimental evidence in the literature, either based on individual locus studies or on experimentally validated subsets from larger-scale studies, such as the positive subset of VISTA enhancers. The dataset is publicly available for FTP download (ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq/vertebrate_mammalian/Mus_musculus/latest_assembly_versions/) and can be visualized in the 'Biological regions' track available in NCBI graphical displays, including the Genome Data Viewer. These known elements are valuable for basic discovery of gene regulatory regions, or as known positive controls for genome-wide studies aimed at new discovery. This presentation will encompass analyses of mouse RefSeq Functional Elements, including their overlap with genes and a variety of predicted gene regulatory, chromatin state segmentation and conserved element datasets. The results reveal how these known elements are organized relative to genes, and indicate that this dataset incorporates gene regulatory elements that have not been found in epigenomic predicted datasets, including several developmental-specific elements that have been elucidated in the literature.

893B Long Noncoding RNA Interactions as Functional Determinants in Stem Cells *Keriayn Smith*[1], Sarah Miller[1,2], Joshua Starmer[1], Terry Magnuson[1] 1) UNC-Chapel Hill; 2) Johns Hopkins University School of Medicine.

Long noncoding RNAs (lncRNAs) are key regulators of many cellular functions in developmental and disease processes. They can adopt diverse structures, which facilitates their cooperation with transcriptional, translational and signaling regulators that are determinants of cell fate. Many lncRNAs have been implicated in stem cell maintenance and differentiation, and yet precise mechanistic understanding of each individual function remains poorly defined. Additionally, the molecular interactions that mediate each lncRNA's function action are not well understood. We have studied the developmentally relevant lncRNA, *Oip5os1* (aka *Cyrano)* and have shown that it supports gene expression network maintenance, cell adhesion and cell survival in embryonic stem cells. *Oip5os1*'s interactome assessment revealed its participation in diverse molecular networks. These include a developmentally important cell-signaling hub, RNA regulatory networks, and other nuclear and cytoplasmic localizing hubs. We propose that these networks individually drive Cyrano's unique functions. These interactome data will also provide a useful resource for investigations into more general interactions that regulate lncRNA function.

916A Effect of aromatic amino acid starvation-induced by glyphosate-based herbicides on splicing efficiency in *Saccharomyces cerevisiae* *Tulika Sharma*[1], Apoorva Ravishankar[1], Jennifer E.G. Gallagher[1] 1) West Virginia University, Morgantown, WV.

While it is well understood that cells regulate growth during starvation, molecular and genetic factors that contribute to this response is widely not clear. In yeasts, starvation leads to exit from the cell cycle into a quiescent state until the availability of nutrients returns. During short-term starvation, autophagy is induced to recycle building material until nutrients are replenished; however, long term starvation is detrimental for cells. The availability of nutrients activates the TOR pathway which is the central regulator of cell growth including transcription, ribosome biogenesis, translation, and cell cycle progression. One of the cellular responses to increasing survivability during starvation is to modulate splicing which leads to the stabilization of a subset of diverse introns. Most of the introns in yeasts are in the ribosomal protein-encoding genes. One possible function for these stabilized introns during starvation is to sequester splicing factors which further reduces splicing. Production of ribosomes is energy-intensive and is rapidly downregulated during any stress including starvation. During growth, TOR inhibits the accumulation of stable introns. Yeasts exposed to glyphosate-based herbicides (GBH), such as RoundUp™, undergo starvation and inhibit the TOR pathway. GBH inhibits the shikimate pathway which is responsible for the production of aromatic amino acids i.e., phenylalanine, tryptophan, and tyrosine. Quantitative Trait analysis identified a splicing factor that when knockout out increased yeast tolerance to GBH, which led to our hypothesis that downregulation of splicing increases survival during nutrient limitation. Comparison of RNA-seq from the sensitive and resistant strains found hundreds of differentially express transcripts and future analysis will quantify the changes in splicing efficiency. We expect to see modulation in the splicing profile of various ribosomal protein genes. We will then manually curate the list to look for candidate genes that are previously known to be associated with starvation and how modulation of splicing provides survival benefits to cells during starvation.

967A Analysis of epigenetic gene regulation using a novel zebrafish epigenetic reporter transgenic line *Miranda Marvel*[1], Kiyohito Taimatsu[1], Daniel Castranova[1], Andrew Davis[1], Aniket Gore[1], Brant Weinstein[1] 1) Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), NIH, Bethesda, MD.

Epigenetic mechanisms including DNA methylation, histone modifications, chromatin remodeling, and others play crucial roles during development. Human epigenome sequencing projects and other studies have shown that tissue-specific epigenetic marks can specify cell identity and tissue-specific gene expression during development. While large-scale genetic screens performed in fruit flies and nematode worms have been very successful in identifying epigenetic regulators in invertebrates, comparable screens have not been carried out in vertebrates, and mechanisms of vertebrate tissue-specific epigenetic regulation are still not well understood. We have developed a novel zebrafish transgenic epigenetic reporter line that reliably reports changes in tissue-specific epigenetic silencing based on the dynamic expression of destabilized green fluorescent protein (GFPd2). Using this line, we are performing the first ever large scale F3 genetic screen in a vertebrate to identify recessive mutants that regulate epigenetic silencing or activation. One of the mutants isolated through the forward genetic screen is a largely uncharacterized histone modifying gene. Preliminary analysis of the mutant phenotypes show that the mutated gene likely plays crucial roles in brain and neural tissue development. We are comprehensively characterizing the roles and epigenetic modifications regulated by this gene.

1926C Meiotic mutations impact lifespan and healthspan in *C. elegans* *Julia Loose*[1], Thayjas Patil [1], Judith Yanowitz[1], Arjumand Ghazi [1] 1) University of Pittsburgh, Pittsburgh, PA.

Meiotic chromosomal defects increase with age and are a major cause of miscarriages and age-related fertility loss in women. While the link between increased maternal age and fertility deficits is well established, it is unclear how the decline of germline integrity impacts animals' rate of aging. In this study we are using the nematode model *Caenorhabditis elegans* to address the cause-and-effect relationship between germline health and somatic aging. We examined genes that govern meiotic fidelity in the worm germline and addressed their role in somatic aging. Meiosis is a germline-restricted process and since *C. elegans* soma are post-mitotic, this allows us to selectively disrupt germline health and address the impact on the rate of aging of the whole organism. We found that 14 of the 38 mutations we examined, in genes that govern different steps of meiosis demonstrated a significant lifespan reduction. We found that germline-specific RNAi knockdown of four selected candidates, *spo-11, dsb-2, chk-2* and *htp-3,* also reduced lifespan. We then studied mutants of these four meiotic genes to investigate the healthspan consequences of meiosis disruption. We found that all four mutants exhibited deficits in at least one healthspan feature, and often more, including early loss of mobility, muscle pumping and neurological function, suggesting accelerated somatic aging. Overall, our data demonstrate that genes that govern meiotic fidelity in the *C. elegans* germline impact the physiological health of the somatic tissues and aging of the whole organism.

1954A *Caenorhabditis elegans* PIEZO channel coordinates multiple reproductive tissues to govern ovulation *Xiaofei Bai*[1], Jeffrey Bouffard[3], Avery Lord[2], Katherine Brugman[4], Paul Sternberg[4], Erin Cram[2], Andy Golden[1] 1) National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; 2) Department of Biology, Northeastern University, Boston, MA; 3) Department of Bioengineering, Northeastern University, Boston, MA; 4) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA.

The PIEZO proteins are involved in a wide range of developmental and physiological processes. Human PIEZO1 and PIEZO2 are newly identified excitatory mechano-sensitive proteins; they are non-selective ion channels that exhibit a preference for calcium in response to mechanical stimuli. Dysfunctional mutations of PIEZOs cause multiple diseases, such as Dehydrated Hereditary Stomatocytosis (DHS) and Generalized Lymphatic Dysplasia (GLD). However, the cellular and molecular mechanisms of PIEZOs in these diseases are less understood. To further understand the function of these proteins, we investigated the roles of *pezo-1*, the sole PIEZO ortholog in *C. elegans*. *pezo-1* is expressed throughout development in *C. elegans*, with strong expression in reproductive tissues. A number of deletion alleles as well as five putative gain-of-function mutants caused severe defects in reproduction. A reduced brood size was observed in the strains depleted of PEZO-1. *In vivo* observations show that oocytes undergo a variety of transit defects as they enter and exit the spermatheca during ovulation. Post ovulation oocytes were frequently damaged during spermathecal contraction. Due to PIEZOs channels' preferable permeability to Ca^{2+} and the importance of calcium signaling in regulating spermatheca contractility, we imaged the calcium indicator GCaMP3, which was specifically expressed in the spermathecal cells in *pezo-1* mutants. Calcium signaling in the spermatheca is normal during ovulation in *pezo-1* mutants, however, *pezo-1* interacts genetically with known regulators of calcium signaling. Depletion of Ca^{2+} channels *itr-1* and *orai-1*, and Ca^{2+} transporting ATPase *sca-1*, by RNAi substantially enhanced the reproductive deficiencies in *pezo-1* mutants. Additionally, the *pezo-1* mutants displayed a sperm navigational defect as well as a reduced ovulation rate. Sperm that were readily washed out of the spermatheca during ovulation failed to migrate back to the spermatheca, thus depleting the spermatheca of sperm early in the reproductive lifecycle. Mating with males rescued these reproductive deficiencies in our *pezo-1* mutants. Lastly, genetic interaction between *pezo-1* and the genes encoding the connexin hemichannel proteins, INX-14 and INX-22 which function in oocyte maturation and sperm attraction are consistent with the hypothesis that both *pezo-1* deletion and *inx-14/inx-22* RNAi inhibit oocyte-sperm communication. Using an auxin-inducible degradation system, we depleted PEZO-1 in somatic tissues, sperm, and the germline. Reduced brood sizes were observed in each tissue-specific

degradation strain, suggesting PEZO-1 may act in different reproductive tissues to promote proper ovulation and fertilization in *C. elegans*. To our knowledge, this is the first report of a functional role for PEZO-1 in *C. elegans* reproduction.

1962C Transcriptional response of aged *Drosophila melanogaster* following infection with an RNA virus *Noah Sciambra*[1], Lakbira Sheffield[1], Alysa Evans[1], Megan Delfeld[1], Janna Fierst[1], Stanislava Chtarbanova[1] 1) University of Alabama.

The fruit fly *Drosophila melanogaster* mounts potent innate immune defenses against a variety of microorganisms including viruses and serves as an excellent model organism for studying host-pathogen interactions. With its relatively short lifespan, the fruit fly is also an organism of choice for aging studies. In humans, advanced age is associated with greater susceptibility and higher mortality rates to infections including infections with some RNA viruses. Aged fruit flies also succumb more rapidly to infection with the RNA-containing virus Flock House Virus (FHV); however, we have only a limited knowledge about the interactions of the aged host with the virus and the factors responsible for the host's increased mortality.
We investigated how aging impacts *Drosophila*'s ability to respond to FHV infection. We demonstrate that wild type 30-days-old *Drosophila* succumb more rapidly to infection in comparison with younger (5-days-old) adults and that the increased mortality is not accompanied by an increase in virus load. These results suggest that mechanisms different from those in control of pathogen burden affect survival to FHV infection of the aged organism. We next used RNA sequencing (RNAseq) to compare transcriptional responses of young and aged *Drosophila* hosts that have been infected with FHV or injected with a control solution. We found that two days post-infection, older hosts exhibited larger transcriptional responses than younger individuals, upregulating ~2 times more genes and downregulating ~2.8 times more genes than young flies. Among differentially regulated genes for both age groups, we found 93% and 57% overlap between upregulated and downregulated genes, respectively. KEGG pathway analysis for differentially regulated genes specific to older FHV-infected flies revealed strongest downregulation for genes encoding components of metabolic pathways and strongest upregulation of genes encoding for purine metabolism, pyrimidine metabolism and RNA transport.
Our findings will set the stage for future analysis of the processes that underlie the increased mortality of older flies and the identification of novel age-specific factors associated with this process.

1985B dFOXO, a novel regulator of stress inducible Hsp70 drives Hippo-mediated tumorigenesis *Gunjan Singh*[1], Subhash Lakhotia[1] 1) Cytogenetics Lab, Institute of Science, Banaras Hindu University, Varanasi, India.

Cancers develop in hostile milieu of complex genetic interactions. Therefore, adaptive re-wiring of cellular signaling pathways help the cancer cells to mount a robust stress response for their survival. Stress inducible Hsp70 is overexpressed in different human cancers where its levels often correlate with poor prognosis. However, how the growing tumors regulate expression of Hsp70 remains poorly explored. To identify its regulator/s, Yorkie mediated tumorous clones were generated in *lgl*$^{-}$ background in *Drosophila*. Interestingly major HSPs (Heat shock protein) except Hsp70, showed elevated expression concomitant in parallel to progression since the beginning of tumor growth. However, expression of Heat Shock Factor (HSF) remained unaltered and Hsp70, downstream target of HSF contrarily expressed in tumor clones at later stage but only in a few cells of clones. Surprisingly, while HSF is majorly responsible for Hsp70 expression, HSF-RNAi did not affect its expression in tumors, clearly indicated another regulatory mechanism. Additionally, we found unlike the HSF-independence, the *lgl*$^{-}$ clones were Hsp70 dependent for tumor growth since down-regulation of Hsp70 reduced the clone size and tumorigenic potential. We therefore checked another regulator, dFOXO which follows cellular stresses specifically, oxidative stress to activate stress genes and has binding sites in Hsp70 promoter. We observed that dFOXO was elevated within the niche where Hsp70 expressing cells were present in tumors. Though evidences indicate tumor suppressor role of FOXO in cancer but in this study depleting dFOXO reduced the number of Hsp70 expressing cells and also caused tumor regression. Our work thus stablishes a novel pro-oncogenic role of dFOXO via regulation of Hsp70 in Hippo pathway mediated tumor growth.

2035A Evaluating TDP-43 Targets in Amyotrophic Lateral Sclerosis using Drosophila and Patient Spinal Cords *Alexander Blythe*[1], Erik Lehmkuhl [1], Suvitha Loganathan[1], Eric Alsop[2], Dianne Barrameda [1], Tina Kovalik [3], Bhavani Siddegowda[1], Archi Joardar[1], Robert Bowser [3], Kendall Jensen [2], Daniela Zarnescu [1] 1) University of Arizona; 2) Translational Genomics Research Institute ; 3) Barrow Neurological Institute .

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that is characterized by the progressive loss of motor neurons which results in paralysis and ultimately death. While there is no single established genetic or environmental cause of ALS, analyses on ALS patient tissue have revealed insoluble complexes containing highly ubiquitinated and phosphorylated TAR DNA-binding (TDP-43) proteins arranged in prion-like aggregates in the cytoplasm of affected motor neurons. These aggregates have been found in 97% of ALS cases and are recognized as a pathological hallmark of numerous neurodegenerative diseases, ALS included. Subsequent studies on these insoluble cytoplasmic complexes suggest that certain mRNA transcripts may be sequestered by TDP-43 into the complexes, potentially dysregulating global translation and inhibiting critical cellular processes. Among several proteins currently being investigated as a potential target during TDP-43 proteinopathy is dally-like protein (DLP), a glypican expressed on the plasma membrane and in the extracellular matrix at the neuromuscular junction. The focus of my project is to assess the change in DLP protein abundance in motor neurons and spinal cord tissue and evaluate the physiological impact of DLP abundance on locomotor function and lifespan. Using a *Drosophila* model of ALS generated by overexpressing wild-type or TDP-43^{G298S}, we found that DLP knockdown exacerbates locomotor dysfunction in both disease models while overexpressing DLP results in a rescue of locomotor function. Immunofluorescence and western blots showed that DLP levels are significantly reduced at the neuromuscular junction and significantly increased in the ventral nerve cord in larvae expressing TDP-43^{G298S}. Interestingly, western blots of human spinal tissue similarly show that levels of DLP human orthologs, GPC4 and GPC6, are altered in spinal tissue from ALS patients. These results show that our findings in flies can be recapitulated in patient spinal cords and suggest that the expression of DLP in ALS may be altered by defects in axonal transport or translation. We are currently investigating other TDP-43 targets in fly models of ALS and patient spinal cords.

2066B Heterogeneity in the fat body tissue revealed using single-cell RNA sequencing *Vanika Gupta*[1], Brian Lazzaro[1] 1) Cornell University.

Fat body tissue in *Drosophila* dynamically controls systemic immune responses, metabolism and detoxification, and provisioning of developing eggs. The constraints imposed by using the same tissue for multiple purposes can result in sub-optimal performance of each process, and it is largely unknown how tissues balance their roles. We hypothesized that the fat body achieves its multiple functions through a division of labour resulting in cellular subpopulations performing specific functions. We further hypothesized that physiological perturbations affect specific subsets of the tissue, including the robust systemic immune response triggered by bacterial infection. We used single-cell RNA sequencing to test for heterogeneity in the fat body tissue. To understand the dynamic nature of the tissue, we used flies which were either challenged with a Gram-negative bacteria *Providencia rettgeri* or unchallenged, under both reproductively active and inactive conditions. The nuclei prepared from these fat body tissues were

used for bead encapsulation on the 10X Chromium platform and the libraries were sequenced using Illumina. The results supported our hypothesis that the fat body tissue in fruit flies consists of transcriptionally heterogeneous cell subpopulations marked by unique gene expression signatures. Our results consistently showed that the ten most abundant cell subpopulations represented about 90% of all the cells assayed across replicates and treatments. Across treatments, we found that the two most abundant subpopulations were marked by the expression of genes encoding yolk proteins, which play an important role in egg provisioning. While upon bacterial challenge, the expression of immune system genes was significantly upregulated ubiquitously across all the cells in the tissue, as opposed to being restricted to particular subpopulations. Nevertheless, the specific immune genes expressed differed across subpopulations. Our findings demonstrate that the polyfunctional fat body achieves its multiple roles both through the specialization of cellular subpopulations and dynamic tissue-wide change in gene expression.

2078B Growing a thicker heart wall under *ELAC2*-linked cardiomyopathy condition in *Drosophila* *Ekaterina Migunova*[1], Edward Dubrovsky[1] 1) Fordham University, Bronx, NY.

Hypertrophic cardiomyopathy (HCM) is a pathological condition characterized primarily by the thickening of the left ventricular heart wall. It affects 1 in 500 people and is the most common cause of sudden cardiac death among adolescent children. HCM has a heterogeneous etiology, as it may be caused by mutations in 30 different genes. Certain alleles of human gene *ELAC2* (*RNaseZ*) have been associated with an especially severe form of HCM. Patients carrying these mutant alleles have a median life expectancy of 4 months. *ELAC2* encodes the RNaseZ protein, an endonuclease essential for tRNA maturation.
Previously we have reported creating a Drosophila model of RNaseZ-linked HCM. The fly model recapitulates major symptoms observed in human patients such as heart wall hypertrophy and reduced longevity. Here we investigate the underlying processes leading to the heart wall thickening. Fly heart is a tube composed by single layer of 52 pairs of cardiomyocytes. These cells are fully differentiated and they do not proliferate. We hypothesized that heart wall thickening is caused either by an increase in the heart cell number, heart cell growth or both. To test this, we created a cardiomyocyte specific marker with strong expression at all stages of fly development. We used this marker to count the number of cardiac cells in fly heart and for estimating cardiomyocyte ploidy, as an indicator of cell growth. Our data show that RNaseZ mutant flies experience both cardiomyocyte hypertrophy and hyperplasia. Given that cardiomyocyte hypertrophy is a common feature of HCM in humans, a similar phenotype in flies was expected. However, finding hyperplasia was a surprise. Though an increase in cell number in human hypertrophic heart has been reported, this is the first time it has been documented in the fly HCM model. It opens an opportunity for us to find the underlying mechanism of cell over-proliferation.
Currently, using immunostaining, we are also testing if RNaseZ mutations cause increased deposition of extracellular matrix, a phenomenon similar to fibrosis in humans. These findings will allow us to study the mechanisms leading to heart hypertrophy due to RNaseZ mutations.

2079C A Novel Role for Micos Complex *CHCHD3/6* in Cardiac Function and Structure *Katja Birker*[1], Georg Vogler[1], Sreehari Kalvakuri[1], Maria Azzurra Missinato[1], Jeanne Theis[2], Timothy Olson[2], Rolf Bodmer[1] 1) Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA; 2) Mayo Clinic, Rochester, MN.

Hypoplastic left heart syndrome (HLHS) is a severe birth defect that accounts for up to 4% of congenital heart diseases (CHDs). HLHS is thought to be a complex, multifactorial genetic disease, however, our ability to understand the genetic complexities and pathogenic mechanisms leading to this disease is limited. A candidate list of ten genes was generated based on whole genome sequencing of a patient with sporadic HLHS and their unaffected family. Of the candidate HLHS genes tested, cardiac-specific knockdown (KD) of *CHCHD3/6* in *Drosophila* results in drastically compromised heart contractility. Additionally, filamentous (F-) actin-based sarcomeric structures are greatly altered and other myofibrillar proteins (Myosin, Sallimus, α-actinin, Obscurin) are slightly misaligned, indicative of a potentially critical role for CHCHD3/6 in the adult heart. CHCHD3/6 is part of the MICOS complex, important for maintaining cristae morphology and respiratory complex assembly and is suspected to be critical for *Drosophila* heart and muscle function. KD of *CHCHD3/6* in muscle cells caused mitochondrial dysfunction and mislocalization in the indirect flight muscles, likely due to disruption of cristae morphology and reduced ATP production. KD of other MICOS complex and related components also exhibit significantly reduced contractility, however, sarcomeric F-actin appears normal. In developmental studies, *CHCHD3/6* function was particularly required at late pupal/early adult stages, consistent with a later stage metabolic shift from glycolysis to oxidative phosphorylation. Extended bioinformatic analysis of 183 HLHS patients revealed an additional 15 variants in *CHCHD3*, *CHCHD6*, and other MICOS complex subunits. To further understand the mechanism of *CHCHD3/*6 in the adult heart, we have generated a cardiac-specific driver line combined with UAS-*CHCHD3/6*^{RNAi} or a *CHCHD3/6* loss-of-function allele. Using these sensitizer lines, we are currently examining interactions between *CHCHD3/6* and other MICOS subunits, as well as with other genes that were mutated in the HLHS patients with *CHCHD3/6* variants. Additionally, we are studying the interaction of mitochondrial genes with *CHCHD3/6* by knocking down or overexpressing mitochondrial genes involved in a variety of processes (e.g. fission/fusion, mitophagy, OXPHOS). Further elucidation of novel candidate genes, genetic interactions, and potentially causal pathways could lead to the targeted prevention of HLHS and other CHDs.

2091C Flies in A MinE, FLAME: exploring the biology of deep underground mining *Thomas Merritt*[1], John Hick[1] 1) Laurentian University.

Mining is a physically and mentally strenuous profession. Underground miners are often exposed to environmental extremes, including temperatures of 35°C or more, air filled with mine dust, and atmospheric pressure exceeding 20% greater than surface pressure. These environmental challenges can lead to changes in biology such as accelerated tiring, increased appetite, and difficulty thinking. While we know that the mining environment leads to these biological changes, we do not know the molecular and biochemical basis of these changes. We are using fruit flies, *Drosphila melanogaster*, and a deep underground laboratory to explore the molecular basis of these biological changes. Using the facilities at SNOLAB, a controlled clean laboratory facility located 2 kilometers underground, and a novel fly exercise machine, the Flygometer 2.0, which uses the negative geotaxis behavior of fruit flies to exercise flies, we simulate mining conditions of high atmospheric pressure and physical activity. We measured the longevity of both sexes in a pair of *D. melanogaster*lines when exposed to this mining environment proxy and found a significant decrease in their average lifespan, in some, but not all flies. To further study the overall effects of the mining environment, we are using a broad-spectrum approach analyzing both the transcriptomic and metabolomic response. We measured the metabolomic response using liquid chromatography - mass spectrometry to separate and identify a broad suite of metabolites and quantify changes in response to exposure to our simulated mining conditions. We have also measured the broad transcriptomic changes using next generation mRNA sequencing (RNA-seq) and differential expression analysis to quantify changes in gene expression. Changes in the metabolic pathways, determined by associations between metabolomic and transcriptomic analysis, will provide a basis for future targeted exploration of specific metabolic pathways, genes and metabolites that are altered under under-

ground mining conditions. Ultimately, we hope to make suggestions to limit or mitigate the negative impacts of working deep underground.

2146A Zevatars; the future of personalized cancer medicine *Shaila Mudambi*[1], Jessica Miller[2], Saumya Kasliwal[1], Seray Er[1], Kamden Gray[1], Ceylan Metin[1], Russell Sillmon[1], Anna Zdunek[1], Mary Pasquale[1], Michael Pishvaian[1], Stephen Byers[1], Eric Glasgow[1] 1) Georgetown University; 2) Vanderbilt University Medical Center.

Personalized medicine offers a more informed strategy to treat cancer patients. Although promising, the efficacy of targeting therapy based on a patient's molecular profile is still difficult to predict. Currently, *ex vivo* models such as patient derived mouse xenograft (mPDX), organoid culture, and conditionally reprogrammed cells (CRC) are being developed to improve the prediction of chemotherapy response. These models require significant expansion of tumor cells for drug testing requiring relatively long wait times, which introduces potential for changes in genetic and epigenetic characteristics of the tumor. Moreover, these models do not consider the tumor microenvironment, including the immune system which is important for cancer therapy. Therefore, to compliment these models, we have developed patient-derived xenografts in zebrafish embryos or 'Zevatars' that directly samples the patient's tumor, is rapid enough to benefit patients with aggressive disease, allows for high-throughput drug screening, and provides a readout that is not simply a surrogate for cell growth. The assay is performed by **implanting** labelled pieces of patient tumor tissue (fresh or cryopreserved) into 2 dpf embryos, treating with drugs, monitoring tumor behaviors, including size changes, cell migration, and metastasis through imaging. The best treatment for a given patient can be determined in less than one week. In addition, we can generate Zevatars from multiple tumor types including pancreatic cancer and liver metastasis, and are expanding our repertoire to breast, lung, ovarian and squamous epithelial cancers. Our results show that tumor biopsy tissue differentially responds to standard of care drugs. Importantly, the response varies greatly from patient to patient, thus **recapitulating patient tumor behavior in the clinic**. However, one major limitation of the Zevatar is that we cannot study adaptive immune responses or cancer immunotherapy in this model, though we have observed macrophage homing to cancer xenografts suggesting a role for the innate immune system. Hence we are developing a humanized fish in which we replace the zebrafish hematopoietic system with a human hematopoietic system via stem and progenitor cell transplantation (HSPC). If successful we can then predict chemotherapy and immunotherapy effectiveness by using a Zevatar with a patient's own HSPCs coupled with their tumor biopsy. In developing this technology we can provide a **rapid and relatively cost-effective** method for personalized cancer diagnostics.

Wednesday, April 29 12:00 PM - 3:00 PM

Intracellular Dynamics/Genome Integrity 1 - Poster Q&A 1734C Ran-GTP regulation of cytokinesis in the early *C. elegans* embryo *Imge Ozugergin*[1], Karina Mastronardi[1], Alisa Piekny[1] 1) Concordia University.

Cytokinesis is required to complete division, and must be tightly regulated to prevent aneuploidy and cell fate changes. Microtubule-dependent and –independent mechanisms regulate cytokinesis, and reliance on a pathway varies based on cell size, shape or fate. We found that Ran-GTP regulates the localization of human anillin (*C. elegans* ANI-1), a core component of the cytokinetic ring. During mitosis, a Ran-GTP gradient is maintained with high levels around chromatin and low levels in the cytosol. This is because the RanGEF RCC1 (*C. elegans* RAN-3) is tethered to chromatin, while RanGAP (*C. elegans* RAN-2) is cytoplasmic. Our model is that importin-α/-β binds to the nuclear localization signals of cortical regulators to facilitate their localization and function for cytokinesis, and position the ring away from chromatin. To determine if requirements for the Ran pathway differs depending on cell fate, we studied cytokinesis in the early *C. elegans* embryo. The fertilized embryo divides asymmetrically to give rise to an anterior AB daughter fated to be multiple tissues, and a posterior P_1 daughter fated to be germline. Imaging with high temporal resolution revealed that each cell has unique ingression kinetics, supported by differences in the accumulation of contractile proteins. Lowering Ran-GTP levels via RAN-3 RNAi increased ingression kinetics in both AB and P_1 cells, which was suppressed by co-depletion of the contractility regulators ECT-2 (RhoA-GEF) or LET-502 (Rho Kinase). Interestingly, co-depletion of ANI-1 suppressed RAN-3 phenotypes in AB, but not P_1 cells, suggesting that they have different pathway requirements. This is supported by different requirements for importin-α (IMA-3) and -β (IMB-1) in AB vs. P_1 cells. We are currently using CRISPR to generate mutations in ANI-1 that disrupt importin-binding. Our findings reveal differences in mechanisms regulating cytokinesis in cells with different fates and emphasize the need to study cytokinesis *in vivo*.

1738A TOM-1/Tomosyn is an inhibitor of growth cone protrusion and works with the UNC-6/Netrin receptor UNC-5. *Snehal Mahadik*[1], Erik Lundquist[1] 1) University of Kansas.

Previous work from the Lundquist lab showed that the UNC-6/Netrin receptors UNC-40 and UNC-5 regulate growth cone protrusion. UNC-40 stimulates protrusion whereas UNC-5 inhibits protrusion, and asymmetric distribution of protrusive activity across the growth cone results in directed growth cone migration away from UNC-6/Netrin (the Polarity/Protrusion model). *unc-5* mutant VD growth cones display unpolarized and excessive protrusion. UNC-5 inhibits protrusion using the FMO flavin monoxygenases, likely via actin inhibition, and by restricting growth cone microtubule entry via UNC-33/CRMP. MTs deliver vesicles into the growth cone. To explore the role of vesicle fusion in growth cone protrusion, we analyzed *tom-1/tomosyn* mutants. Tomosyn normally occludes the formation of the SNARE complex by interacting with and inhibiting syntaxin-1. VD growth cones of *tom-1* mutants were similar to wild-type and showed no apparent protrusion defects. However, loss of *tom-1* suppressed the effects of constitutively-activated MYR::UNC-5, which alone causes small growth cones with little protrusion. This suggests that TOM-1 is normally required for the inhibitory effects of MYR::UNC-5 on growth cone protrusion. Transgenic expression of wild-type *tom-1* resulted in small and non-protrusive growth cones in both wild-type and *unc-5* mutant backgrounds, consistent with a role of TOM-1 in inhibiting protrusion downstream of UNC-5. Future studies will involve analysis of actin and MTs in TOM-1 mutant growth cones, and an analysis of other regulators of vesicle fusion, including UNC-64/Syntaxin and UNC-13. In the polarity/protrusion model of growth cone outgrowth, UNC-6/Netrin inhibits growth cone protrusion via the UNC-5 receptor. Previous studies showed that UNC-5 inhibits protrusion via the FMOs and actin destabilization, and by preventing MT entry via UNC-33/CRMP. These results suggest that UNC-5 inhibits protrusion via a third pathway, employing TOM-1/tomosyn to prevent vesicle fusion.

1754B *C. elegans* surface barrier lipid composition is regulated by the DBL-1/BMP signaling pathway *Bhoomi Madhu*[1], Lionel Faure[1], Tina Gumienny[1] 1) Texas Woman's University, Denton, TX.

All living organisms possess mechanisms to protect themselves from their environment. For nematodes, the cuticle and surface coat are physical barriers that protect them from their external environment. The protein composition of the cuticle is well studied; however, the lipid composition of the surface barrier lacks characterization. To understand how the surface barrier of *C. elegans* confers physical protection, we are studying its

lipid composition. We previously showed that the protective surface barrier is affected by DBL-1/BMP (Bone Morphogenetic Protein) signaling in a dose-dependent manner. Previously published microarray analyses indicate that DBL-1 regulates lipid metabolism genes. We developed a protocol to extract surface-enriched lipids and remaining (internal) lipids. To determine if DBL-1 affects the general lipid composition of surface and internal lipids, we used thin layer chromatography and solid-phase extraction followed by gas chromatography-mass spectrometry (GC-MS) analyses. We observed common lipid classes (*e.g*., phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid, and triacylglycerols) in both surface and internal lipid extractions regardless of DBL-1 expression. However, we observed a decrease in the internal triacylglycerol and phospholipid levels between wild-type and *dbl-1(nk3)* animals, supporting previously published data. Next, to determine if the DBL-1 pathway affects the fatty acid composition of surface and inside lipids, we used GC-MS to identify, quantitate, and compare surface lipids to internal lipids of wild-type and *dbl-1* knockout populations. We determined that relative levels of specific surface-enriched lipids are reduced by loss of DBL-1. We also observed a difference in the acyl moieties of the inside lipids of *dbl-1* knockout populations compared with wild-type populations. We conclude that while the same lipid classes are represented in both wild-type and *dbl-1(nk3)* populations, the composition of the lipid classes is different. Lastly, to identify lipid metabolism genes regulated by DBL-1, we performed RNA-seq of wild-type and *dbl-1(nk3)*. We will validate the expression of candidate lipid metabolism genes by real-time PCR. This study 1) identifies the specific lipid composition of the *C. elegans* surface barrier of both wild-type and *dbl-1* mutant animals, and 2) will help characterize underlying molecular mechanisms for synthesis of lipids regulated by DBL-1.

1759A Slogging through Mud: Isoform Expression and Function Nicholas Lowe[1], Ruiyue Tan[1], Nicholas Weeks[1], Sifan Yang[1], Dan Bergstralh[1] 1) University of Rochester, Rochester, NY.

The conserved protein Mud/NuMA/Lin-5 (flies, vertebrates, worms) has long been implicated in the orientation of cell division. Together with Dynein, Mud acts to produce a pulling force that reels astral microtubules, and therefore the metaphase spindle, into alignment. This force is anchored by another conserved factor, Pins/LGN/GPR1-2 (flies, vertebrates, worms), in most cell types examined. We and others have found that multiple isoforms of Mud do not include the Pins-binding domain. Mud is less well-studied outside of spindle orientation, but is implicated in diverse processes, including migration of the oocyte nucleus and spindle cohesion at meiosis II. We are currently investigating the expression and function of Mud isoforms, and in particular whether they participate in Mud function outside of spindle orientation.

1840A Neuronal ribosomal protein function regulates *Drosophila* growth and development *Lisa Deliu*[1, 2, 3, 4], Deeshpaul Jadir[1, 2, 3, 4], Abishek Ghosh[1, 2, 3, 4], Savraj Grewal[1, 2, 3, 4] 1) Clark H. Smith Brain Tumour Center, Calgary, AB; 2) Arnie Charbonneau Cancer Institute, Calgary, AB; 3) Alberta Children's Hospital Research Institute, Calgary, AB; 4) Cumming School of Medicine- University of Calgary, Calgary, AB.

Stimulation of ribosome biogenesis is a conserved mechanism of growth control. Studies, mostly in yeast and cell culture, have shown how ribosome synthesis controls cell growth. However, less is know about how ribosome synthesis promotes body growth and development. We have been studying this issue by studying the *Minutes*. These are a class of dominant ribosomal protein (*rp/+)* mutants that exhibit a characteristic delay in larval development – a phenotype classically thought to due to an overall reduction in ribosome numbers and protein synthesis. However, when we examined three *Minutes* (*rpS13/+, rpS26/+* and *rpL38/+*) we saw little or no change in either global ribosome numbers or in protein synthesis rates, when compared to wild-type controls. Instead, as discussed below, we found evidence of a cell type-specific function for one RP (S13) in the control of development.
Termination of the larval period is controlled by a neuroendocrine circuit that leads to a pulse of secretion of the steroid hormone ecdysone from the prothoracic gland (PG) in response to signals from specific CNS neurons. We found that *rpS13/+* animals had a delayed ecdysone pulse as seen by delayed expression of the 'Halloween' genes, *spooky* and *phantom*, which are required for ecdysone synthesis in the PG. Ecdysone feeding also partially reversed the delay in development therefore we postulated that these effects might reflect a specific role for S13 in the CNS-PG neuroendocrine circuit. To test this, we used the GAL4/UAS system to see if tissue selective expression of S13 could rescue the delayed development seen in *rps13/+* animals, expression of S13 in the PG had no effect. However, we found that expression of S13 in neurons (using either elav-Gal4, or nSyb-Gal4) could rescue the delay in development in *rpS13/+* animals by ~40%. Furthermore, we discovered that expression of S13 in serotonergic (5-HT) neurons alone (TRH-Gal4) lead to the same rescue in developmental timing as pan-neuronal S13 expression, while re-expression in other neuronal subtypes had mostly negligible effects. Three pairs of 5-HT innervate the PG to control ecdysone release, and S13 expression in these neurons (using the R29H01-GAL4) driver also partially rescued the delay in development in *rpS13/+* animals. While axonal projection growth of these neurons do not appear to be affected, electrical activation of *rpS13/+* animals with TRPA1 channel expression partially rescues adult eclosion delay. Our model suggests that RpS13 is required for proper synaptic activity in the serotonergic neurons that signal to the prothoracic gland to produce ecdysone. A reduction in RpS13 in these neurons thus leads to delayed ecdysone production and delay in development.

1842C The role of intestinal TOR signaling in metabolic responses to bacterial infection. *Rujuta Deshpande*[1,2,3,4], Andee Qiao[1,2,3,4], Savraj Grewal[1,2,3,4] 1) Clark H. Smith Brain Tumor Center, University of Calgary, Calgary, AB; 2) Arnie Charbonneau Cancer Institute, Calgary, AB; 3) Alberta Children's Hospital Research Institute, Calgary, AB; 4) Cummings School of Medicine- University of Calgary, Calgary, AB.

Upon oral infection with pathogenic bacteria, Drosophila adults mount organism-wide immune and physiological responses in order to both provide infection resistance and to promote tolerance. The intestine plays a central role in mediating these effects. For example, upon infection, damaged intestinal epithelial cells trigger a local cytokine response to promote stem cell-mediated regeneration in order to promote repair and maintain tissue integrity. In addition, the intestine functions as an endocrine organ to signal to other tissues such as the fat body and brain to control host metabolism and physiology. We have begun exploring the role for TOR kinase signaling in these infection responses. We found that *P.e.* feeding induced a rapid increase in TOR kinase signaling in the intestine. This increase was also seen with feeding with *Ecc* and *Vibrio*, two other gram-negative bacteria, but not with stressors such as DSS or bleomycin. These infection induced increases in intestinal TOR signaling were particularly pronounced in the enterocytes (ECs) but were also apparent in enteroendocrine (EE) and stem cells. TOR signaling is a well-established regulator of cell, tissue and whole body metabolism. We therefore explored whether the intestinal TOR induction might contribute to metabolic responses to infection. We found that oral *P.e.* infection led to upregulation of many lipid metabolic genes in the intestine and also lead to reduced levels of total body lipids. We found that inhibition of intestinal TOR by feeding with rapamycin blocked many of the infection-mediated changes in gut lipid gene expression. In contrast, overexpression of the TOR activator, Rheb specifically in ECs was sufficient to induce many of the metabolic gene expression changes caused by infection. We also found that EC-specific Rheb overexpression mimicked the reduction in whole body lipids levels caused by infection. These data suggest that intestinal TOR induction may mediate both local and whole body metabolic responses to infection. EE cells secrete many different endocrine hormone peptides that can signal both locally and remotely to control metabolism. We found that *P.e.* infection induced

TOR-dependent upregulation in the expression of several of these EE-derived peptides. We are currently exploring whether signaling through these peptides may couple the infection induced intestinal TOR activation to host lipid metabolic changes, and how these changes may contribute to infection tolerance.

1848C Splice isoforms of Alan shepard with distinct roles in fat metabolism *Claire Gillette*[1], Kelsey Hazegh[1], Tânia Reis[1] 1) University of Colorado, Anschutz Medical Campus .

Mounting evidence points to an important yet poorly understood role for genetic background in the control of organismal fat. We previously used an unbiased genetic screen to identify 66 genes that when mutated increase body fat in larvae. Here, we explore the first known characterization of the RNA binding protein Alan shepard (Shep) in organismal fat storage in *Drosophila melanogaster*. We found that knockdown of *shep* in neurons phenocopied the high-fat phenotype of the *shep* mutant and drives changes in the complex metabolic behaviors of feeding and activity. In contrast, knockdown of *shep* in the fat body resulted in a lean phenotype. We further investigated this uncharacterized role of Shep in the fat body. The Shep genomic locus codes for eight mRNA and six protein isoforms. The fat body endogenously expresses five of these mRNAs: *shep-RA*, *RB*, *RE*, *RF*, and *RH*. Thus far, we have shown that fat body-specific overexpression of the Shep-RE increases organismal fat. Neither overexpression of Shep-RA nor RB generated an adiposity phenotype. Next, we found that the RNA-binding activity is necessary to generate the fat phenotype in Shep-RE FB overexpression. Additionally, we found that Shep transcript and protein levels are regulated in a nutrient-dependent manner, and that the relative proportions of the isoforms are nutrient sensitive. As nutrient content of the diet increases, Shep isoform expression at both the mRNA and protein levels increase. This suggests that Shep is regulated by a nutrient sensing pathway. We continue to explore the functional differences between these endogenously expressed isoforms to understand how Shep functions in an isoform-specific manner to ultimately regulate whole organism fat storage.

1861A Alternating polarity in a linear array of *Scaptodrosophila* follicle cells *Miriam Osterfield*[1] 1) UT Southwestern.

Epithelial cells can exhibit multiple types of polarity. In addition to the apical-basal polarity generally found in epithelia, polarization within the plane of the epithelium can also be present. Examples include the planar cell polarity (PCP) pathway, in which cells are all oriented in a single direction within the plane of an epithelial sheet, or the planar polarity seen during Drosophila axis elongation, where Bazooka (Par-3) is localized to dorsal-ventral cell-cell interfaces and Myosin II is localized to anterior-posterior interfaces. Here we examine the in-plane polarity in the floor cells of the follicular epithelium of *Scaptodrosophila pattersoni* (which is conspecific with *Scaptodrosophila lebanonensis*). During stage 10 of oogenesis, these cells form a single curved line within the epithelium. In later stages, these cells deform to create the underside of the dorsal appendages, through a process that involves the substantial lengthening of alternating floor-floor interfaces. In other words, there is an alternating left/right pattern, so if a given floor cell specifically lengthens on its left edge, its neighboring floor cells lengthen on their right edges. The floor-floor interfaces that are fated to lengthen show a strong enrichment of Par-3, aPKC, and F-actin localization at the onset of lengthening. Pharmacological approaches suggest that alternating polarization of F-actin, or possibly the maintenance of this polarization, is not dependent on aPKC localization. We are continuing to examine the molecular basis underlying this unusual example of planar polarity.

1865B Loss of F-box motif Encoding Gene *SAF1* and Chromatin Associated factor *CTF8* together contributes to MMS Resistance and HU Sensitive phenotype in *S. cerevisiae* *Meenu Sharma*[1], Vijeshwar Verma[1], Narendra Kumar Bairwa[1] 1) Shri Mata Vaishno Devi University, katra, Jammu and Kashmir, India.

Abstract: Cohesion related replication factor-C complex constitutes, three subunits called Ctf18, Ctf8 and Dcc1. These three subunit complex assist the loading of PCNA onto the chromosome. None of the replication factor C components are essential for cell viability. The null mutant of *CTF8* in *S. cerevisiae* shows high frequency of chromosome loss. The *SAF1* gene product of *S. cerevisiae* involves in recruitment and degradation of adenine deaminase factor Aah1p through SCF-E3 ligase mediated ubiquitination. Here we have investigated the genetic interaction between *SAF1* and *CTF8* genes. The single and double gene deletions of *SAF1* and *CTF8* were constructed in BY4741 genetic background and evaluated for growth fitness, genome stability and cellular growth response to genotoxic stress caused by Hydroxyurea (HU) and Methyl methane sulfonate (MMS). The *saf1Δctf8Δ* strain showed the increased growth phenotype in comparison to WT, *saf1Δ* and *ctf8Δ* strains on YPD medium. However, *saf1Δctf8Δ* strain when grown in the presence MMS showed resistance and HU sensitive phenotype when compared with *saf1Δ, ctf8Δ.* The frequency of *Ty1* retro-transposition was also elevated in *saf1Δctf8Δ* in comparison to either *saf1Δ* or *ctf8Δ*. The number of cells showing the two or multi-nuclei phenotype was also increased in *saf1Δctf8Δ* cells when compared with the either *saf1Δ* or *ctf8Δ*. Based on these observations, we report that the absence of both *SAF1* and *CTF8* genes together contributes to MMS resistance, HU sensitivity, and genome instability phenotype. This report warrants the further investigation into the mechanisms of differential growth phenotype due to loss of *SAF1* and *CTF8* together in presence of genotoxic stress.
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1867A Differential expression of tRNAs may shape maize response to environmental stress *Sarah Jensen*[1], Edward Buckler[1,2] 1) Cornell University, Ithaca, NY; 2) USDA-ARS, Ithaca, NY.

Transfer RNAs are a ubiquitous but often overlooked piece of the central dogma of biology. Recently however, the importance of tRNAs has become more apparent. In yeast, tRNAs were shown to be differentially expressed in response to oxidative and temperature stress, while human studies have shown that tRNA profiles vary significantly across tissues. Our lab has shown that tRNA sequences are highly correlated with bacteria and archaea optimal growth temperatures (OGTs), and can predict OGT for a range of prokaryotes using only tRNA sequence data. Taken together, these results suggest that tRNAs are exquisitely adapted for specific environmental conditions, and that tRNA expression can have an important downstream effect on diverse characteristics, including stress response. We hypothesize that tRNA differential expression is particularly important in maize, which has adapted to a wide range of environmental conditions. In agreement with this, maize has 3-5 times more tRNA loci than humans, and tRNA quantity and isoacceptors vary across maize tissues. We describe how tRNA expression in maize changes over time and in response to temperature stress, and hypothesize that tRNA differential expression could shape maize response to the environment by quickly changing the translation efficiency of existing mRNA transcripts and promoting fast translation of specific stress-response genes.

1871B *THI73* Mediates Regulation of *CLN3* G1 Cyclin Activity in *Saccharomyces cerevisiae*. *Monroe McKay*[1], Jacob Menke[1], Alex Richards[1], Mary Miller[1] 1) Rhodes College.

Cell cycle regulation in *Saccharomyces cerevisiae* is tightly controlled, especially when external stimuli have the capacity to induce stress. G1 cyclins are the primary regulatory factors regarding the G1/S transition that commits a cell to replication of its DNA and subsequent cell division. Cln3—one of the three G1 cyclins—binds Cdc28, initiating transcription of genes necessary for the progression to S phase. In order for Cln3 to direct transcription, it must localize to the nucleus (Miller and Cross, 2000). Nuclear localization of Cln3 requires the presence of its C-terminal bipartite nuclear localization sequence (NLS). The NLS of Cln3 supports movement to the nucleus via Ran dependent hydrolysis of GTP. An analysis of 79 targeted genes showed twenty with defects in Cln3 NLS function when deleted. Twenty of these genes exhibited defects in Cln3 localization when knocked out. Of these twenty genes, nine showed functionally relevant growth defects in the context of full length Cln3 activity. Of these twenty genes identified as important for Cln3 NLS activity, *THI73* was the only to demonstrate specificity for a Cln3 NLS in comparison to the monopartite NLS from the SV40 Large T protein and a bipartite NLS from the nucleoprotein (NP) of *Homo sapiens*. Thus, we postulate that *THI73* maintains a role in Cln3 nuclear import. *THI73* exhibits nutrient dependency on thiamine (Vitamin B_1) and is controlled by the *THI* regulon, which manages expression of all genes responsive to thiamine (Mojzita and Hohmann, 2006). *THI73* shows dynamic growth defects in a strain where *CLN3* is the only cyclin present in the genome. Our data are consistent with the idea that Thi73 plays a physiologically relevant role in Cln3 activity, and therefore, cell cycle progression by altering *CLN3* dependent growth. Ongoing research assesses the distinct phenotypes found in *thi73D* strains that indicate complex regulation of cyclin proteins.

1872C Coordinating spindle disassembly and cytokinesis during meiotic exit *Brian Seitz*[1], Xheni Mucelli[1], Linda Huang[1] 1) UMass Boston.

In the budding yeast *Saccharomyces cerevisiae*, meiosis is coupled with spore formation to create haploid gametes, a process called sporulation. Sporulation occurs under starvation conditions, and results in the remodeling of the mother cell to form four haploid spores within the ascus. Successful completion of this process requires the coordination of multiple events as cells exit meiosis to form four separate haploid gametes. These events include the timely disassembly of the spindle apparatus after meiosis II, the division of the nuclear envelope, and the closure of the prospore membranes. Previous work has shown that the sporulation specific STE20-family GCK III kinase Sps1 functions in parallel to the APC/C subunit Ama1 to promote timely prospore membrane closure, the meiotic cytokinetic event. Both *sps1Δ* and *ama1Δ* also exhibit meiosis II spindle disassembly defects. We would like to better understand how the events of meiotic exit are regulated and to better understand the relationship between spindle disassembly and cytokinesis. We have been conducting experiments to ask whether prospore membrane closure is dependent on spindle disassembly or vice versa. We have also examined the meiotic role of proteins involved in the process of spindle disassembly in mitosis. We find that the spindle midzone protein Ase1 persists on spindles after anaphase II in *ama1 Δ* mutants but appears to be removed normally in *sps1Δ* cells. This difference in Ase1 localization is consistent with our observations that *sps1Δ* and *ama1Δ* mutants exhibit distinct spindle disassembly defects, suggesting that *SPS1* and *AMA1* represent two mechanistically distinct pathways important for meiosis II spindle disassembly.

1884C Regulation of an Intrinsic Polarity Establishment Pathway by a Differentiation-Type MAPK Pathway *Aditi Prabhakar*[1], Jacky Chow[1], Alan Siegel[1], Paul Cullen[1] 1) University at Buffalo, Buffalo, NY.

All cells establish and maintain an axis of polarity that is critical for cell shape and progression through the cell cycle. A well-studied example of polarity establishment is bud emergence in yeast, where the Rho GTPase Cdc42p regulates symmetry breaking at bud sites and the establishment of polarity by interacting with effector proteins. The prevailing view of bud emergence does not account for regulation by extrinsic cues or signal transduction pathways. Here, we show that the MAPK pathway that controls filamentous growth (fMAPK pathway), which also requires Cdc42p and the effector p21 activated kinase (PAK) Ste20p, regulates bud emergence under nutrient-limiting conditions that favor filamentous/invasive growth. The fMAPK pathway regulated the expression of polarity targets that included the gene encoding a direct effector of Cdc42p, Gic2p. The fMAPK pathway also stimulated GTP-Cdc42p levels, which is a critical determinant of polarity establishment. The fMAPK pathway activity was spatially restricted to bud sites and highest at a period in the cell cycle that coincided with bud emergence. Time-lapse fluorescence microscopy showed that the fMAPK pathway stimulated the rate of bud emergence during filamentous growth. Unregulated activation of the fMAPK pathway induced growth at multiple sites that resulted from multiple rounds of symmetry breaking inside the growing bud. Collectively, our findings identify a new regulatory aspect of bud emergence that sensitizes this essential cellular process to external cues.

1886B Function of the C-terminal domain of Hkr1p, a signaling mucin of *Saccharomyces cerevisiae* in the context of bud-site selection and resistance to the cell wall integrity disruptor HM-1 killer toxin *Shin Kasahara*[1], Ukyo Suzuki[1], Toshihiro Kondo[1] 1) Miyagi University.

Lindnera mrakii (syn. *Williopsis saturnus* var. *mrakii* or *Hansenula mrakii*) produces a proteinous killer toxin called HM-1 which kills sensitive yeasts including *Saccharomyces cerevisiae*. *HKR1* (*Hansenula mrakii* killer toxin-resistant gene 1) was originally isolated from the genome of *S. cerevisiae* as a gene whose overexpression overcame the cytocidal effect of HM-1. The gene product Hkr1p is a large, highly glycosylated mucin-like type I transmembrane protein containing an N-terminal signal peptide sequence, Ser/Thr-rich repetitive sequences and a putative transmembrane domain. Calcium-binding EF hand and leucine zipper motives were found in its cytoplasmic tail. It also functions as an osmosensor in the high-osmolarity glycerol (HOG) MAP kinase pathway. We previously reported that only the partial sequence of Hkr1p endowed HM-1 resistance to the cells, then first in this study, the minimum sequence of Hkr1p required for HM-1 resistance was determined by serial deletions. Apparently the extracellular HMH (Hkr1p-Msb2 Homology) domain in addition to the cytoplasmic tail was indispensable for HM-1 resistance. Also we observed that the cells overexpressing partial *HKR1* showed altered budding patterns. The haploid *S. cerevisiae* cells mainly select bud sites in an axial pattern, but bipolar and randomized patterns were often observed in the presence of HM-1. The mutant cells lacking the cytoplasmic part of Hkr1p showed an aberrant budding pattern, too. It is well studied that a series of *BUD* gene products, Bud1p/Rsr1p, Bud2p, Bud5p and so forth are required for proper bud-site selection in *S. cerevisiae*. Since both overexpression and disruption of *HKR1* gave rise to abnormal budding patterns, Hkr1p might regulate budding coordinately with those proteins, or possibly interacting with some other factors. Moreover, we found that the budding pattern of haploid *S. cerevisiae* cells grown under the influence of HM-1 was also affected, suggesting that HM-1 perturbed the bud-site selection process. HM-1 has been studied as a cell wall integrity disruptor and believed to inhibit the synthesis of cell wall polysaccharides, but we now postulate that it may target other cellular events such as bud-site selection and cell polarity regulation. Our observations could provide important pieces of information to understand the mechanism of the cytotoxicity of HM-1 and the function of Hkr1p, especially its cytoplasmic domain in bud-site selection as well as cell wall integrity of *S. cerevisiae*.

1890C *C. albicans* Tor1 N-terminal HEAT repeats are required for modulation of TORC1 activity during stress resistance *Wanjun Qi*[1], Maikel Acosta-Zaldívar[1], Ning-ning Liu[2], Maria Andrés[3], José Fierro[3], Julia Köhler[1] 1) Boston Children's Hospital / Harvard Medical School, Boston, MA, USA; 2)

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Persistence and growth of *C. albicans* as a commensal or a pathogen require resistance to constant nutritional, oxidative, heat and other stresses, many of which are actively imposed by the host immune system or the competing bacterial microbiome. Given the key importance of the TOR signaling pathway in controlling eukaryotic cell growth and proliferation, *C. albicans* TOR signaling is predicted to play an important role in stress resistance. Although Tor1 regions that respond to specific stressors have not yet been defined, models of the mammalian Tor (mTor) kinase show that its 20 N-terminal HEAT repeats are exposed at the surface of TORC1 and accessible to interaction with regulatory molecules. In this study, we found that this N-terminal region of Ca.Tor1 is essential for TORC1 activity modulation as well as specific stress resistance.
Comparing *C. albicans* strains that express either full-length Tor1 (Tor1-full) or a truncated Tor1 (Tor1-trunc), which lacks 9 N-terminal HEAT repeats, we found defective growth of cells expressing Tor1-trunc during heat-, oxidative- or cell wall stress, but not plasma membrane stress. Oxidative stress signaling was weak in Tor1-trunc expressing cells, while their TORC1-activity remained abnormally elevated. Concurrently, cells expressing Tor1-trunc showed low oxygen consumption and low intracellular ROS accumulation under optimum growth conditions. Our findings highlight the importance of TORC1 activity downregulation in specific stress responses, and a requirement for the Tor1 N-terminal protein-protein interaction domains in these adaptations. They also indicate a regulatory role of Tor1 kinase in carbon source catabolism. These processes are required for C. *albicans* fitness during interaction with the human host.

1922B Hair-Cell Presynaptic Activity Contributes to Ototoxin Susceptibility in Zebrafish *Daria Lukasz*[1, 2], Katie S. Kindt[2] 1) National Institutes of Health - Johns Hopkins University Graduate Partnerships Program, NIH, Bethesda, MD; 2) National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, MD.

The powerful gram-negative aminoglycoside antibiotic neomycin specifically targets not only life-threatening bacterial infections but also sensory hair cells. Hair-cell death causes irreversible and often isolating hearing loss in humans. Though much is known about the process of neomycin-induced cell death, factors that underlie hair-cell neomycin susceptibility have not been fully elucidated. Current work suggests that persistent metabolic activity resulting from the near constant stimulation of apical mechanotransduction channels in an aqueous environment may sensitize cells to neomycin by contributing to a slow buildup of toxic metabolic byproducts. Additionally, mechanosensation has been shown to stress mitochondria and subsequently boost the production of reactive oxygen species (ROS). Neomycin itself triggers ROS overproduction because it targets mitochondria and thus may pose a direct threat to metabolically stressed cells. Our work aims to understand the relationship between hair-cell metabolic activity, ROS production, and neomycin susceptibility.
To explore this further, we utilized a larval zebrafish model which offers external lateral-line hair cells that express membrane-localized GCaMP6s and allows for close monitoring of individual cells in an *in vivo* and *in toto* context. Hair cells rely on the influx of calcium through basal voltage-gated $Ca_v1.3$ channels for signal transduction, and so we began by acutely blocking these channels with an antagonist isradipine during neomycin bath treatment. We found that acute calcium block did not prevent cell death relative to control. However, examination of $ca_v1.3^{-/-}$ mutants revealed augmented cell survival, suggesting that the chronic block of metabolic activity does mitigate neomycin susceptibility. Preliminary data utilizing the cytosolic ROS indicator cellROX Orange also shows a reduction in oxidative stress in these mutants. Furthermore, examination of $otof^{-/-}$ mutants, which lack the calcium sensor necessary for the coupling of calcium influx with vesicle fusion, revealed that the loss of exocytosis alone reduces neomycin susceptibility. These findings suggest that metabolic activity related to the exocytosis of glutamate-containing vesicles contributes to oxidative stress that weakens hair cells. We are currently examining other activity-related mutations and more specific ROS indicators with the ultimate goal of identifying an otoprotective therapeutic target and preventing hearing loss.

2195B Identification of Telomere Regulating Genes in *Drosophila melanogaster* *Patrick Elysee*[1], Sydney Sieh-Takata[1], Billy Nguyen[1], Isabella J. Hanesworth[1], Murad Kaid[1], Chad E. Jacob[1], Allaysia Bradley[1], Bobbi Kennedy[1], Steena Samuel[1], Besjan Kelmendi[1], Chun Zhou[1] 1) Mercy College.

Telomeres are the natural ends of linear chromosomes and contribute to the maintenance of chromosome stability. Without the capping effect of telomeres, broken chromosomes can undergo the breakage-fusion-bridge cycle which may cause cancer. In *Drosophila melanogaster*, telomeres are extended by telomere-specific non-LTR retrotransposons which serve as an alternative, yet similar approach to the telomerase. Previously, a genetic factor called *Telomere elongation* (*Tel*) was discovered on the third chromosome of fruit flies that can enhance telomere elongation. Another telomere-elongating gene was also identified in the similar chromosomal region in *D. melanogaster*. In the present study, we used a bioinformatic approach to identify the genes in this chromosomal region that have been indicated to influence chromosomal stability in Drosophila. We hypothesized that the genes that can modulate chromosomal structure or remodeling have the potential to regulate the telomere length or structure. We extracted genomic DNA from various mutant strains of these candidate genes. Using real-time PCR, we have analyzed the telomere length among different mutant strains with the Oregon-R wild-type strain and the *Tel* mutant strain as the controls. In addition, to probe whether or not disruption of these gene candidates causes a structural defect of telomeres, we are performing polytene chromosome staining. Currently, we have identified two new genes, *Ino80* and *CG6026*, whose mutation can lead to elongated telomeres while other tested chromatin-remodeling genes do not affect the telomere length. We are currently analyzing whether the elongated telomeres in those mutant strains are related to any abnormal telomere structures or chromosomal behaviors. This research can help understand the molecular regulatory mechanisms of telomere elongation and structure in fruit flies.

2249B Telomere and telomerase evolution in *Saccharomyces cerevisiae* *Melissa Mefford*[1], Ethan Chandler[1], Brianna Haynes[1], Nadia Richardson[1], Brice Smith[1], Abbey Whaley[1] 1) Morehead State University.

Telomeres, composed of repetitive DNA sequences at the termini of linear chromosomes, serve as protective caps. However, telomeres cannot be fully replicated without the ribonucleoprotein enzyme telomerase. Understanding the balance between telomere length and telomerase activity has important implications for human health concerns: aging and cancer. Telomeres shorten with age, acting as a “biological clock” limiting the rounds of division a cell can undergo. To circumvent this proliferative limit, >85% of cancers aberrantly up-regulate telomerase expression. The goal of our work is to understand the evolution of telomeres and telomerase, and how these contribute to the health and lifespan of an organism.

To address these questions, two projects are underway. First, we are screening for gain-of-function mutations in telomerase RNA to identify novel alleles that lengthen telomeres. These mutants will shed light on how the RNA component of telomerase contributes to enzyme action. We are currently optimizing our selection strategy and creating a library of mutations. Second, we are genetically engineering yeast to circularize each of

their 16 linear chromosomes, allowing experimental investigation of the advantages and disadvantages of circular chromosomes in a eukaryotic organism. We have built DNA cassettes with selectable markers and inserted these into the ends of four different chromosomes. We are currently selecting for recombination events that generate circular chromosomes. These novel yeast strains will build a foundation for creating an innovative eukaryotic organism with all linear chromosomes circularized, allowing experimental exploration of telomere and telomerase evolution.

2250C The core-enclosing helix in yeast telomerase RNA is essential for binding to the TERT catalytic protein subunit and for telomerase activity *in vivo* and *in vitro* *David Zappulla*[1], Melissa Mefford[2], Evan Hass[3] 1) Lehigh University; 2) Morehead State University; 3) HHMI - University of Colorado at Boulder.

Telomerase countervails the chromosome end-replication problem, completing genome replication to prevent cellular senescence. Increased telomerase function is linked to 90% of cancers, while reductions are associated with premature aging and telomere syndromes that tend to cause organ failure. At its core, the telomerase RNP is composed of a dedicated reverse transcriptase (TERT) and a long noncoding RNA. Although the majority of the 1157-nt *Saccharomyces cerevisiae* telomerase RNA, TLC1, is rapidly evolving, the central catalytic core secondary structure is conserved, even sharing features with humans. Consistent with the importance of the conserved structural elements, TLC1 can be pared down to "Mini-T" RNAs one-third the size of wild-type that maintain short, stable telomeres *in vivo*. Mini-T's contain the catalytic core (with the template, template-boundary helix, pseudoknot/base-triples, and core-enclosing helix) as well as holoenzyme subunit-binding sites for Est1, Ku, and Sm_7, which normally reside on the tips of TLC1's three long arms.

Telomerase RNA is likely to contain multiple binding sites for TERT. We hypothesize that the core-enclosing helix (CEH) is one of the elements needed for this critical interaction. To test this, we used circularly permuted Mini-T RNAs to avoid disrupting the Area of Required Connectivity in the core of TLC1, allowing us to precisely evaluate how CEH structure relates to telomerase activity and TERT binding. With the RNA ends relocated to multiple alternative locations, we consistently observed that a 4-bp CEH is necessary for telomerase to be active *in vitro* and maintain yeast telomeres *in vivo*, whereas ΔCEH, 1-bp, and 2-bp alleles were catalytically dead and senesced.

We then used our CRISPR dCas9-based "CARRY two-hybrid" assay to assess binding of our circularly permuted Mini-T RNAs to TERT. The results of these tests showed that the 4-bp CEH RNA bound to TERT, but the shorter-CEH constructs did not, consistent with the telomerase activity and *in-vivo* complementation results. We conclude that a major reason why the CEH is essential in yeast telomerase RNA is because it is needed to bind TERT to form the core RNP enzyme. Although the 8 nucleotides that form this 4-bp stem at the base of the CEH are nearly invariant among *Saccharomyces* species, our results with sequence-randomized and truncated-CEH helices strongly suggest that this binding interaction with TERT is dictated more by secondary than primary structure.

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Intracellular Dynamics/Genome Integrity 2 - Poster Q&A 1766B Upregulation of Store Operated Ca^{2+} Entry pathologically impairs *Drosophila* cardiac function *Courtney Petersen*[1], Jeremy Smyth[1] 1) Uniformed Services University of the Health Sciences, Bethesda MD.

Store operated Ca^{2+} entry (SOCE), whereby Ca^{2+} influx is triggered upon depletion of endo/sarcoplasmic reticulum (ER/SR) stores, is an essential Ca^{2+} signaling and homeostatic mechanism present in nearly all animal cells. SOCE is mediated by STIM proteins, which function as Ca^{2+} sensors in the ER/SR, and Orai Ca^{2+} influx channels in the plasma membrane. Numerous animal studies have demonstrated that upregulation of SOCE in cardiomyocytes induces pathological cardiac hypertrophy and heart failure. Interestingly however, humans with gain-of-function mutations in STIM1 or Orai1 exhibit varying degrees of skeletal muscle myopathy and platelet dysfunction, but not cardiac hypertrophy or other cardiac complications. To better understand how upregulated SOCE alters cardiomyocyte physiology, we generated a transgenic *Drosophila* model that expresses a Stim mutant which confers constitutive Ca^{2+} influx activity ($Stim^{CA}$) due to two aspartate to alanine changes in the Ca^{2+} binding EF-hand domain. Surprisingly, ubiquitous expression of $Stim^{CA}$ resulted in embryonic lethality. By comparison, ubiquitous expression of wildtype *Stim* or *Orai* transgenes significantly delayed pupariation and eclosion, suggesting that even modest upregulation of SOCE is deleterious. Heart-specific expression of $Stim^{CA}$ also significantly impaired animal development, as 80% of these animals died as larvae and pupariation of surviving larvae was delayed by approximately two days compared to W^{1118} driven $Stim^{CA}$ controls. Intravital imaging of adult heart contractility revealed that heart specific expression of $Stim^{CA}$ reduced end-diastolic and end-systolic dimensions and decreased heart rate, consistent with significantly impaired heart function. Myofibrils in $Stim^{CA}$-expressing hearts were highly disorganized, suggesting SOCE upregulation may cause cardiac remodeling that contributes to contractile dysfunction. Conversely, heart-specific expression of transgenic wildtype *Stim* or *Orai* resulted in decreased heart rate but did not affect cardiac dimensions or animal development. Collectively, these results suggest that SOCE upregulation pathologically impairs cardiac function in *Drosophila*. Moving forward, we plan to directly measure cardiomyocyte Ca^{2+} *in vivo* to determine how $Stim^{CA}$ expression affects Ca^{2+} physiology and dynamics.

1798A Planar Cell Polarity Regulates Cell Competition and Tumor Progression in *Drosophila melanogaster* *Pablo Sánchez Bosch*[1], Bomsoo Cho[1], Jeffrey D. Axelrod[1] 1) Stanford University, Stanford, CA.

Morphogenesis and physiology of tissues and organs require polarization of subcellular structures. In addition to the apical-basal polarization of epithelial tissues, planar cell polarity (PCP) signaling polarizes cells within the plane of the epithelium. PCP orients cellular structures, cell divisions, and cell movements during development and homeostasis. Polarity is achieved by the asymmetric subcellular distribution of a set of core PCP proteins: in the distal side of a cell, Frizzled (Fz), Dishevelled (Dsh), and Diego (Dgo) organize a complex and connect with Van Gogh (Vang) and Prickle (Pk), the proximal proteins of the adjacent cell, via the atypical cadherin Flamingo (Fmi).

Defects in PCP proteins have been linked to severe developmental defects, disease, and cancer, although the mechanisms are only partially understood. Recently, our lab found that Fmi has a key role in tumor malignancy and cell competition in *Drosophila*. Cell competition is a process in which cells compare 'fitness,' as decided by intercellular communication, resulting in the elimination of less fit cells (losers) at the expense of more fit cells (winners). This ensures that unfit or potentially tumoral cells are rapidly eliminated from the tissue. We observed that the loss of *fmi* decreases tumorigenesis and blocks cell competition. Tumors lacking *fmi* cannot compete well against surrounding WT cells with Fmi, whereas *fmi* mutant cells are unable to eliminate unfit neighbors during cell competition. We hypothesize that Fmi and PCP are required to establish communication between neighboring cells to evaluate their fitness and allow more "fit" cells to grow at the expense of their surrounding neighbors.

Our current research focuses on deciphering the mechanisms by which PCP regulates cell-cell communication, using *Drosophila* eye tumors and cell competition models. Ultimately, understanding the role of PCP in intercellular signaling and cell competition can open the door to new therapies to limit tumor progression and invasion.

1803C Reorganization of the nuclear architecture in the *Drosophila melanogaster* Lamin B mutant lacking the CaaX box Semen Bondarenko[1], *Igor Sharakhov*[1] 1) Virginia Tech.

Lamins are major components of the nuclear lamina, a protein meshwork adjacent to the inner nuclear membrane. Lamins interact with the nuclear membrane and chromatin but the precise players and mechanisms of these interactions are unknown. Here, we tested whether the removal of the CaaX motif from Lamin B disrupts interaction of the protein with the nuclear periphery and affects chromatin distribution inside the nucleus. For this study, we used *Drosophila melanogaster* Lam^{A25} homozygous mutants that lack the CaaX box. The Lam^{A25} adult flies are viable, although sterile, with reduced survival, meaning that the mutant protein still performs some of its essential functions. Localization of Lamin B and chromatin density distribution were analyzed in the salivary gland and the proventriculus nuclei of the mutant and wild-type larvae using high-resolution confocal microscopy and ImageJ software. We found that mutant Lamin B is not confined to the nuclear periphery but is distributed throughout the nuclear interior colocalizing with chromosomes in both cell types. The fluorescence intensity of the peripheral chromatin significantly decreases, but that of the central chromatin significantly increases in the proventriculus nuclei of Lam^{A25} flies compared to wild type. However, the mutation had little effect on chromatin density distribution inside the salivary gland nuclei. These results demonstrate that the removal of the CaaX motif abrogates the interaction of Lamin B with the nuclear membrane but not with the chromatin. Our data also indicate that tethering of the chromatin to the nuclear periphery is impaired in nuclei with the mutant lamin as is clearly seen in the proventriculus cells. The effect of the mutation on chromatin density distribution inside the nucleus is cell-type specific and likely depends on the level of polyteny. The higher levels of polyteny in salivary gland nuclei could be responsible for preserving the chromatin architecture in this cell type.

1843A Privileged immune cell upon activation – how it changes its own metabolism and metabolism of the whole organism *Tomas Dolezal*[1], Pavla Nedbalova[1], Gabriela Krejcova[1], Michalina Kazek[1], Katharina Lehr[1], Lenka Chodakova[1], Lukas Strych[1], Tereza Dolejskova[1], Adam Bajgar[1] 1) Faculty of Science, University of South Bohemia in Ceske Buejovice, Czech Republic.

Activated immune cells initiate a number of previously silenced processes that are associated with significantly altered metabolism, in particular increased glycolysis and the pentose phosphate pathway and re-wired TCA cycle. We have shown that the regulation of this complex metabolic switch in an activated immune cell is evolutionarily conserved between insects, *Drosophila melanogaster* in particular, and mammals and is dependent on Hif1alpha (doi: 10.7554/eLife.50414). This switch makes the immune cells dependent on an increased supply of nutrients and therefore the immune system becomes privileged within organism. The immune cells produce signals that suppress the metabolism of other tissues, ensuring that the immune system receives sufficient energy / nutrients upon activation (doi: 10.1371/journal.pbio.1002135). We are now revealing the molecular mechanisms by which an immune cell measures its activity and energy state, and accordingly informs other tissues to ensure a sufficient supply of energy. Within these mechanisms, adenosine is a central player that is produced in the methylation cycle (activity measurement system) and metabolized back to AMP and subsequently to ADP and ATP via adenylate kinase, depending on energy state and glycolysis. We therefore propose that coupling the methylation cycle to adenylate kinase and glycolysis through adenosine metabolism is an extremely sensitive relay mechanism that measures the balance between energy supply / glycolysis and immune cell activity. If supply is insufficient, increased release of adenosine from immune cells informs surrounding tissues to leave more energy for the privileged immune system. We also reveal an interesting solution of insect immune cells for a privileged access to nutrients through the specific metabolism of trehalose (the most prevalent carbohydrate in insects), which could represent an alternative evolutionary solution as compared to mammalian immune cells. Overall, we would like to present a complex interaction of processes that alter the metabolism of immune cells after activation, ensuring their balance and sufficient energy supply, which during immune activation make the immune system privileged, using a fruit fly as a model and comparing it with mammalian models.

1844B The importance of trehalose metabolism for proper Drosophila immune response during parasitoid infection. *Michalina Kazek*[1], Tomas Dolezal[1] 1) University of South Bohemia in České Budějovice, Czech Republic.

The primary circulating sugar in insects is trehalose, which is usually present in hemolymph in much higher concentration than glucose. Trehalose is a non-reducing sugar, which can be rapidly cleaved to two glucose molecules by the trehalase enzyme. In Drosophila, there are two versions of trehalase, cytoplasmic and secreted. The secreted version is used to maintain circulating glucose, by hydrolyzing trehalose, at a stable level and appears to be part of systemic regulation of carbohydrate metabolism. However, this systemic regulation cannot distinguish the supply of different organs. The activated immune system becomes privileged in the acquisition of nutrients, and this raises an extremely interesting question of whether activated insect immune cells (hemocytes) favor direct uptake of trehalose and conversion to glucose by cytoplasmic trehalase, thereby being independent of systemic regulation. Therefore, we test the role of trehalose and its metabolism during the immune response. Experiments with 13C-labeled trehalose show that hemocytes during parasitoid infection increase the uptake of trehalose, which is partially metabolized by glycolysis but mainly by the pentose phosphate pathway. In addition, transcript-specific expression analysis, immune cell-specific RNAi and mitotic recombination have shown us the importance of cytoplasmic trehalase for immune cells. The absence of the trehalase enzyme, specifically in hemocytes, significantly reduces resistance to parasitoid. These results are in agreement with the privileged access of immune cells to nutrients during infection, which is required for efficient resistance, and demonstrate an alternative evolutionary strategy for such a privileged access in insects.

1882A New Regulators of the Mitogen-Activated Protein Kinase (MAPK) Pathway that Controls Filamentous Growth in Yeast *Sheida Jamalzadeh*[1], Paul Cullen[1] 1) State University of New York at Buffalo.

Signal transduction pathways control the response to stress and cell differentiation into specialized cell types. In yeast and other fungal species, signalling pathways control filamentous growth (FG), which in pathogens is critical for host–cell attachment, invasion into tissues, and virulence. As commonly seen during cell differentiation, multiple pathways operate in an integrated network to regulate filamentous growth. A MAPK pathway, called the FG (or fMAPK) pathway is among the pathways that regulate filamentous growth. To identify new regulators of the fMAPK pathway in yeast, a genome-wide overexpression screen (of 6000 yeast genes) was performed using an fMAPK pathway-dependent growth reporter. Bioinformatics analysis and target validation identified proteins not previously implicated in MAPK signalling, including *MRS6* and *KAR2*. *MRS6* is a Rab escort protein that forms a complex with the trafficking GTPase Ypt1p. In mammals, loss of the *MRS6* homolog REP1/CHM leads to choroideremia,

which causes progressive vision loss. Intriguingly, REP1/CHM regulates EGFR, which is a major regulator of the Grb-SOS-RAS-MEK-ERK pathway. We found that *MRS6* interacts with the PAK kinase *STE20* and MAPKK *STE7* by two-hybrid analysis. The fMAPK pathway shares components with other pathways (HOG and MATING). *MRS6* did not impact the activity of the HOG pathway which requires a separate MAPKK, *PBS2*. Therefore, *MRS6* may promote fMAPK (*STE20->STE11->STE7*) pathway signaling over HOG (*STE20->STE11->PBS2*). The screen also identified *KAR2*, the ER chaperone BiP, as a positive regulator of the fMAPK pathway. The fact that protein chaperones regulate FG suggested that stimuli that impact protein folding (e.g. temperature) might impact this differentiation response. We identified an extended FG (EX-FG) response that occurred in response to nutrient limitation when combined with high temperatures, similar to that seen in fungal pathogens. The receptor that detects unfolded proteins in the ER, *IRE1*, was also required for EX-FG. Unexpectedly, EX-FG occurred in cells lacking several key pathways that control FG. This allowed us to identify the RAS, MAPK, and retrograde (RTG) mitochondria-to-nucleus pathways as key regulators of the EX-FG response. Our findings identify new MAPK pathway regulators that control microbial differentiation. Given that these proteins are conserved throughout eukaryotes, we suggest that these proteins may function as general regulators of MAPK pathway signalling.

1887C Uncovering the Role of Eaf1 in the Delicate Balance of Lipid Droplet Synthesis and Membrane Composition in *Saccharomyces cerevisiae* *Sarah Laframboise*[1, 2], Kristin Baetz[1, 2] 1) Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada; 2) Ottawa Institute of Systems Biology, Ottawa, Ontario, Canada.

The yeast lysine acetyltransferase, NuA4, has been implicated in the regulation of various aspects of metabolism, including a poorly defined role in lipid homeostasis. Surprisingly we have discovered a new role for NuA4 in regulating phospholipid availability for organelle morphology. Upon deletion of *EAF1*, the main scaffolding subunit of NuA4, over 70% of *eaf1Δ* cells displayed nuclear flares or extension of the nuclear membrane, compared to only 7% in wild type (WT) cells. In addition to nuclear flares, the loss of the NuA4 complex resulted in defects in vacuole fusion, with over 60% of all *eaf1Δ* cells containing more than 10 vacuolar lobes, instead of an average of two to five vacuoles found in WT cells. The nuclear flares and vacuole fusion defects of *eaf1D* cells suggest a gross dysregulation of phospholipid production in the absence of NuA4. How is NuA4 regulating phospholipid homeostasis? Recent studies have shown that the phosphatidic acid phosphohydrolase 1 (Pah1) is an acetylation target of NuA4. Sitting at the cross-roads between lipid droplet formation and membrane phospholipid production, Pah1 converts phosphatidic acid (PA) into diacylglycerol (DAG), which is then subsequently processed to form TAG and stored in lipid droplets. However, in the absence of Pah1 activity, excessive PA is converted to membrane phospholipids and, similar to *eaf1Δ* cells, *pah1Δ* cells display nuclear flares. Here we present genetic and cell biology studies that show that the nuclear flare and vacuolar defects of *eaf1Δ* cells are due to mis-regulation of Pah1. Surprisingly we determined that in the absence of Eaf1, the subcellular localization of Pah1-GFP changes from cytoplasmic to punctate structures. In agreement with a change in Pah1 subcellular localization, through the use of lipid biosensors, we detect gross changes in subcellular pools of phospholipids, DAGs and lipid droplets in *eaf1D* cells. Taken together, our work shows that NuA4 is critical in establishing the balance between lipid droplet formation and phospholipid availability for organelle and cell membranes.

1918A Functional studies of a conserved protease-like extracellular matrix protein, Tinagl1, in the zebrafish developmental model *Ellen LeMosy*[1], Brooklyn Zwinklis[1,2], Helena Blackburn[1,2], Hannah Neiswender[1] 1) Augusta University, Augusta, GA; 2) Augusta University Honors Program, Augusta, GA.

Tinag (tubulo-interstitial nephritis antigen) and its related protein Tinagl1 (aka LCN7 or AZ-1) are variably expressed in basement membranes of mature tissues of mammals, while a single ortholog is present in other genetic model organisms. *Drosophila* Tinagl1 binds to a Wnt, Wg, in a palmitate-dependent manner and is required for Wg stability and signaling *in vitro*. In contrast, mouse Tinagl1 reduces metastatic potential of mammary tumor cells through inhibitory binding to integrins and EGF-R. The pathways by which Tinagl1 functions *in vivo* in development and disease remain to be determined. We have focused on establishing phenotypes and tools for examining Tinagl1 in the zebrafish developmental model. Using acute knockdown approaches, we observed defects in heart looping, pronephric and KV cilia, pharyngeal arch cartilages, and spinal cord; these results are consistent with *tinagl1* mRNA expression patterns, and suggest roles for Tinagl1 in motile cilia function and in neuronal and neural crest cell survival and migration. Preliminary data hinted that Tinagl1 may act via Wnts. Early results will be reported from our ongoing work to test CRISPR knockout lines, interrogate the requirement for a putative Wnt-binding lipocalin motif in Tinagl1, and characterize the protein's sub-cellular and tissue distribution during development. Our broad goals are to contribute to understanding this conserved but enigmatic protein, and how it interacts with Wnts and/or other signals in the extracellular matrix to regulate cell behavior.

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2171B Defining the roles of conserved DNA repair complexes in maintenance of *C. elegans* meiotic genome integrity *Alina Salagean*[1], Erik Toraason[1], Diana Libuda[1] 1) University of Oregon.

Most organisms utilize meiosis, a specialized form of cell division, to produce haploid gametes such as sperm and eggs. Failure to maintain genomic integrity during meiosis can result in infertility and serious diseases, such as cancer and birth defects. Despite these risks, double strand DNA breaks (DSBs) are intentionally induced during meiotic prophase I. Meiotic cells must repair a specific subset of DSBs through interhomolog crossover recombination to ensure accurate chromosome segregation, while the remainder are resolved through alternative repair pathways to maintain genome integrity. Interhomolog recombination has been studied extensively, but the mechanisms of alternative meiotic DNA repair remain elusive. The Structural Maintenance of Chromosomes 5/6 complex (SMC-5/6), its E3 SUMO ligase subunit NSE-2, and the BRC-1/BRD-1 heterodimer are conserved proteins required for homolog-independent meiotic DSB repair and have been shown to genetically interact. However, the specific mechanisms by which these proteins function together to preserve meiotic genome integrity is unknown. To determine the NSE-2 specific and NSE-2 independent meiotic functions of the SMC-5/6 complex in meiotic DSB repair, we utilized immunofluorescence imaging and a mortal germline phenotype assay to assess *smc-5* and *nse-2 C. elegans* mutants. Both *smc-5* and *nse-2* mutants exhibit persistent DNA damage, suggesting that both SMC-5/6 and NSE-2 are required for efficient meiotic DSB repair. However, we find that SMC-5, but not NSE-2, is required for germline immortality. These data suggest a separation of function for SMC-5/6, which performs NSE-2 dependent and independent functions to maintain meiotic genome integrity. Finally, to define epistatic relationships between BRC-1/BRD-1, SMC-5/6, and NSE-2 in DNA repair, we assessed the germline sensitivity to ionizing radiation by brood viability of pairwise *brc-1*, *smc-5*, and *nse-2* double mutants. These data suggest that exogenous DSB repair is differentially regulated within meiotic prophase I and implicate SMC-5/6 as a central regulator of both NSE-2 and BRC-1 dependent DSB repair. Taken together, our research defines fundamental genetic mechanisms and interactions preserving genomic integrity.

2201B Recombination Rate Plasticity and Interchromosomal Effect in *Drosophila Pseudoobscura* Ulku Huma Altindag[1], Laurie Stevison[1] 1) Auburn University, Auburn, AL.

Extrinsic (temperature) and intrinsic (age) factors influence recombination rate, impacting the ability of the population to respond to selection pressures. Similar to plasticity, chromosomal inversions act as global modifiers of recombination by stalling checkpoints and provide more time for double strand breaks known as, interchromosomal effect, (ICE). Here, we used *D. pseudoobscura* to study the interaction between recombination rate plasticity and ICE. For plasticity, we used both temperature and age to induce changes in recombination rates. For ICE, we crossed flies with inversion differences of the treeline vs arrowhead arrangement of the 3rd chromosome of *D. pseudoobscura*. Visual markers spanning the X chromosome: *cut*, *scalloped*, *yellow*, and *sepia* were used to measure recombination rates. First, homokaryotic F_1 females with the treeline arrangement, were reared in high temperature, 26˚C and control of 21˚C. Second, the same strains were aged 35 days into adulthood and compared to 7 days old flies. These two experiments were repeated for heterokaryotic flies to examine the interaction of plasticity and ICE. Females in all experiments were transferred every 72 hours to compare temporal variation in plasticity. Results indicate a saturation in the recombination rates due to age; the heterokaryotype control and homokaryotype age were largely similar. Aging increases the recombination rates 10% and 13% in the 1-3 and 4-6 time points respectively (p=0.00027 and p=0.0017). Surprisingly, for temperature, ICE showed a subtractive effect in recombination rates. Temperature stress decreases recombination rate 60% in the heterokaryotypes compared to the homokaryotypes. Fecundity results reveal that ICE caused a more severe effect in fecundity relative to age and temperature stresses. Our future plans are to repeat the heterokaryotype experiment with 24h transfers to target the now known peak of plasticity in this system of 9-days post-mating. This experimental design should provide a better design for the interaction of ICE and plasticity. We are also investigating the coincidence and crossover control mechanisms. While plasticity and ICE have been studied independently, this is the first experiment to examine their interaction. Thus, our study shows preliminary results for the interaction between plasticity and ICE using a novel design.

2202C HP1 Gene Family Proteins and Their Impact on Meiotic Crossovers *Andrew Halza*[1], K Nicole Crown[1] 1) Case Western Reserve University.

Genome architecture, composed of a highly diverse yet exquisitely arranged set of proteins, dictate a wide variety of cellular function. Among the starkest modifications of the genome architecture is the tightly-wound heterochromatin region, which maintains a distinct collection of proteins that dictate the region's physical structure. Of these, the HP1a gene is known for its profound impact on preserving regions of centromeric heterochromatin that suppress meiotic crossovers. However, the mechanism by which heterochromatin suppresses meiotic crossovers is still unknown. The HP1 gene family has 4 other highly conserved genes – HP1b, HP1c, HP1d (*rhino*) and HP1e – that have varying degrees of functional understanding in the cell. Noting their common lineage, we are looking to further clarify what role, if any, each of the primary members of the HP1 gene family have on meiotic recombination. Using *Drosophila melanogaster*, we are analyzing crossover rates and their distribution through recessive marker scoring. This data will contribute to our understanding of the centromere effect and mechanisms of crossover patterning during meiosis.

2240B The role of the TCA cycle enzyme fumarase and the metabolite fumarate in response to DNA replication stress in *Saccharomyces cerevisiae.* *Faeze Saatchi*[1,2,3,4], Ann Kirchmaier[2,3,4] 1) Department of Internal Medicine, UTSouthwestern Medical Center, Dallas, TX; 2) Department of Biochemistry, Purdue University, West Lafayette, IN; 3) Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN; 4) Bindley Bioscience Center, Purdue University, West Lafayette, IN.

Our study uncovers links between the metabolic enzyme fumarase plus the metabolite fumarate and chromatin modifications during DNA damage response in *Saccharomyces cerevisiae*. Fumarase is a well-characterized TCA cycle enzyme that catalyzes the reversible conversion of fumarate to malate. Fumarase also acts as a tumor suppressor, and defects in the gene encoding fumarase (FH) in humans are commonly found in hereditary leiomyomatosis and renal cell cancer, glioblastomas and neuroblastomas. Recent studies have provided a link between fumarase (and other metabolic enzymes) and genome integrity, revealing a previously unappreciated pathway by which metabolic defects could promote tumorigenesis. Fumarate is structurally related to α-ketoglutarate (α-KG) and acts as competitive inhibitor of α-KG-dependent dioxygenases including histone demethylases. Inactivation of FH and accumulation of fumarate has been previously shown to increase the levels of methylated histone H3. In mammalian cells, fumarase becomes enriched at sites of double stranded break (DSB) through interaction with the histone variant H2A.Z. At DSBs, fumarase promotes DNA repair by nonhomologous end joining (NHEJ) by local production of fumarate which in turn acts as an inhibitor of KDM2B, a H3 K36-specific histone demethylase. Using plasmid re-ligation assay, we have shown that both Fum1p (yeast fumarase) and Htz1p (yeast ortholog of H2A.Z) promote NHEJ indicating a conserved role for fumarase and H2A.Z in DNA repair. We have also demonstrated that treatment with hydroxyurea (HU), which creates stalled replication forks by depletion of nucleotides, leads to upregulation as well as nuclear enrichment of Fum1p. Our results indicate that increased cellular levels of fumarate (upon deletion of *FUM1* or addition of exogenous fumarate) suppresses the sensitivity to HU in *htz1*Δ mutants in a manner that is independent of modulating nucleotide levels. In fact, fumarate confers resistance to HU in *htz1*Δ mutants by inhibition of the H3 K4-specific histone demethylase Jhd2p, and increasing H3 K4 methylation levels. Sensors and mediators of the DNA replication checkpoint were required for fumarate-dependent resistance to HU in *htz1*Δ, whereas factors involved in processing of regressed replication forks were dispensable. Together, our findings imply that high cellular levels of fumarate support processing of replicative intermediates by regulation of histone methylation, thereby promoting genome integrity (Saatchi and Kirchmaier, *GENETICS*, 2019).

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Intracellular Dynamics/Genome Integrity 3 - Poster Q&A 1855A Tagging Juvenile Hormone receptors in Drosophila *Saathvika Rajamani*[1], Li Ying Wei[1], Edward Dubrovsky[1] 1) Fordham University, Bronx, NY.

Juvenile Hormone (JH) is a key component of the insect endocrine system that regulates metamorphosis, mating behavior and yolk protein uptake, among others. In Drosophila, JH elicits its action through the paralogous receptors, Methoprene tolerant (Met) and Germ cell-expressed (Gce). Being members of the bHLH family, the receptors become active transcription factors upon heterodimerizing with proteins from the same family. The current model for hormone action implies that upon binding JH, Met heterodimerizes with a partner Taiman (Tai), and binds to JH response elements in target genes to regulate transcription. Despite the discovery of the receptors, the repertoire of the primary targets and hence the underlying genetic programs of JH regulated processes are not entirely understood.

An important aspect of receptor functioning lies in its subcellular localization, and efforts have been taken to study it. It is hypothesized that recep-

tor localization is regulated by both hormone dependent and independent mechanisms. Endogenously expressed Met was observed to be exclusively nuclear, irrespective of hormone presence. However, ectopically expressed Met was cytoplasmic when JH titers were low, and the presence of JH induced its nuclear import. These contradictory data and a lack of endogenous Gce localization studies warrants further study of endogenous receptor localization.

We generated two epitope tagged receptor fly lines using CRISPR/Cas9 technology. The receptors were tagged at their carboxy termini with different epitopes, Met with V5 and Gce with 3xFLAG. Western blot analysis revealed Met protein at the expected size of 78.7 kDa, but Gce at a higher size of ~140 kDa. We hypothesize that this may be due to posttranslational modifications or it is a previously unidentified transcript. We performed immunostaining of different tissues and observed localization of both receptors to be predominantly nuclear. Being paralogous receptors we expected similar expression patterns, and were surprised that while Gce expression was observed in male accessory glands and spermatheca, Met expression was not. The tagging opens up the opportunity to study the subcellular localization of the receptors in a tissue specific manner, and can be used to identify the primary targets of JH, which will immensely improve our understanding of JH action.

1859B Microtubule Associated Proteins affect stomatal aperture size *Jessica Lucas*[1], Elias Flors[1], Jessica Frey[1] 1) University of Wisconsin Oshkosh.

Climate change threatens the productivity of our agricultural plants. Increased heat and drought conditions challenge plants and reduce crop yield. Stomata are epidermal structures on the surface of plant leaves that mediate multiple physiological processes including photosynthesis, thermoregulation and water homeostasis. Hundreds of stomata are found on each leaf, and each stoma is composed of two identical guard cells that surround a central aperture. Guard cells regulate the flow of gases from inside the leaf to the atmosphere through the aperture. The current paradigm is that water pressure within the guard cells determine the size of the aperture and thereby adjust gas exchange to maintain physiological homeostasis. Recent data show a correlation between microtubule organization within the guard cells and aperture size. To further understand the role of microtubules in stomata, we studied the model flowering plant *Arabidopsis thaliana* harboring genetic mutations in two microtubule associated proteins, KATANIN and ZWICHEL. Cellular observations of wild type stomatal apertures show smaller apertures in the dark than in light, due to the increased photosynthetic activity during the day light. However, stomatal apertures in the *katanin* and *zwichel* mutants differed from wildtype and from each other in both the dark and light growth periods. Both *KATANIN* and *ZWICHEL* proteins are known to be important for organizing the interphase cortical array of microtubules in *Arabidopsis.* Therefore our data suggest that microtubule organization is needed for regulating aperture size.

1891A Determining the Effects of Pab1 Acetylation at K131 on Stress Granule Dynamics in *Saccharomyces cerevisiae* *Sangavi Sivananthan*[1], Sylvain Huard[1], Kristin Baetz[1] 1) University of Ottawa.

Eukaryotic cells form cytoplasmic RNA-protein aggregates, or stress granules under a variety of stress conditions. Their formation is associated with both neurodegenerative diseases and cancer. For each stress condition, distinct stress-activated signaling pathways regulate stress granule formation; however, the molecular details of these pathways remain largely unknown. We and others have shown that lysine acetyltransferases and deacetylases are a key signaling axis regulating stress granule formation and large scale acetylome studies have detected acetylation sites on many proteins found in stress granules, including the Poly(A) Binding protein - Pab1. We systematically mutated all known sites of lysine acetylation on Pab1 and identified one site, K131, essential for stress granule formation upon glucose deprivation. When Pab1-K131 is mutated to Q to mimic the acetylated state, glucose deprived stress granule formation is inhibited. In contrast, when Pab1-K131 is mutated to R to mimic the unacetylated state, there was no impact on stress granule formation. Remarkably, neither Pab1-K131Q or Pab1-K131R had any impact on cellular fitness or stress granule formation upon other stresses such as heat shock and ethanol. This suggests that acetylation of Pab1-K131 is a post translational modification, specifically regulating glucose deprived stress granule formation. We are presently performing genetic screens to identify the KAT/KDACs regulating the acetylation state of Pab1-K131, and biochemical assays to assess the impact of acetylation of Pab1-K131 on Poly(A) RNA binding and polyribosome response to glucose deprivation. As K131 is a conserved site found in many eukaryote Poly(A) binding proteins, we anticipate lysine acetylation at this position maybe a conserved mechanism to control stress granule dynamics.

1896C G body nucleation, mobility and localization, and degradation in *Saccharomyces cerevisiae* *Joy Huang*[1], Gregory Fuller[1], John Kim[1] 1) Johns Hopkins University.

Under prolonged periods of hypoxic stress, glycolysis enzymes and RNA, which are normally diffuse in the cytoplasm, coalesce to form nonmembrane bound structures called glycolytic bodies, G bodies[1]. In hypoxia, wild type *S. cerevisiae* were depleted of upstream glycolysis intermediates when compared to *snf1Δ* mutants that could not form G bodies. This observation suggests that the localization of cytoplasmic glycolytic enzymes to G bodies enhance glycolysis rates by reducing reaction diffusion time. Overall, the mechanisms that induce G body formation and G body localization patterns are poorly understood. Here, we investigate the nucleation, localization, and degradation mechanisms of G bodies. To identify a potential factor that nucleates G body formation we focused on Glucokinase 1 (Glk1), the enzyme catalyzing the first step in glycolysis, as a candidate marker for the nucleating structure in G body formation. *glk1Δ* mutants were unable to form G bodies in hypoxia and Glk1-GFP is punctate in both normoxia and hypoxia. Furthermore, Glk1 has been copurified with mitochondria[2]. Interestingly, we identified that a number of mitochondrial genes were required for G-body formation. To further investigate this relationship, the physical association of G bodies using Pfk2 and Glk1 tagged with GFP and MitoTracker red stained mitochondria was analyzed. The colocalization results reflect similar levels of mitochondrial association for both proteins. Unlike mitochondria, where G bodies associate and disassociate dynamically, G bodies are stably associated with the vacuolar membrane. Finally, to determine if G bodies can be degraded through bulk cytoplasmic degradation, macroautophagy was stimulated via nitrogen starvation, and PMSF (a vacuolar protease inhibitor) was added to analyze its effect on the degradation of G bodies targeted to the vacuolar lumen. Under varying conditions, G bodies accumulate in vacuoles at higher frequencies in PMSF treated cells vs. untreated cells, suggesting that autophagy may influence traffick of G bodies to vacuoles for degradation under starvation conditions.

1. Jin M, Fuller GG, Han T, Yao Y, Alessi AF, Freeberg MA, et al. Glycolytic Enzymes Coalesce in G Bodies under Hypoxic Stress. Cell Reports. 2017 Jul 25;20(4):895–908.
2. Morgenstern M, Stiller SB, Lübbert P, Peikert CD, Dannenmaier S, Drepper F, et al. Definition of a High-Confidence Mitochondrial Proteome at Quantitative Scale. Cell Reports. 2017 Jun 27;19(13):2836–52.

1910B The ABCF gene family facilitates disaggregation during animal development *Sydney Skuodas*[1], Amy Clemons[1], Michael Hayes[2], Ashley Goll[1],

Betul Zora[1], Daniel Weeks[2], Bryan Phillips[1], Jan Fassler[1] 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Department of Biochemistry, University of Iowa, Iowa City, IA.

Amyloids are notorious for their role in human neurodegenerative conditions included Huntington's, Alzheimer's, and Parkinson's Diseases. However, beneficial functions of amyloids have also been reported in a variety of organisms. Here we show a role for amyloid-type aggregation in early development of *Xenopus laevis* and *C. elegans*. We detect significant staining/reactivity with amyloid specific dyes and antibodies during the animal gametogenesis and embryogenesis, and we find that indiscriminate solubilization of these structures leads to developmental phenotypes. We hypothesize that developmental amyloids must be solubilized in a temporal and spatially specific manner by dedicated disaggregases encoded by animal genomes and expressed maternally and/or embryonically. The *HSP104* gene encoding a potent fungal disaggregase is absent from animal genomes, so we sought candidate animal disaggregases with a role during early development. We identified the *ABCF* gene family by its close phylogenetic relationship to *NEW1*, a fungal-specific disaggregase with Hsp104-like activity. We find that yeast and animal Abcf proteins are essential to the normal processing, propagation and/or inheritance of amyloid aggregates in yeast, and for normal disaggregation of heat-denatured aggregates. Animal Abcf orthologs complement these disaggregation phenotypes, suggesting that Abcf chaperone activities are conserved. Knockdown of *ABCF* mRNA during early animal development results in amyloid-positive aggregate disorganization and developmental arrest. The co-localization of yeast Abcf2 with heat-denatured proteins and native yeast amyloids suggests a possible direct interaction with aggregated clients. The identification of novel chaperone functions for the ABCF protein family with essential roles in early development suggest that amyloid aggregates and their regulated disaggregation may constitute a novel strategy for compartmentalizing developmentally important clients

1917C Visualizing non-canonical translation events using single molecule reporters in live yeast and human cells *Kelsey Bettridge*[1], Agnes Karasik[1], John Taylor Hosmer-Quint[1], Nicholas Guydosh[1] 1) NIDDK.

While it is known that improper protein regulation contributes to disease, the mechanisms that maintain protein homeostasis remain unclear. In particular, over 50% of human mRNA transcripts contain upstream open reading frames (uORFs) that are translated, and thus dissociate ribosomes from mRNAs before they can encounter the main ORF, inhibiting translation of that gene. However, some uORFs can lose their inhibitory status under certain activating conditions and allow ribosomes to "reinitiate" translation at the main ORF following uORF translation. The classic example is the yeast gene *GCN4* (or *ATF4* in humans), a master transcription factor for the integrated stress response, which is essential for cell survival under a variety of common cellular stress. Further, two-thirds of uORF-containing genes are known oncogenes. Despite its clear importance, the mechanisms of uORF regulation and reinitiation are not well understood, due to the difficulty in detecting these non-canonical events using bulk assays. In addition, reinitiation can also occur at ORFs downstream of the main ORF (dORFs) though it remains unclear how the cell regulates these events. Here, we present current efforts to develop single molecule reporters that enable direct visualization of nascent translation events within both yeast and human cells. These reporters enable direct evidence of uORF translation and main ORF reinitiation, dORF translation, and a detailed biophysical characterization of these non-canonical translation events. Using this system in concert with knock downs of translation factors, we plan to uncover which factors are likely involved and subsequently, the mechanisms of uORF regulation.

2185A Genetic analysis of the *Rad51D* gene. *Alexander Konev*[1], Il'ina Yulia[1], Ukraintsev Vladislav[1] 1) Petersburg Nuclear Physics Institute of NRC «Kurchatov Institute».

The key event in recombination repair of DNA double strand breaks (DSB) is the formation of the Rad51 nucleoprotein filament, which is necessary for the homology search and exchange of DNA strands. An important role in the regulation of the assembly, stabilization, and disassembly of Rad51 filaments is played by the Rad51 paralogs — proteins structurally similar to Rad51. Recently, an interest Rad51 paralogs has greatly increased due to their significant role in carcinogenesis. Mammals have six paralogs of the Rad51: Rad51B, Rad51C, Rad51D, XRCC2;, XRCC3 and the SWSAP1 (RadA homolog). The *Drosophila* genome contains the Rad51 ortholog spnA and four Rad51 paralogs: spnB (XRCC3 homolog), spnD (hRad51C), XRCC2 and Rad51D. However, the functions of these proteins remain largely unclear.
The *rad201G1* mutation (radiation sensitive 201) was isolated from a natural population by its larval hypersensitivity to ionizing radiation (Khromykh, Zakharow, 1981). Subsequently, the *rad201G1* mutation was extensively characterized genetically in respect of its effects on meiotic and mitotic recombination, spontaneous and radiation-induced chromosome aberrations, mutagenesis, radiation induced effects in oogenesis and in the development. Here we show that the *rad201G1* mutation is caused by the insertion of the *Opus* retrotransposon at the 5 ' untranslated region of the *Rad51D* gene. In addition to the *Opus* insertion in the site of mutation, the "rad201G1" chromosome contains a number of nucleotide changes, which cause K61E, V93A and Y108H subtitutions in the Rad51D and F50L in the protein encoded by the overlapping *CG42382* gene. We isolated spontaneous reversions of the *rad201* radiation sensitivity phenotype. All reversions are associated with the loss of *Opus*, leaving the nucleotide substitutions in *Rad51D* and *CG42382* genes intact. In the *rad201G1* mutant embryos the *Rad51D* transcription is 30-fold reduced by contrast with the wild type or revertants, while the level of the *CG42382* transcription does not differ . Thus, the *rad201G1* mutation is a *Rad51D* allele. By contrast with the other studied members of the Rad51 family in Drosophila, *Rad51D* mutant has a very weak spindle phenotype which appears only with age. The results of the studies of genetic interactions between *Rad51D[rad201]* and *spn-A* mutations will be presented. A known genetic effects of the *rad201G1* mutation will be reviewed in a light of the fact that they reflect the functions of the Drosophila Rad51D gene.

2190C A genetic screen for mechanisms that counter extra centrosomes *Erin Jezuit*[1], Don Fox[1] 1) Duke University Medical Center, Durham, NC.

Metazoan cells carefully regulate centrosome number. With the exception of multiciliated cells, the presence of more than two centrosomes per cell can be detrimental, causing a host of disease phenotypes including cellular aneuploidy and aberrant cell motility. Understanding the cellular mechanisms that counter extra centrosomes and associated phenotypes can reveal fundamental centrosome regulation and potential therapies of centrosome-related diseases. We previously found a tissue, the *Drosophila* rectal papillar cells of the hindgut, that are extremely tolerant to extra centrosomes and multipolar divisions. When the centrosome duplication regulator Polo-like kinase 4 (Plk4) is overexpressed in this tissue, ~100% of cells contain extra centrosomes, and ~75% of these cells in this developing organ undergo tripolar division. Despite this aberrant centrosome production, no cell death occurs and the tissue develops and functions normally. In order to better understand the mechanism(s) by which these cells tolerate extra centrosomes and the extreme aneuploidy generated by tripolar cell division, we are conducting a forward genetic screen for mutants that are lethal in papillar tissue only in the presence of extra centrosomes. To do so, we have generated 1300 recessive mutations on the X chromosome and are generating mosaic homozygous clones in papillar tissue to screen for lethality specifically in a Plk4-overexpression background. This screen takes advantage of the ability of wild type rectal papillar tissue to excrete excess salt. Animals with compromised papillae, including large

patches of mosaic mutant cells, die rapidly on a high-salt diet. Using this simple feeding assay, we have already screened 484 EMS-generated lines and identified four mutants that reproducibly yield phenotypes only when centrosomes are amplified. To further analyze mutants, we will evaluate wild type and mutant clones during mitosis using centrosome, chromatin and microtubule markers. Results from this screen are beginning to reveal new, physiologically relevant responses to centrosome amplification.

2216B Natural variation in radiation tolerance among nematodes from Chernobyl *Sophia Tintori*[1], Patrick Ortiz[1], Timothy Mousseau[2], Matthew Rockman[1] 1) New York University, New York, NY ; 2) University of South Carolina, Columbia, SC.

Background levels of ionizing radiation range naturally by orders of magnitude across the globe, with severe spikes in some areas due to human activity. Our ability to cope with such environmental exposure is partially due to broadly conserved cellular mechanisms such as DNA damage repair. While key molecular players of these mechanisms have been identified, their natural variation in existing organisms remains poorly understood. By identifying and investigating diversity in animals' sensitivities to radiation, we can uncover genetic variants that allow individuals to be better or worse at tolerating radiation in their environment.

To do this, we have assembled a panel of nematodes from diverse environments — some previously isolated from locations around the world and archived in the C. elegans Natural Diversity Resource, and several hundred newly isolated nematode lines that we have collected from the Chernobyl Exclusion Zone. We are using this array of genetic backgrounds to ask the following questions: (1) Do observed variations in tolerance depend on preventing, repairing, or coping with DNA damage? And (2) which elements of the DNA repair pathways are naturally most variable and what are the genetic and cellular signatures of their variants?

We are currently characterizing our panel of hundreds of wild nematodes isolates. This includes sequencing molecular barcodes to assign species identities to the new isolates from Chernobyl, whole genome sequencing to identify genetic similarity between each pair of isolates, and assaying each isolate for radiation tolerance in a controlled lab environment. The comparative genomic resources that we are currently generating will allow us to identify sets of worms that illustrate evolutionary transitions between different radiation responses in relatively few steps. We hypothesize that major differences will be due to variation in broadly conserved DNA repair mechanisms, with implications for genetic predisposition to cancer in humans.

2225B Genome integrity and the heritability of somatic mutations in clonal, colonial corals *Elora Lopez*[1], Rebecca Albright[2], Stephen Palumbi[1] 1) Stanford University, Stanford, CA; 2) California Academy of Sciences, San Francisco, CA.

Organisms maintain genome integrity by protecting cells from DNA damage and committing resources to DNA repair. In many animals, germ cells give rise to offspring, and are set aside early in development to limit cell divisions and protect those cells from accumulating many mutations. Somatic cells, in contrast, undergo far more cell divisions, are less protected from exogenous DNA damage, and accumulate far more mutations. However, it has long been thought that plants and many basal animal taxa lack germ-soma distinction, and these taxa deserve careful analysis of mutation patterns, rate, and inheritance. There are a few such analyses in plants, but these studies in animals are severely lacking. Clonal, colonial corals are a group of animals for which the matters of senescence as well as the presence of a dedicated germline are unclear. Genome integrity research may answer these questions, and thereby better clarify the evolution of aging and germline-soma distinction. We first analyzed single nucleotide variants from transcriptomes of 17-22 branches from each of four *Acropora hyacinthus* colonies. There was no signature of mutation caused by UV damage, and the coral somatic mutation spectrum was most similar to the human germ line spectrum. The somatic mutation frequency per nucleotide in *A. hyacinthus* is on the same order of magnitude (10^{-7}) as noncancerous human somatic cells. Loss of heterozygosity variants outnumber gain of heterozygosity mutations about 2:1, suggesting reliance on homologous recombination in corals to repair double stranded breaks. In order to test inheritance of coral somatic mutations, we sequenced full genomes for 10 parent coral branches as well as the sperm spawned by each of those branches. From ~1000 mutations in the somatic tissue of each parent branch, we found that the majority do not appear in the sperm produced by the branch. Yet 10-20% of these unique somatic mutations do appear in the sperm. These data suggest that there is some germline segregation in corals, but it may be incomplete. This study yields insight into genome maintenance dynamics and somatic mutation inheritance in a colonial organism with a position in the tree of life that suits it to be key in understanding the evolution of aging and germline-soma distinction.

2226C Targeted next-generation sequencing reveals complex mutation signatures in *rnr1 msh* genetic backgrounds *Natalie Lamb*[1], Jonathan Bard[1], Michael Buck[1], Jennifer Surtees[1] 1) University at Buffalo, Buffalo, NY.

Multiple pathways contribute to maintaining high-fidelity DNA replication, including the regulation of free dNTP levels and both the selectivity and exonuclease domains of the replicative polymerases. Elevated free dNTP levels are a well-established source of mutagenesis due to increased DNA polymerase error and decreased proof-reading, and are likely a hallmark of cancer cells. Replication errors are typically substrates for the mismatch repair (MMR) system, which recognizes misincorporation and insertion/deletion errors and targets them for repair. Here, we developed a high-throughput, targeted deep-sequencing approach to examine the consequences of elevated dNTP pools and DNA polymerase error repair via MMR, both alone and in combination. The combination of altered dNTP pools and compromised MMR together has the potential to alter the mutational landscape. Importantly, mutation signatures of human tumors are used to tailor cancer treatment and inform clinical outcome. We used *Saccharomyces cerevisiae* as a model system to document how mutational signatures are modified by different combinations of elevated dNTP levels and reduced MMR. We sequenced pools of mutated (canavanine-resistant) colonies with 1) altered dNTP pools (*rnr1* alleles), 2) with deletions in MMR recognition factors (*msh* alleles) and 3) combinations of *rnr1* and *msh* alleles.

The depth of sequencing allowed us to delineate *CAN1* regions that are systematically susceptible to mutagenesis. We combined variant type and positional information to develop genotype-specific mutation signatures. We developed computational methods to quantify the contribution of two different genotypes to the underlying mutation signature in double mutants, to assess additive, epistatic or synergistic effects. Individually, altered dNTP pools, even very modest changes, and compromised MMR led to distinct mutational signatures. Furthermore, the increased and altered mutation signatures in *rnr1* backgrounds allowed us to identify novel specificity of Msh2-Msh3 for single base deletions in repetitive GC runs, mutations commonly observed in MMR-deficient cancers. Notably, the mutation signatures of double mutants were not a simple combination of the single mutant signatures, indicating a more complex effect on mutagenesis. We propose that establishing mutation signatures from the ground up will provide useful information when interpreting mutational signatures in human tumors.

2256C Investigating the Role of Introns in Transcription-Associated Mutagenesis in Budding Yeast *Cedric Lansangan*[1] 1) California State University - San Marcos, California, United States of America.

A key feature required for cell survival is genomic stability, which is the ability of a cell to prevent mutations in its genome and repair them if they do occur. Genomic mutations can be generated in several ways, including transcription of a gene via transcription-associated mutagenesis (TAM). Introns are known to be able to enhance transcription *in vivo*, but the influence of introns on TAM is not well-understood. Here, our work investigates whether introns modulate the rate of TAM in a *URA3* reporter gene using the budding yeast *Saccharomyces cerevisiae* as a model organism. Our preliminary results suggest that the presence of introns in the natively-intronless *URA3* gene is associated with elevated mutation rates of that gene. Interestingly, there appears to be an intron-length-dependent effect on this trend (*i.e.* the yeast strain with a long intron in the *URA3* reporter demonstrated elevated *URA3* gene mutation rates compared to the other two strains with either a short intron or no intron). Our findings suggest that the length and presence of introns in a gene are associated with increased mutagenesis of that gene. Furthermore, the long-intron-containing strains in our study grew slower in the absence of uracil than the short-intron strains and no-intron controls, possibly indicating an effect of intron length on splicing of the *URA3* primary transcript. We are currently investigating if elevated transcription rate of a *URA3* gene containing an intron causes an increase in TAM rate. In addition, *URA3* reporter gene expression levels are being quantitated in each strain. We expect that *URA3* mRNA transcript levels will be elevated in the intron-containing *URA3* yeast strains relative to those of the no-intron controls. In future work for this study, we will determine the effect of the presence of an intron located near the promoter of an induced *URA3* gene on the rate of TAM. Our future results will contribute to better understanding the impact of introns on the mutagenesis of highly-expressed genes, which may play a role in mechanisms underlying mutagenesis in the human genome.

Wednesday, April 29 12:00 PM - 3:00 PM

Ethical, Legal, Social Issues/Best Practices in Training and Education - Poster Q&A 449B Undergraduate research in epigenetics using *Drosophila melanogaster* *Sarah Elgin*[1], Andrew Arsham[2], Catherine Reinke[3], Sidney H. Wang[4], Elena Gracheva[1], Wilson Leung[1] 1) Washington University in St. Louis, St Louis, MO; 2) Bemidji State University, Bemidji, MN; 3) Linfield College, McMinnville, OR; 4) The University of Texas Health Science Center, Houston, TX.

Position Effect Variegation – the silencing of a gene in some of the cells in which it is normally expressed – occurs when a gene normally found in euchromatin is juxtaposed with heterochromatin through rearrangement or transposition. This silencing is due to stochastic assembly of heterochromatin over the reporter gene, an epigenetic phenomenon. Because modifications of PEV phenotypes of reporter genes such as *white* are easily scored, undergraduates can use PEV to study epigenetic mechanisms. We have available three sets of lines for such studies. 1) Lines carrying a *P* element reporter with *hsp70-white* in different heterochromatic domains (pericentric heterochromatin, telomeres, 4th or Y chromosome) can be used to examine the impact of genetic modifiers, environmental conditions (diet, temperature), etc. on the different types of heterochromatin. Selection for high and low levels of expression can be used to identify background modifiers and reveal distinctive patterns of variegation, potentially providing new mechanistic insights. 2) Lines with that reporter inserted at sites along the 4th chromosome allow investigation of genes present, and active, in a heterochromatic domain, both those dominated by H3K9me2/3-HP1a and those dominated by H3K27me3-Pc. 3) Lines exhibiting ectopic silencing induced by a *1360* element or by a tandem array of repeats, either $[GAA]_{310}$ or $[lacO]_{256}$, display different sensitivities to modifiers, implying different mechanisms for heterochromatin targeting and assembly; this suggests multiple investigations. Important studies can be done using genetic crosses, with the eye phenotype scored by a pigment assay (quantitative results; requires a spectrophotometer) and pattern characteristics (requires an iphone with jpeg software). Many modifiers of PEV [Su(vars)s and E(var)s] as well as the group 1 lines are available from the Bloomington Stock Center. Group 2 and 3 lines are available through May 30 from S Elgin lab, and thereafter from A Arsham or C Reinke. The excellent annotation of *D. melanogaster*, including ChIP results for many chromosomal proteins and histone modifications, plus extensive annotation of transcripts, from modENCODE and others, enables students to frame sophisticated questions (browsers at FlyBase and GEP http://gander.wustl.edu). Examples of student work using these resources will be shown. Come chat with us if you think your students might enjoy a project in epigenetics!

453C Fly-CURE: A consortium of undergraduate genetics laboratory courses mapping and characterizing Drosophila EMS mutants *Jacob Kagey*[1], Kayla Bieser[2], Danielle Hamill[3], Julie Merkle[4], Alysia Mortimer[5], Kenneth Saville[6], Jamie Siders[7], Joyce Stamm[4], Melanie Hwalek[8], Victoria Straub[8] 1) University of Detroit Mercy, Detroit, Michigan; 2) Nevada State College, Las Vegas, Nevada; 3) Ohio Wesleyan University, Delaware, Ohio; 4) University of Evansville, Evansville, Indiana; 5) Illinois State University, Normal, Illinois; 6) Albion College, Albion, Michigan; 7) Ohio Northern University, Ada, Ohio; 8) SPEC Associates, Detroit, Michigan.

A Flp/FRT EMS screen was conducted in the Drosophila eye in the context of blocked apoptosis to screen for conditional mutants that altered control of cell growth and development. Using the mutants from this screen, we have established a classroom-based approach to the mapping and characteriziation of each mutant identified in the initial screen. Mutants are mapped and characterized by undergraduate students at one of seven diverse institutions currently participating in the Fly-CURE consortium. The Fly-CURE consortium allows the data to be independently generated and confirmed by groups of undergraduate researchers at different institutions. From this project, undergraduate researchers have successfully mapped a number of EMS mutants, including alleles of Shn, Egfr, and Cos2. This data has led to local and national scientific presentations by students, as well as four peer-reviewed publications with student-generated data. In addition, we have established a protocol to use whole-genome sequencing in combination with deficiency mapping to identify mutations that do not fail to complement known alleles within the region that fails to complement. This project has involved over 300 undergraduate researchers within a classroom setting, providing research opportunities to students who may not have otherwise had exposure to authentic research experiences. Recently, we piloted a pedagogical study to measure the impact of the Fly-CURE on students' attitudes toward research and to measure the impact of research dosage and gains in learning objectives for students who take this course. Current analysis demonstrates, that Fly-CURE was the first research experience for over half of the students participating at 4 institutions and students reported significant gains in research self-efficacy and belonging to a scientific community. We believe that the establishment of CURE consortiums, such as this one, will help create a large network of undergraduate researchers.

454A Effects of a cancer model organisms course on student self-efficacy and attitudes about science *Christopher Abdullah*[1] 1) SPIRE Postdoctoral Fellowship Program, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Incorporating primary literature into undergraduate science curriculum is a common goal among biology educators. Implementation of journal articles as part of existing core courses or as stand-alone courses, such as those using the CREATE method, are becoming the norm particularly in biology courses. Recent research has focused on paper selection, research topics, structured reading methodology, and student perceptions and outcomes. One unique aspect of biomedical research, which has not, to my knowledge, been directly addressed, is the use of model organisms. Biomedical research utilizes a variety of common model organisms with unique strengths and weaknesses that make them well-suited for specific approaches and research questions. Here, I describe the novel design of an upper-level undergraduate elective course that uses cancer as a paradigm to explore the use of model organisms in primary literature. Cancer hallmarks (proliferation, genomic instability, apoptosis evasion, and metastasis) were used as course units. Both teacher- and student-selected data from journal articles was used to explore a variety of model organisms. These data were used to discuss the benefits and limitations of each model system in the context of the research. Instructional emphasis was placed on data analysis, data interpretation, and experimental design and methodology. A structured analysis rubric was utilized to facilitate student engagement with the primary literature and data. As a final project, students incorporated their knowledge of cancer model organisms by developing an experimental design to test a hypothesis developed throughout the course. Here we present our findings from a pre- and post-course survey and assessment involving students' attitudes, self-rated abilities and epistemological beliefs.

458B Beyond the CURE: addressing the needs of undergraduates through an advanced independent research program that examines the effects of gene regulation in neurodegenerative diseases Kahealani Bentosino[1], Nicole Chandonnet[1], Jesslyn Connors[1], Myles Freeman[1], Taylor Kring[1], Benjamin Madden[1], Alexis Montague[1], Justin Timmins[1], *Pamela Harvey*[1] 1) University of Colorado Boulder.

Students who engage in research experiences during their undergraduate education exhibit greater long-term retention in science, deeper understanding of the scientific method, and are more scientifically literate. However, most departments at large universities are unable to offer opportunities for novel discovery because one-on-one mentoring relationships are not available at this scale. A solution to this problem is to formalize research experiences within the departmental course curricula. Course-based Undergraduate Research Experiences (CUREs), an innovative pedagogical approach, can efficiently offer research opportunities to many more students. The Molecular, Cellular, and Developmental Biology Department (MCDB) at University of Colorado Boulder committed to providing opportunities for research-based experiences to all of our majors early in the students' education. The department currently offers more than 500 seats in MCDB CUREs per academic year.

Students who have taken CUREs in our department report significant gains in confidence in critical analysis of data, feelings of membership in a department, understanding the impact of the work to the scientific community, and technical skills through iteration. Indeed, about 15% of students exiting these courses continue to express an interest in pursuing research projects in our department. The students are prepared to engage in independent research, yet there remains the problem of providing these students with advanced opportunities that include the honors theses. We have met this need through the creation of a format that provides up to 12 students each semester with the opportunity for increased independence in the laboratory beyond the CURE laboratory courses. Undergraduates involved in this program conceived a long-term project that focuses on understanding the role of abnormal systemic cholesterol homeostasis in demyelinating diseases like multiple sclerosis. In this setting, with the mentorship of a senior instructor, students developed an experimental approach that involves synthesis and injection of dsRNA into adult Drosophila melanogaster. Assessment of resulting phenotypes involves behavior testing, quantification of gene expression, and measurement of myelin production and levels of systemic cholesterol. We present a review of the logistics of this scalable program, a scaffold for adapting the program to other research projects, preliminary data generated by students, and challenges associated with oversight by a non-research faculty member.

461B Learning genetics by doing: "making a baby" with a deck of cards *Tina Gumienny*[1], Lionel Faure[1] 1) Texas Woman's University, Denton, TX.

Genetics can be a difficult topic to master, especially for non-majors students. For some students, the random yet precise segregation of chromosomes during meiosis and what the different inheritance patterns mean are especially hard to grasp. To help students understand these basic genetic concepts, we developed an in-class activity to connect genetics concepts with students' lived experiences. After in-class instruction on meiosis, the diploid life cycle, and basic inheritance patterns, students spent a class period applying what they learned by "making a baby". The student make groups and are given a deck of cards. Each card represents a chromosome. The two black suits are the dad's chromosomes. The two red suits are the mom's chromosomes. Students are instructed to perform meiosis: after laying out cards in order by suit, one student in the group makes the haploid "sperm" chromosome set by selecting one of each card number, randomly choosing clubs and spades. In a similar way, another student makes the "oocyte" chromosome set from heart and diamond cards. The group performs "fertilization" by lining up the two sets of cards, the "sperm" ace with the "oocyte" ace, etc. They confirm no aneuploidies. After students have successfully "made a baby", the instructor makes available a table with the genotypes associated with each chromosome and the inheritance pattern. Real human traits that generally follow autosomal recessive, dominant, sex-linked, incomplete, and polygenic inheritance patterns are provided for the students to work through. The students' goal is, as a group, to interpret the genotype and phenotypes of their "baby" based on the randomly selected card chromosomes their "baby" has. They fill in a paragraph describing their baby's traits based on the genotype and the given inheritance patterns. This activity has been refined over four years with two different instructors. A high percent of students provided positive responses when asked if this activity helped them understand the patterns of inheritance and if this activity was enjoyable.

This activity is adaptable to one's favorite inheritance patterns, traits, and diploid, sexually reproducing, multi-chromosomal organism. Practically, it is fairly inexpensive, doable in 50 or 80 minutes, and scalable to fairly large classes (especially with help from teaching assistant(s)).

462C Departmental mentoring climate guidelines to increase recruitment, retention, and trainee success Jamie L. Lahvic[1], Adriana Bankston[2], Stephanie Davis[3], Gary McDowell[9], Nicole Parker[4], Juan Pablo Ruiz[5], Kaliris Y. Salas-Ramirez[6], Harinder Singh[7], Cara Weismann[8] 1) University of California, Berkeley, CA; 2) University of California Office of Federal Governmental Relations, Washington, DC; 3) National Institute on Aging, NIH, Bethesda, MD; 4) Ripple Effect Communications, Rockville, MD; 5) University of Wisconsin-Madison, WI; 6) CUNY School of Medicine and Hunter College, CUNY, New York, NY; 7) University of California, Irvine, CA; 8) University of Massachusetts Lowell and Medical School, Worcester, MA; 9) Lightroller LLC, Chicago, IL.

Academic research institutions are the cradle for innovation, scientific discovery, and problem-solving. Yet the lack of prioritization and incentivization of ethical and effective mentoring practices at these institutions is partly responsible for preventing early career researchers (ECRs), particularly those from underrepresented minority (URM) backgrounds, from reaching their fullest potential as the next generation of leaders in STEM. Strong

mentorship has been shown to improve the diversity of the biomedical workforce by increasing retention in training and enhancing training experiences. Because stakeholders at all levels recognize the need for improved mentorship standards, Future of Research (FoR) organized a meeting to develop mentoring climate guidelines to increase transparency regarding departmental mentoring efforts and prioritization of training standards. With support from experts and leaders in the field of mentoring, we used the available evidence-based research on mentor/mentee competency training, the practical expertise of departmental leaders, and the experience of early career researchers to develop a set of guidelines to be used as an assessment tool by departmental leaders wishing to commit to actionable improvement. Additionally, local satellite meetings were organized to livestream workshops from the central meeting and allow participation and feedback from ECRs in departments around the nation. Meeting results have been publicly reported and departments now have the opportunity to commit to the guidelines on the Future of Research website (https://mentoringfuturesci.net/). By using these guidelines, departments will increase recruitment, retention, and success of trainees, while decreasing URM attrition from STEM careers.

464B Incubators: Building community networks and developing open educational resources to integrate bioinformatics into life science education *William Morgan*[1], Sam Donovan[2], Hayley Orndorf[2], Sabrina Robertson[3], Elizabeth Ryder[4], Michael Sierk[5], Anne Rosenwald[6], Elizabeth Dinsdale[7], Eric Triplett[8], Mark Pauley[9], William Tapprich[10] 1) College of Wooster, Wooster, OH; 2) University of Pittsburgh, Pittsburgh, PA; 3) University of North Carolina at Chapel Hill, Chapel Hill, NC; 4) Worcester Polytechnic Institute, Worcester, MA; 5) Saint Vincent College, Latrobe, PA; 6) Georgetown University, Washington, DC; 7) San Diego State University, San Diego, CA; 8) University of Florida, Gainesville, FL; 9) National Science Foundation, Alexandria, VA; 10) University of Nebraska at Omaha, Omaha, NE.

The Network for Integrating Bioinformatics into Life Sciences Education (NIBLSE) is an NSF-funded Research Coordination Network that aims to establish bioinformatics as an essential component of undergraduate life sciences education. Thus far, NIBLSE has published a set of bioinformatics core competencies for undergraduate biologists (Wilson Sayres et al., 2018, *PLoS One*) and documented barriers to integrating bioinformatics into life sciences education (Williams et al., 2019, *PLoS One*; see accompanying abstract by Rosenwald et al.). In addition, NIBLSE is working to make existing bioinformatics learning resources more accessible to non-specialists and to increase their use across undergraduate biology courses. To this end, NIBLSE has partnered with the Quantitative Undergraduate Biology Education and Synthesis (QUBES) project to develop and implement a novel model for supporting the refinement, publication, and dissemination of high quality bioinformatics teaching resources. NIBLSE Incubators are small, short-lived, online communities that work with an existing learning resource to (1) introduce and teach important bioinformatics learning outcomes, (2) improve its usability across diverse life sciences classrooms, and (3) move the learning resource toward publication and broader dissemination. Incubated learning resources are available to the broader community through the NIBLSE Learning Resource Collection hosted by QUBES and cross-referenced to the NIBLSE Bioinformatics Core Competencies. In addition, some resources are linked to adaptations that faculty have tailored to specific courses or specific sub-disciplines (see accompanying abstract by Kleinschmit et al.). This presentation will highlight the current status of the NIBLSE Learning Resource Collection and discuss the opportunities and challenges associated with the Incubator approach for refining learning resources. NIBLSE and QUBES are supported by grants from the National Science Foundation (DBI 1539900 and DUE 1446269, respectively). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

466A Planning and developing a state-level policy fellowship: The Virginia Academy of Science, Engineering, and Medicine Commonwealth of Virginia Engineering and Science (COVES) Policy Fellowship Matthew Diasio[1], *Danielle DaCrema*[1], Holly Mayton[1], Michaela Rikard[1], James Aylor[1,2] 1) University of Virginia; 2) Virginia Academy of Science, Engineering, and Medicine.

The Commonwealth of Virginia Engineering and Science (COVES) Graduate Policy Fellowship is a new state-level STEM policy fellowship program. In developing COVES, we wanted to balance the effectiveness and usefulness of COVES fellows with the fellows' professional development. We met with Virginia legislators, university officials, and PhD students and postdoctoral trainees to assess the needs of the groups we hope COVES will impact, and assessed the structural advantages and disadvantages of other STEM policy fellowships. We concluded that a 1-year program would be useful to policy-makers, but the nature of Virginia's part-time legislature means that work for a science advisor would be sporadic. Therefore, COVES will be a 12-week program in the style of the Christine Mirzayan Science and Technology Policy Fellowship at the National Academies of Sciences, Engineering, and Medicine. COVES fellows will work in the summer when bills are drafted so they can better advise the content of the bills. The 12-week structure opens COVES to current graduate students and reduces costs. For the inaugural class in 2020, we approached university officials directly for support. University graduate affairs offices are only willing to support graduate students since postdoctoral trainees are employees, although we hope to apply for philanthropic support and open future years to recent graduates (within 5 years) as well as current PhD students.

Fellows will serve primarily as advisors in willing offices across Virginia in government, industry, or NGOs. As these offices are spread throughout Virginia, COVES will not have a central location. Therefore, it is essential that the fellowship program provides a method to unify the fellows. It is also essential that we recognize that the fellowship is educational - fellows must receive specific training in the functions of policy-making in Virginia and will have a mentor they can work with to learn about science policy. The natural pairing for COVES was with the Virginia Academy of Science, Engineering, and Medicine (VASEM), which is made of National Academies members in Virginia, and has worked over the last 5 years to provide scientific advice to Virginia's government. COVES fellows will have a VASEM mentor. The program will also provide a bootcamp-style workshop on science policy generally and in Virginia through VASEM and the state universities.

477C A mobile technology-based cooperative learning platform for undergraduate biology courses in common college classrooms *Chun Zhou*[1], Matthew Lewis[2] 1) Mercy College, Dobbs Ferry, NY; 2) CUNY School of Professional Studies, New York, NY.

As a high-impact educational practice, cooperative learning uses a structured group study to promote students' active learning. Currently, it lacks economical yet effective tools to facilitate the interactive nature of structured cooperative learning in regular classrooms. Here, we have established a mobile technology-based cooperative learning (MBCL) platform that comprises the 2018 iPad, Apple Pencil, LiveBoard, Google Forms, Google Drive, and Blackboard. We tested the MBCL platform in three undergraduate biology courses: Genetics, Microbiology, and Immunology. During semester-long MBCL studies, the students engaged in cooperative learning to discuss a real-life issue. With the MBCL platform, the students' group study processes were shown on shared electronic whiteboards that were updated in real-time, generating visible thinking and instant, interactive communication. The instructor was able to guide the students promptly to conduct knowledge integration and knowledge synthesis using tables and diagrams. The deep learning outcome was evident in the examples and quantitative analyses of students' whiteboard study results and team

presentations. Thus, integrating innovative mobile technologies with high-impact teaching practices, exemplified by the MBCL platform, promotes deep learning in higher education.

480C The Mastery Rubric for Bioinformatics: supporting design and evaluation of education and training across the life sciences Rochelle E Tractenberg[1], Jessica M Lindvall[2], Teresa K Attwood[3], Allegra Via[4] 1) Collaborative for Research on Outcomes and –Metrics, and Departments of Neurology, Biostatistics, Biomathematics and Bioinformatics, and Rehabilitation Medicine, Georgetown University; 2) National Bioinformatics Infrastructure Sweden (NBIS)/ELIXIR-SE, Science for Life Laboratory (SciLifeLab), Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden; 3) Department of Computer Science, The University of Manchester, Manchester, England, United Kingdom; The GOBLET Foundation, Radboud University, Nijmegen Medical Centre, Nijmegen, The Netherlands; 4) ELIXIR Italy, National Research Council of Italy, Institute of Molecular Biology and Pathology, Rome, Italy.

As the life sciences have become more computational and data intensive, the pressure to incorporate the training required to effectively utilize these technologies into life-science education and training programs has increased. To facilitate curriculum development, various sets of bioinformatics competencies have been articulated; however, these have proved difficult to implement in practice. Addressing the implementation difficulties, we have created a curriculum-design and -evaluation tool – the Mastery Rubric for Bioinformatics (MR-Bi) – to support the development of specific Knowledge, Skills and Abilities (KSAs) that promote bioinformatics practice and the achievement of competencies. The KSAs represent scientific thinking and their development over stages from novice to experienced independent scientist is described in the MR-Bi. The MR-Bi can be used for biology, genetics, or bioinformatics instruction – wherever the integration of computation and biological knowledge are intended in curricula that seek to promote the development of independence and scientific reasoning. It can be used to map instruction for Vision and Change concepts and competencies, as well as similar curricular objectives from other sources. It can also be used by practicing scientists at all career stages to direct their (and their team's) acquisition of new, or to deepen existing, KSAs relating to bioinformatics and computational life sciences. It can be used to strengthen teaching and learning, and for curriculum building. It can thereby contribute to the cultivation of a next generation of computationally-literate biologists and life scientists who can design reproducible and rigorous research, and can critically analyze results from their own, and others', work. This presentation will describe the MR-Bi and outline how it can be utilized to support instructional and curriculum design for biology, genetics, or bioinformatics instruction in higher education and training contexts.

481A RT-qPCR analysis of putative mutants of the Arabidopsis karrikin signal transduction pathway as a Course-based Undergraduate Research Experience *John Stanga*[1] 1) Mercer University.

Course-based Undergraduate Research Experiences (CUREs) are beneficial to students and faculty alike. Here I describe a one-semester CURE implemented in an upper division molecular genetics class at Mercer University. Students developed and applied skills in sterile technique, DNA isolation, PCR, gel electrophoresis, RNA isolation, RT-qPCR, statistics, investigative research, and literature analysis. In many plants, seed germination, photomorphogenesis, and other developmental processes are responsive to the presence of karrikins, chemicals found in smoke that may mimic an as-yet-unidentified endogenous hormone. Mutant screens in Arabidopsis have identified several components of the karrikin signal transduction pathway, including the receptor for karrikins, KAI2; an F-box protein, MAX2; and the probable targets for MAX2-mediated proteolysis, SMAX1 and the related SMXL proteins. The molecular mechanism by which SMAX1 and SMXL2 regulate gene expression in response to karrikins remains unresolved. *smax1 smxl2* double mutants were used as the basis for a suppressor screen to identify novel signaling components. Thirteen putative suppressors were isolated, some but not all of which may have the same mutation. Students were tasked with genotyping the suppressors and using RT-qPCR to classify them based on the relative mRNA abundance of three genes known to be responsive to karrikin, *IAA1*, *KUF1*, and *DLK2*. Student data helped to classify putative mutants based on expression patterns and narrowed the list of putative suppressors to be used for linkage mapping.

483C The Yeast ORFan Gene Project: Finding a Place for Uncharacterized Genes to GO *Jill Keeney*[1], Pamela Hanson[2], Tammy Tobin[3], Erin Strome[4], Mary Miller[5], David Aiello[6], Steve Johnston[7], David Kushner[8] 1) Juniata College Huntingdon, PA; 2) Furman University, Greenville, SC; 3) Susquehanna University Selinsgrove, PA; 4) Northern Kentucky University Highland Heights, KY; 5) Rhodes College Memphis, TN; 6) Austin College Sherman, TX; 7) North Central College Naperville, IL; 8) Dickinson College, Carlisle, PA.

Course-based undergraduate research experiences (CUREs) have numerous positive impacts on students, including increased knowledge of course content, independence, and interest in related subject matter. We describe the development, assessment, and propagation of an easily transferable, collaborative CURE that aims to determine the function of as yet uncharacterized *Saccharomyces cerevisiae* genes. More than 20 years after the sequencing of the budding yeast genome, nearly 10% of open reading frames (ORFs) are still considered uncharacterized. We hypothesize that CURE modules for study of yeast genes of unknown function can be effective tools to teach undergraduates basic bioinformatics, gene discovery tools and experimental design. Thus, we formed and are growing a consortium of undergraduate researchers and faculty at primarily undergraduate institutions (PUIs) to collaborate in assigning functions to these orphan genes (ORFans). Summer workshops for faculty and students introduce attendees to the ORFan bioinformatics work-flow and basic laboratory techniques, and provide faculty with the knowledge and materials to incorporate the modules into courses at their home institution. Pre- and post-test assessments provide data on the effectiveness of the modules. Assessment results (n>300) confirm that students gained an understanding of the Gene Ontology (GO) system for describing gene function and knowledge in the use of bioinformatics to assign gene function.

484A The Build-a-Genome Network: a Course-Based Undergraduate Research Experience for the Design and Modular Assembly of Bacteriophage Genomes *Lisa Scheifele*[1], Robert Newman[2], Eric Cooper[3], Franziska Sandmeier[4], Jennifer Roecklein-Canfield[5] 1) Loyola University Maryland; 2) North Carolina A&T University; 3) Hartwick College; 4) Colorado State University-Pueblo; 5) Simmons University.

The Build-a-Genome network seeks to integrate the teaching of synthetic genomics into undergraduate curricula. The network provides professional development, access to shared resources, and standardized workflows that can be implemented as course-based undergraduate research experiences (CUREs). To date, these workflows have included synthesis of designer yeast chromosomes, yeast neochromosomes containing metabolic pathways, and bacteriophage genes. We now describe expansion of this last workflow to include synthesis and cloning of a semi-synthetic phage genome.

Mycobacteriophage Giles is a 53kb temperate phage that infects *Mycobacterium smegmatis*. It falls into one of thirty distinct clusters of mycobacteriophages, each of which has little sequence similarity to the others. More than half of its proteins remain uncharacterized, and few have orthologs

in other mycobacteriophages outside of their cluster. There are therefore numerous questions that remain to be answered about genome structure and function of this phage, offering students endless opportunities for hypothesis-driven genomics research.

To enable these questions to be addressed at multiple institutions, we have chosen to clone the Giles genome so that it can serve as a template for students to create semi-synthetic phage genomes. The Giles genome was designed as 11 overlapping genomic fragments with homology to the pCC (containing both BAC and yeast centromeric plasmid sequences). Transformation-associated recombination (TAR) was then used to clone the 11 individual fragments in yeast. The 11 clones are then released from the vector and joined into a complete genome using a second round of TAR.

Construction of synthetic genome fragments up to 5 kb has been a central workflow for Build-a-Genome and has been accomplished by undergraduates at several undergraduate institutions. By cloning the 55kb wild-type phage genome as 11 modular fragments, we now enable assembly of wild-type and synthetic fragments in various combinations. Undergraduate students can therefore design and construct synthetic fragments with targeted genomic changes and combine those fragments with the remaining wild-type sequences by TAR cloning in yeast to generate full phage genomes whose infectivity and properties can be assayed. We expect that this workflow would be especially attractive to schools participating in HHMI SEA-PHAGES phage hunting program.

485B Understanding our scientific past: What became of eugenics course offerings? Richard Tam[1], Jasmeen Kaur[1], Cynnie Tam[1], Milton Reynolds[2], *Rori Rohlfs*[1] 1) San Francisco State University; 2) Milton Reynolds Consulting.

Our field of genetics is historically entangled with the political movement and scientific study of eugenics. This is clear from the overlap in early leaders of both fields (eg: Francis Galton, Karl Pearson, R.A. Fischer, J.B.S. Haldane), and from society and journal name changes (eg: *The Annals of Eugenics* became *The Annals of Human Genetics* in 1954). It is tempting to argue that our current projects have entirely departed from the scientific study of eugenics. However, such claims are unsubstantiated without considering the history of eugenics and subsequent changes in our scientific fields. Here we begin to examine this process through historical course offerings from the Department of Biology at our home institution, San Francisco State University (SFSU). We have no reason to believe that SFSU was exceptional in its eugenics course offerings, rather we chose to start here as a self-reflection and because it is more feasible to locate historical records on our own campus. By inspecting historical SFSU course bulletins, we found that 'Eugenics' was an upper-division elective offered by the Department of Biology from 1926 to 1951. We also noticed that in 1952 a new course, with a strikingly similar course description, was offered: 'Human genetics.' The timing of these course offerings and similarities in course descriptions seems to suggest that 'Human genetics' replaced 'Eugenics.' Despite our efforts, we did not find any data on the motivation behind this course change, nor about the specific content for either course. However, we can better understand exactly why academia stepped away from eugenics, and clarify how scientific ideas evolved through this transition through parallel studies that can be implemented at other universities (with more fastidious record keeping). The design of our study is feasible for a project leader (faculty member, post-doc, or grad student) working with a few motivated undergraduates. These studies are particularly crucial as now tremendous genetic datasets enable investigation into questions that may be rooted in persistent ideas dating from the era of intentionally eugenic science. Critical re-examination of our field's history will better position us to lessen harmful societal impacts and better align our field's positive intentions and actual impacts.

488B Addressing the Threat in the Air: Reducing stereotype threat in science environments Leticia Marquez-Magana[1], *Audrey Parangan-Smith*[1] 1) San Francisco State University.

Pseudoscience in the form of eugenics studies have led to the unfortunate stereotyping of people of color as less intelligent than their white counterparts. As a result, science environments often invalidate scientists of color and/or render them invisible, thereby triggering stereotype threat. This situational threat leads to the underperformance of trainees of color in science domains. To overcome this barrier to equity, diversity, and inclusion in science, leaders of the SF BUILD project at San Francisco State University (SF State) have developed a workshop for science faculty and research mentors. The workshop enables participants to recognize and reduce stereotype threat in their classrooms and research labs. It was developed by a transdisciplinary team of researchers that included cognitive and social psychologists, basic scientists, and science educators, and is based on nearly 50 scholarly articles. Since its development in 2016 the workshop has been delivered to hundreds of faculty and research mentors at SF State and at the University of California, San Francisco (UCSF). UCSF is the research partner for the SF BUILD project, and works with SF State, to "*Enable Full Representation in Science*." Thus, the stereotype threat workshop can meet the needs of faculty and mentors at research-intensive universities (e.g., UCSF), as well at a comprehensive, minority-serving institution (e.g., SF State) who are committed to equity, diversity, and inclusion.

491B Development and Retention of Female Academics Via a Peer-led Book Club Intervention Amanda L. Zacharias[1,2,3], Deborah Y. Kwon[1] 1) Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2) Cincinnati Children's Hospital Med Ctr, Cincinnati, OH; 3) University of Cincinnati College of Medicine, Cincinnati, OH.

Purpose: In response to concerns from fellow postdocs that there was insufficient institutional and individual support to encourage women to remain in the academic "pipeline", we created a peer-led book club intervention for female graduate students and postdocs. This intervention has operated successfully for over 4 years with support from the Department of Genetics at the Perelman School of Medicine at the University of Pennsylvania.
Design: Membership expands widely beyond the Department of Genetics to anyone on the department seminar listserv and is composed of 56% postdocs, with a sizable international contingent. Books are nominated and ranked in preference by the membership; selections have ranged from self-help titles aimed at women in business targeting common issues (negotiation, Imposter syndrome), the history of women in science, to new research on biological gender differences. Meetings are held monthly in several small groups that meet over lunch or dinner at a consistent time to foster group cohesion and accommodate different schedules. These meetings not only provide education and awareness on gender-related issues in science, but also offer a safe environment where female scientists are able to share personal and professional issues. Once a semester, a joint session is held to hear a female faculty describe their career path, which serves to provide relatable examples and opportunities for trainees to network with faculty. Experts from across the Penn campus have also been brought in to lead interactive workshops on relevant topics.
Results: Based on Likert scale surveys of 52 participants over 4 years, 94% reported the group helped them feel part of the university community, 80% reported increased confidence, 75% reported the group had helped them identify solutions to problems in their professional life, and 98% would recommend participating in the group to other women. All women departing the group thus far (n=14) have continued their scientific careers in academia, pursuing graduate education, postdoctoral fellowships, and faculty positions, or have attained other academic positions of scientif-

ic leadership. These results indicate that an intervention of only 12 hours per year may have significant impact on retaining women in academic science.

Wednesday, April 29 12:00 PM - 3:00 PM

Intracellular Dynamics/Genome Integrity 4 - Poster Q&A 1794C Evaluating the Effect of Extracellular Gaps on Border Cell Migration in *Drosophila* *Alexander George*[1], Bradford Peercy[1], Michelle Starz-Gaiano[1] 1) University of Maryland Baltimore County.

Elucidating the underlying mechanisms governing collective cell migration is imperative, given their role in development, wound healing, and diseases like cancer. Many cell migration studies, however, are conducted in vitro, which neglects to consider the chemical and biophysical complexities of the in vivo tissue environment. Collectively migrating populations of cells traverse through the physically diverse architecture of a tissue, often along a concentration gradient of diffusible chemical attractants (chemoattractants). Using the border cells, which navigate through the three-dimensional cellular terrain of the *Drosophila* egg chamber during oogenesis, we can study the impact of physical architecture on collective cell migration in vivo. We observe extracellular gaps within the egg chamber and hypothesize that they directly affect the migration behaviors of the border cells, potentially by disrupting the local distributions of secreted chemoattractants. In silico, our lab has demonstrated these gaps affect the distribution of the morphogen that specifies border cell fate and preliminary results in vivo has shown that diffusible chemical signals concentrate between cells at potential gaps. Therefore, the underlying tissue architecture might affect chemical distribution. Additionally, genetically inducing a uniform concentration of the potent chemoattractant, Platelet Derived Growth Factor/Vascular Endothelial Growth Factor (PVF1), in the egg chamber stalled border cells at what may be an extracellular gap between cells. Furthermore, we are using mutant fly lines that perturb the egg chamber's innate physical architecture and lines that manipulate the chemoattractant gradient shape to evaluate the impact on border cell migration. This study identifies a role for surrounding tissue architecture in affecting collective cell migration and aims to explore how this can alter the distribution of chemical cues.

1816A ReepA is Required for Endoplasmic Reticulum Clearance from Chromosomes but not Endoplasmic Reticulum Partitioning to Spindle Poles in Dividing *Drosophila* Cells *Darya Karabasheva*[1], Jeremy Smyth[1] 1) USU.

The endoplasmic Reticulum (ER) is essential for biogenesis of lipids, proteins, and steroid hormones, as well as calcium signaling. The ER cannot be formed *de novo* and must be inherited during cell division. In animal cells, ER inheritance depends on partitioning of the organelle to the poles of the mitotic spindle via direct association with astral microtubules. Therefore, defining the specific mechanisms that link the ER to spindle microtubules is essential to our understanding of developmental and physiological processes that depend on ER functions. It was recently shown in mammalian cells that Receptor Expression Enhancing Proteins (REEPs) 3 and 4 are required for clearance of the ER from chromosomes in mitotic cells, via a mechanism that involves direct association of the ER with spindle microtubules and focusing of the ER around spindle poles. Thus, in addition to ensuring exclusion of the ER from nuclei, REEP proteins may also play a role in microtubule and spindle pole-dependent ER inheritance in dividing cells. To test this in an animal model, we generated *Drosophila* with a targeted deletion of the *REEPA* gene, the fly orthologue of human REEPs 3 and 4. *REEPA* homozygous mutant flies were fully viable and fertile, suggesting *REEPA* is not essential for normal animal development or overall physiology. *In vivo* analysis of syncytial embryonic nuclear divisions and dividing spermatocytes revealed that ER partitioning to spindle poles and association with astral MTs were largely unaffected in *REEPA* mutants, suggesting ReepA is not required for ER inheritance in these tissues. Surprisingly, however, close examination revealed abnormal strands of ER within the spindle, extending from the spindle poles towards aligned chromosomes, in both *REEPA* mutant embryos and spermatocytes. We also observed abnormal accumulations of ER that were associated with chromosomes in interphase nuclei of *REEPA* mutant embryos and spermatocytes. These findings are consistent with results from human cells with both REEPs 3 and 4 suppressed, suggesting a highly conserved mechanism whereby REEP proteins are required to sequester ER membranes away from chromosomes during cell division and prevent ER entrapment in newly formed interphase nuclei. Importantly though, our findings also suggest that REEP proteins are not the key factors that universally associate the ER with spindle microtubules and ensure proper partitioning of the organelle to daughter cells.

1821C Regulation of Polyamine Transport by Chmp1 Overexpression in *Drosophila melanogaster* *Shannon Nowotarski*[1], Coryn Stump[1], Erik Avis[1], Justin DiAngelo[1] 1) Pennsylvania State University Berks Campus.

Polyamines are small organic cations that are essential for a number of biological processes such as cell proliferation and cell cycle progression. High concentrations of polyamines are often associated with diseases like cancer. While the metabolism of polyamines has been well studied, the mechanisms by which polyamines are transported are poorly understood. Previous research has described lung carcinoma cells (H157), which are devoid of polyamine transport activity. It has been hypothesized that these cells exhibit high expression of Chmp1. Chmp1 has been shown to be involved in vesicular trafficking, which makes Chmp1 a valid potential player in the polyamine transport system (PTS). *Drosophila melanogaster* larvae were used in these experiments because the overexpression of Chmp1 in tissue culture was unsuccessful. In these studies, imaginal discs from wild type (WT) and Chmp1 overexpressing (o/e) larvae were harvested and incubated for 18 hours at 25°C in the presence or absence of 1µg/mL 20-hydroxyecdysone (hydroxy) as it is known that discs develop in the presence of hydroxy and not in its absence. In order to study whether Chmp1 overexpression regulates polyamine transport, a cytotoxic drug known to enter cells via the PTS, Ant44 (a generous gift from Dr. Otto Phanstiel) was added to the leg imaginal discs. It was proposed that if Chmp1 o/e down-regulates the PTS, then the addition of Ant44 would not inhibit leg development because it cannot enter the cell through the polyamine transporter. Polyamine rescue experiments were also performed in the presence of hydroxy and difluoromethylornithine (DFMO), an inhibitor of polyamine metabolism, to study whether the addition of different polyamines could rescue development of imaginal leg discs in Chmp1 o/e flies. It was proposed that if Chmp1 o/e down-regulates the PTS, then adding polyamines in the presence of hydroxy and DFMO would decrease leg development in Chmp1 o/e flies. If Chmp1 is found to be a down-regulator of the PTS, this may provide insight into the underlying mechanisms that are involved in polyamine transport. These studies are important because a better understanding of the players involved in the PTS could provide a vital target for cancer drug development.

1824C A novel role of presynaptic periactive zone proteins in extracellular vesicle trafficking Cassandra R. Blanchette[1], Amy L. Scalera[1], Zechuan Zhao[1], Kathryn Harris[2,3], Kate Koles[1], Anna Yeh[1], Julia K. Apiki[1], Bryan A. Stewart[2,3], Avital A. Rodal[1] 1) Brandeis University, Biology, Waltham, MA; 2) University of Toronto Mississauga, Biology, Mississauga, Canada; 3) University of Toronto, Cell and Systems Biology, Toronto, Canada.

Extracellular vesicles (EVs) are small, endosomally-derived, membrane bound vesicles that can transport protein, lipid, and nucleic acid cargoes

between cells, and are important for cell-cell communication in the nervous system. However, it remains unclear how specific endosomal cargoes are sorted to the EV pathway. Through a directed genetic screen, we identified an unexpected role for presynaptic periactive zone (PAZ) membrane remodeling proteins in regulating the traffic of EV cargoes at the *Drosophila* neuromuscular junction (NMJ). Mutants of synaptojanin, dynamin, endophilin, and the F-BAR/SH3 protein Nervous Wreck (Nwk) exhibit a local and dramatic decrease in the levels of the EV cargoes Synaptotagmin-4 (Syt4) and Amyloid Precursor Protein (APP) at presynaptic terminals. Interestingly, this novel EV cargo traffic defect is genetically separable from the well-established functions of PAZ proteins in synaptic vesicle recycling and synaptic growth. Further, this decrease is sufficient to abolish Syt4 function and reduce APP toxicity, suggesting that loss of specific EV cargoes may play unrecognized roles in canonical phenotypes of PAZ mutants. We restored EV cargo levels in *nwk* mutants by shifting EV cargo traffic to a recycling route, suggesting that PAZ membrane remodeling machinery may protect EV cargoes from a degradative fate, therefore allowing for their release in EVs.

1827C Importins regulating cytoplasmic histone dynamics in Drosophila Asmita Dutta[1], Roxan Stephenson[1], Jonathon Thomalla[1], Petra Kolkhof[2], Mathias Beller[2], *Michael Welte*[1] 1) Univ Rochester; 2) Heinrich Heine Univ Düsseldorf.

Following synthesis in the cytoplasm, histones are handled by an elaborate network of chaperones before being incorporated into chromatin. Because nuclear import is typically rapid, the earliest cytoplasmic steps are not well understood. However, for the maternally supplied histones of early embryos, synthesis and nuclear import occur during different developmental stages. In Drosophila, massive amounts of the histone variant H2Av are synthesized in nurse cells and oocytes and stored on lipid droplets (LDs), cytoplasmic fat storage organelles, via the LD protein Jabba. Storage is dynamic as H2Av constantly exchanges between LDs; after fertilization, these H2Av stores are used to package the newly synthesized DNA in the nuclei of the embryo. As mammalian importin-9 is cytoplasmic and can bind H2A/H2B dimers, we analyzed the contribution of its Drosophila ortholog Importin-9/Ranbp9 to H2Av dynamics. Using luciferase complementation, we find that Importin-9 can physically interact not only with H2A and H2B, but also H2Av. We compared H2Av dynamics in wild-type egg chambers and egg chambers lacking Importin-9, using H2Av-Dendra and Fluorescence Recovery After Photobleaching. In the mutant, the rate of recovery is much reduced, and similar to what is observed due to bulk motion of LDs, suggesting that Importin-9 mediates most H2Av transport through the cytoplasm. By luciferase complementation, we detect no interaction between Importin-9 and Jabba; however, there is a robust interaction between Jabba and Importin α2 (Impα2)/Pendulin. Impα2 is indeed recruited to LDs, in a Jabba-dependent manner, as determined by immunostaining and proteomic analysis. Intriguingly, Impα2 is largely cytoplasmic in early-stage embryos but becomes a major LD protein by the cellularization stage. Temporally, this relocalization correlates with Impα2 dephosphorylation as well as with a major transition in H2Av dynamics from rapid exchange between LDs to static LD binding. To test whether these processes are functionally related, we have generated a version of Jabba that normally interacts with LDs and H2Av, but fails to bind to Impα2. We are also testing whether a form of Impα2 that cannot be phosphorylated still interacts with Jabba. We will examine whether H2Av dynamics is altered in flies expressing these mutant constructs. In summary, we have identified two different importins as candidate regulators of cytoplasmic histone H2Av.

1903A Uip4, a novel endoplasmic reticulum protein, maintains nuclear shape and cellular homeostasis in *S. cerevisiae* *Pallavi Deolal*[1] 1) University of Hyderabad.

Nucleus is one of the most prominent cellular organelles and changes in the structural organization of the nucleus are known to be associated with ageing, several laminopathies and muscular diseases. Nucleus has multiple dynamic connections with other organelles of the cell and thereby influences a plethora of cellular processes. These include metabolite exchange, signal transduction, membrane re-modelling and autophagy-related processes. Proper coordination of function and efficient communication between the organelles is therefore essential for the maintenance of cellular homeostasis. In order to understand the nuclear organization and its communication with other organelles, genome–wide screen using a library of deletion mutants for non-essential genes in *Saccharomyces cerevisiae* was initiated in our lab. Loss of several non-nuclear resident proteins was found to affect the distribution of nuclear membrane proteins and nuclear pore complexes. One such uncharacterized endoplasmic reticulum (ER) resident protein, Uip4p, was studied for its effect on the assembly and distribution of nuclear pore complexes.
Our results suggest that Uip4p although is non-essential for survival, plays an important role in maintaining the integrity of yeast nucleus. Loss of Uip4p leads to nuclear blebbing and nuclear import defects. Further, over-expression of Uip4p results in severe mislocalization of several nucleoporins. This phenotype is dependent on nuclear protein quality control pathways. Our studies show that Uip4p localizes to NE/ER membrane in addition to being present in certain cytosolic foci. Additional biochemical characterisation shows nutrient-dependent expression and localization of Uip4p. Our findings related to the effect of altered levels of Uip4p on the organelle structure, function and homeostasis will be presented.

1904B Mitochondrial inheritance in *Saccharomyces cerevisiae* septin mutants Kala Anderson[1], Sophia Porras[1], Sonia Giyanani[1], Nisha Giyanani[1], Matthew Goldstein[1], *Patricia Melloy*[1] 1) Fairleigh Dickinson University.

Septins are scaffolding proteins, conserved from yeast to humans, forming a ring at the bud neck during cytokinesis in budding yeast. These proteins are known to act as key regulators of cytokinesis, including the distribution of macromolecules in the mother and daughter cell during cell division. Given the many roles of septins, we were interested in determining if they participated in organelle inheritance. In our study, we examined mitochondrial localization in wild type budding yeast cells as well as septin mutants including *cdc3*, *cdc11* and *cdc12*. Mitochondria were tracked at permissive and non-permissive temperature using a fluorescent mitochondrial marker. In addition, we used a septin mutant with a GFP-tagged septin marker along with the mitochondrial staining for time-lapse analysis. Cells were observed in G1, S, G2 and M phase and the mitochondrial distribution and appearance were recorded at these stages. At non-permissive temperature, in all three mutants, a change in the mitochondrial localization and appearance was observed along with the expected abnormal bud phenotype and failure of cytokinesis. More punctate spots of mitochondria were visible near the edges of the cells than at room temperature. In addition, these spots were brighter than those spots seen at room temperature or in the wild type cells. The spots were similar in appearance to those seen in an *mgm1* mitochondrial fission mutant using the same fluorescent marker, indicating that the brighter spots could be mitochondrial fragments. The abnormal mitochondrial spot pattern suggested that the typical filamentous network of mitochondria was disturbed in these septin mutants. Studies are underway to compare the mother to daughter mitochondrial distribution in wild type and septin mutants. Defects in mitochondrial inheritance and function have been linked to several neurodegenerative diseases, so we hope that our studies will provide a better understanding of the control of mitochondrial inheritance.

1911C Investigating the AP3 complex: linking a rare syndrome to an ancient molecule *Amanda Bentley-DeSousa*[1, 2], Michael Downey[1, 2] 1) Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada; 2) Ottawa Institute of Systems Biology, Ottawa, ON, Canada.

Polyphosphates (polyP) are long chains of linked inorganic phosphates ranging from 3-1000s of residues in length. They are found in all organisms

and play critical roles in a range of functions including blood clotting and bacterial virulence. Given its diverse roles, polyP is an attractive target for novel therapies. Despite a century of polyP research, there are no uncovered polyP synthetases in mammals – one of the largest challenges to studying polyP in mammalian systems. In yeast, however, polyP is synthesized by the vacuolar transporter chaperone (VTC) complex. This complex is comprised of 4 subunits: Vtc1, Vtc2/3, Vtc4, and Vtc5.

An open question in the field of yeast polyP biology is: How is the VTC complex regulated? Uncovering the molecular details behind its regulation is the focus of my current work. One complex already known to be important for polyP regulation is the conserved heterotetramer Adaptor Protein 3 (AP3) complex that is responsible for protein transport throughout the cell to acidocalcisomes (vacuoles/lysosomes). Interestingly, AP3 complex dysfunction results in decreased polyP levels in yeast and mammals. Mutations in the AP3 in humans give rise to Hermansky-Pudlak Syndrome; a rare genetic disease largely characterized by bleeding diathesis and albinism.

Given these strong links, I hypothesize that the AP3 complex is required for proper VTC localization and protein levels, which in return maintains polyP homeostasis. Current data supports this hypothesis wherein AP3 complex knock-out results in 3 major phenotypes: 1) a severe mislocalization of Vtc5, 2) decreased VTC complex subunit protein levels, and 3) an increase in free GFP:GFP-VTC levels, suggesting the activation of protein degradation. Overall, this suggests AP3 is required for the proper localization and stability of the VTC complex. Moreover, preliminary data suggests that the degradation of Vtc5, in cases of AP3 knock-out, is mediated by the Endosomal Sorting Complexes Required for Transport (ESCRT) pathway. Since both the AP3 and ESCRT transport pathways are conserved between yeast and mammals, elucidating the molecular pathways involved in polyP storage in yeast may ultimately allow us to uncover mammalian proteins critical for polyP synthesis.

2180B Recruitment Mechanism of DNA Polymerases during Homologous Recombination Meaghan Dineen[1], Daniel Kane[1] 1) Le Moyne College.

Unrepaired DNA damage can be detrimental to the integrity of a cell's genomic information leading to diseases and disorders. In particular, double strand breaks (DSBs) are problematic lesions as large amounts of DNA information can be lost. Faithful recovery involves novel synthesis by homologous recombination (HR). Previous research suggests that multiple DNA polymerases may be involved in the recovery synthesis of deleted genetic code during DSB repair. In Drosophila, we have previously used a transposable element excision assay that generates a large 14 kilobase gap to demonstrate a competition between replicative and translesion DNA polymerases for HR repair synthesis. Currently, we are attempting to elucidate mechanisms of DNA polymerase recruitment and coordination at the site of DNA breaks and ultimately polymerase choice during DNA synthesis. We are examining three Drosophila mutants: two mutants eliminating various clamp subunits (*hus1* and *pcna2*) and one polymerase mutant with a deleted C-terminal domain (*rev1ΔCTD*). Of particular interest, preliminary data suggests in the absence of Hus1, full gap repair of the excision event by HR is decreased, with a corollary increase in aborted full HR repair events. This phenotype is distinct from other checkpoint mutants examined in this particular genetic assay. We will be examining these aborted HR events further to determine if, in the absence of Hus1, synthesis tract lengths are reduced. Such a phenotype would indicate whether Hus1 (or other proteins of interest) are serving as a mechanism of DNA polymerase choice during homologous recombination in DSB repair, facilitating synthesis of longer repair tracts with replicative polymerases or shorter repair tracts with translesion polymerases.

2200A Recombination Rate Plasticity in *Drosophila pseudoobscura* *Laurie Stevison*[1], Huma Altindag[1], Hannah Taylor[1], Anna Tourne[1], Adam King[1] 1) Auburn University.

For over a century, recombination rates have been demonstrated to be plastic due to a variety of intrinsic (e.g. age) and extrinsic (e.g. temperature) variables. Here, we use X-linked phenotypic mutants *cut*, *scalloped*, *yellow*, and *sepia* in *Drosophila pseudoobscura* to identify the peak timing of plasticity due to temperature stress (26°C) during development. Further, we investigated the impact of female age on meiotic recombination rate in this system. Our experimental approach used a backcross design, crossing to wildtype to avoid fitness effects of the mutant markers. Control females were aged 7 days to sexual maturity and reared at 21°C. Recombination rate measurements targeted the first 12 days after females were mated, with females transferred every 72 hours and limited to a single mating event to avoid additional stress from male harassment. For the age treatment, a survivorship assay was used to determine an appropriate age for observing age-related plasticity. We selected the age of 35 days, which corresponds to senescence across 32.6% of replicates. For both treatments, we observed a significant effect on overall fecundity (p_{age}= 0.00115; p_{temp}=0.00144) indicative of a fitness effect of our treatments. For temperature stress, we first identified a peak of plasticity 7-9 days post-mating. Using subsequent 24 hour transfers for only 6-10 days post-mating, we narrowed this peak to 9 days post-mating (p=0.00824). This timepoint had an average of 14.54% difference in recombination rate across the three intervals (N_{21}=3424; N_{26}=2082). This result is critical for further experimentation in this system, which is ongoing. We also found an average of 15.73% difference in recombination rate due to age over multiple intervals and timepoints (N_{7}=6217; N_{35}=4506). This result suggests that age permanently alters recombination in a manner distinctly different from temperature stress. Interestingly, we observe plasticity in intervals that are <50cM (y-sd; sd-ct), as well as intervals >50cM apart (y-se), suggesting that plasticity can be detected across large portions of the chromosome. Finally, our results show a large variance in recombination rates among replicates in both control and treatment, but especially in the treatments. This variance bolsters the support for necessary replication in experimental designs studying recombination rate variation more broadly across systems.

2220C Fine scale variation in the recombination landscape of adaptively diverging threespine stickleback fish *Vrinda Venu*[1], Enni Harjunmaa[1], Andreea Dreau[1], Felicity Jones[1] 1) Friedrich Miescher Laboratory of the Max Planck Society.

Meiotic recombination is one of the major molecular mechanisms generating genetic diversity and influencing genome evolution. By shuffling allelic combinations, it can directly influence the patterns and efficacy of natural selection. The rate and the placement of recombination varies substantially within the genome, among individuals, sexes, and different species. Using the threespine stickleback fish, an evolutionary model organism, we investigated the extent and molecular basis of recombination variation to further understand its evolutionary implications. We used both ChIP-sequencing and whole genome sequencing of pedigrees to empirically identify and quantify double strand breaks (DSBs) and meiotic crossovers (COs). High-resolution maps of crossover events were constructed for 36 individuals of diverging marine and freshwater ecotypes and their hybrids. We produced the first genome-wide high-resolution sex-specific and ecotype-specific maps of contemporary recombination events in sticklebacks. We report striking differences in the crossover number and placement between sexes, with male crossovers occurring predominantly near the chromosomal periphery and female crossovers distributed throughout the chromosome. Under conditions of on-going gene flow between diverging forms, theory predicts natural selection will favor recombination modifiers that suppress recombination. In this study, we find empirical support

for a reduction in overall recombination rate in hybrids compared to pure forms – a phenomena that is stronger in females. Further we see that, even though the loci underlying freshwater-marine adaptation fall in regions of low recombination, females recombine considerably in the regions between adaptive loci. This suggests that the sexual dimorphism in recombination phenotype may have important evolutionary implications. The fine scale recombination landscape is highly non-uniform with both recombination hot- and cold-spots. Male crossover landscape broadly mirrors DSB landscape and both DSB as well as crossover hotspots are significantly associated with open chromatin regions like transcription start sites. Whereas, female crossovers do not show an association more than expected by chance. A substantial number of hotspots away from open chromatin marks suggests the possibility of additional novel mechanisms of recombination regulation in sticklebacks. Overall, our study provides insight into the pattern and molecular regulators of recombination variation in the context of adaptive divergence and speciation.

2221A Genetic variation in recombination rate in the pig *Martin Johnsson*[1,2], Andrew Whalen[2], Roger Ros-Freixedes[2,3], Ching-Yi Chen[4], William Herring[4], Gregor Gorjanc[2], Dirk-Jan de Koning[1], John Hickey[2] 1) Swedish University of Agricultural Sciences; 2) The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh; 3) Departament de Ciència Animal, Universitat de Lleida-Agrotecnio Center; 4) Genus PLC.

Recombinations are not uniformly distributed along chromosomes, and this gives rise to a variable recombination rate landscape. Understanding local recombination landscapes and variation in recombination rate can yield insights into the genetic history of a species and may enable increased genetic gain. We used multilocus iterative peeling to estimate recombination rate in 150,000 pigs across 9 different breeding lines, enabling quantitative genetics and genome-wide association of recombination rate. We found that the recombination landscape was repeatable between lines, and at the same time, the lines showed differences in genome-wide recombination rate. The heritability of genome-wide recombination was low but non-zero, and we found three genomic regions associated with recombination rate, one of them harbouring *RNF212*, previously associated with recombination rate in several other species.

2228B Understanding the role of sumoylation in mitotic progression Tori Valachovic[1], *Yee Mon Thu*[1] 1) Allegheny College.

Ensuring the integrity of genome across multiple generations is vital for the success of all organisms. Cells have evolved diverse molecular mechanisms that resist deleterious changes at the nucleotide level, such as point mutations, as well as at the structural level, such as chromosome loss or translocation. One such molecular mechanism is protein sumoylation, a process in which concerted action of E1, E2 and E3 ligases facilitates the covalent linkage of small ubiquitin-like modifiers (SUMO) peptide to target proteins. In *Saccharomyces cerevisiae*, Siz1, Siz2 and Mms21 are the only three E3 SUMO ligases known to date. Functional deficiency of each ligase renders yeast cells sensitive to DNA damaging agents or prone to chromosome instability, implicating the general role of sumoylation in intracellular communication when genome integrity is compromised. Yet, how sumoylation regulates the function of specific proteins remains less understood. Our study dissects the effect of Mms21-dependent sumoylation on mitosis. In *S. cerevisiae*, Bir1 and Sli15, subunits of chromosomal passenger complex (CPC) have been reported to be potential targets of Mms21. During mitosis, CPC activates the spindle assembly checkpoint (SAC) until bi-orientation is achieved. Proper amount of tension between two sister chromatids serves as a molecular indicator of successful bi-orientation. Lack of tension may result from incorrect attachment of mitotic spindles to the chromosome or unresolved replication intermediates resulting from DNA damage accumulation. Bir1 is thought to sense tension, or the lack thereof, subsequently regulating the SAC. Intriguingly, a decrease in sumoylation of Bir1 or Sli15 coincides with SAC deactivation when replication is challenged by a genotoxic agent, methyl methanesulfonate (MMS). The existing evidence led us to hypothesize that Mms21-dependent sumoylation of Bir1 or Sli15 regulates SAC activation when the genome is damaged. Our preliminary data suggests that, when treated with MMS, cells with defective Mms21 E3 SUMO ligase experience less mitotic delay, compared to the wild-type. This data implies that Mms21 sumoylation of Bir1 or Sli15 promotes SAC activation. Nevertheless, cells in which Bir1 cannot be sumoylated do not exhibit any sensitivity to MMS. Our goal is to determine if Mms21-sumoylation truly regulates SAC activation in the presence of DNA damaging agents and if redundant pathways are responsible for the lack of phenotype in Bir1 SUMO mutants.

2243B PCNA promotes cohesion establishment in a context-dependent manner *Caitlin Zuilkoski*[1], Robert Skibbens[1] 1) Lehigh University.

Cellular genomes undergo various structural changes that include *cis* tethering (the tethering together of two loci within a single DNA molecule), which occurs during DNA condensation and transcriptional activation, and *trans* tethering (the tethering together of two DNA molecules), which occurs during cohesion establishment and DNA repair. The protein complex termed cohesin promotes both *cis* and *trans* forms of DNA tethering, but these cohesin functions appear to occur in both temporal and context-specific regulated mechanisms. For instance, cohesion establishment occurs in the context of the DNA replication fork in which PCNA recruits Eco1 to convert chromatin-bound cohesins to a tethering competent state. In support for this model, elevated levels of PCNA rescue cell viability and cohesion defects in *eco1* mutant cells.

Here, we test whether Eco1-dependent chromatin condensation is also promoted in the context of this replication fork component. Our results reveal that overexpressed PCNA does not promote DNA condensation in *eco1* mutant cells, even though levels of Smc3 acetylation are increased. In combination, our data suggests that one cohesin population promotes sister chromatid cohesion in context of the DNA replication fork, whereas an alternate cohesin population participates in chromatin condensation outside the context of the DNA replication fork.

2257A Examination of the DNA damage checkpoint in *Candida glabrata* *Erika Shor*[1,3], David Perlin[1,2] 1) Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ; 2) Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC; 3) School of Health and Medical Sciences, Seton Hall University, Nutley, NJ.

Budding yeast *Candida glabrata* is an opportunistic pathogen closely related to *Saccharomyces cerevisiae* and a leading cause of life-threatening invasive fungal infections. The prevalence of *C. glabrata* infections has been rising, and it is now the second leading cause of bloodstream candidemias in North America and Europe. *C. glabrata* rapidly develops resistance to antifungal drugs and exhibits high genomic heterogeneity among clinical isolates in terms of both nucleotide polymorphisms and chromosomal rearrangements, suggesting that this organism can rapidly generate and tolerate high levels of genetic change. The mechanisms underlying this genetic flexibility are still unclear. In the closely related *S. cerevisiae*, DNA damage checkpoint pathways are critical for maintaining a stable genome. Whether analogous checkpoints are active in *C. glabrata* and function to preserve genome integrity in response to DNA damage is not known. We began investigating the DNA damage checkpoint in *C. glabrata* by analyzing DNA damage-induced phosphorylation of the highly conserved effector kinase Rad53, whose phosphorylation and activation in other organisms help maintain genome stability in the presence of DNA damage. Western blotting and mass spectrometry analysis showed that, although DNA damage induces the expected robust phosphorylation of histone H2A (known as γH2A.X) in *C. glabrata*, Rad53 phosphorylation is greatly reduced in *C. glabrata* compared to *S. cerevisiae*. We also analyzed the *C. glabrata* cell division cycle in the presence of DNA damage and found that

many cells continue to divide under these conditions and that these divisions give rise to cells with aberrant DNA content. Finally, we analyzed DNA damage-induced transcriptomic changes in *C. glabrata*. Together, results from these studies indicate that DNA damage-induced checkpoint activation is attenuated in *C. glabrata*, suggesting a possible molecular mechanism for rapidly generating genetic change, including antifungal drug-resistant mutations, in this organism.

2258B Investigating the role of protein sequestration as a response to DNA damage *Arun Kumar*[1,2], Veena Mathew[1], Peter Stirling[1,2] 1) BC Cancer Agency, Vancouver, Canada; 2) Department of Medical Genetics, University of British Columbia, Vancouver, Canada.

Maintaining proteostasis is essential for cell survival, especially under stressed conditions. In case of DNA damage, the cell mounts a DNA damage response (DDR) which is deployed by proteins. It is therefore in the interest of the cell to avoid misfolding and mislocalization of important proteins during this response. This is achieved through an intricate network of protein quality control (PQC) circuits that aim to refold, degrade or sequester misfolded proteins. Recent studies have unveiled a new PQC circuit wherein, upon genotoxic stress, numerous proteins get sequestered to a quality control site in the nucleus called the Intranuclear Quality control site (or INQ). Here, we use *Saccharomyces cerevisiae* as our model system to establish Rpd3, a histone deacetylase, as an INQ marker and study the role and regulation of sequestration of Rpd3 to INQ. Furthermore, we try to dissect the initial signals upon DNA damage that lead to INQ formation. The work presented aims to shed new light upon this uncharacterized DNA damage stress response and establish a new role for Rpd3 in DDR.

Thursday, April 30 12:00 PM - 3:00 PM

Developmental Genetics 1 - Poster Q&A 1391B The role of sumoylation during vulval morhpogenesis and anchor cell invasion *Aleksandra Fergin*[1], Gabriel Boesch[1], Evelyn Lattmann[1], Charlotte Lambert[1], Alex Hajnal[1] 1) University of Zürich.

The sumoylation pathway is involved in a variety of processes in *C. elegans*, including gonadal and vulval fate specification, cell cycle progression, maintenance of chromosome structure, and chromosome segregation. In a targeted RNAi screen to identify genes regulating cell invasion, we have found that several components of the sumoylation pathway, such as the E2 SUMO-conjugating enzyme *ubc-9*, the SUMO E3 ligase *gei-17* or the SUMO ortholog *smo-1*, are required for the normal anchor cell (AC) invasion. Many transcription factors are known to be sumoylated during the epithelial to mesenchymal transition (EMT) in mammalian cells, highlighting the importance of the sumoylation pathway during cell invasion. To study how the loss of protein sumoylation affects vulval development and AC invasion, we developed tools to block sumoylation in a tissue-specific and temporally controlled manner. For this purpose, we have been using the auxin-inducible tissue-specific protein degradation system to down-regulate a degron-tagged SUMO E3 ligase (GEI-17) or a degron-tagged SUMO (SMO-1) in different tissues of the worm. We generated transgenic animals expressing a modified *Arabidopsis thaliana* TIR1, an E3 ubiquitin ligase specific for the degron tag, under the VPC specific *bar-1* and the AC specific *cdh-3* promoters. The degradation of GEI-17 in the different somatic tissues caused defects in the AC positioning, AC polarity, basement membrane (BM) breaching, and dorsal lumen formation. We also observed that degradation of GEI-17 in the VPCs results in altered cell fate specification. Moreover, tissue-specific degradation of SMO-1 in the VPCs resulted an almost 100% penetrant protruding vulva phenotype (Pvl), while degradation of SMO-1 in the AC caused only in 7% of the animals a Pvl phenotype. In further experiments we will examine if specific transcription factors, such as LIN-1 ETS, EGL-38 PAX2 or FOS-1, require sumoylation for their normal functions during vulval morphogenesis and the AC invasion.

1392C EXC-4/CLIC proteins are conserved regulators of heterotrimeric G-protein-Rac signaling *Anthony Arena*[1], Daniel Shaye[1] 1) University of Illinois - Chicago.

The *C. elegans* excretory canal (*ExCa*), a single-cell tube, is a tractable model to study tubulogenesis. Several conserved proteins required for *ExCa* tubulogenesis also play a role in angiogenesis. One example is EXC-4, a *C. elegans* ortholog of the Chloride Intracellular Channel (CLIC) family of proteins. After the initial discovery that *exc-4* regulates *ExCa* tubulogenesis, others showed that human CLIC1 and CLIC4 function in endothelial cells to promote migration, growth, and tube formation. EXC-4 is localized to the plasma membrane in the *ExCa*, and this localization is critical for its function. In mammalian cells, CLIC1 and CLIC4 are cytoplasmic; however, they are recruited to the plasma membrane upon activation of the S1P family of receptors (S1PRs), which are potent regulators of angiogenesis that act through G_{α}i, G_{α}12/13, RhoA and Rac1. The connection between regulation of CLIC localization and S1PR activity led us to hypothesize that CLICs have a conserved function in G_{α}-Rho/Rac signaling in *C. elegans* and in endothelial cells. We isolated and characterized a new loss-of-function *exc-4* allele, which has allowed us to define genetic interactions between *exc-4/CLIC* and other genes in *ExCa* tubulogenesis. Using this new allele, we found that *exc-4* genetically interacts with *gpa-12/*$G_{\alpha 12/13}$, *egl-30*/$G_{\alpha q}$, *gsa-1*/ $G_{\alpha s}$, and two worm Rac orthologs, *ced-10* and *mig-2*, implicating G_{α}-Rac signaling in *ExCa* tubulogenesis and establishing that EXC-4 interfaces with this pathway in *C. elegans*. Similarly, our collaborators work in endothelial cells indicates that CLIC1 and CLIC4 are required for S1P-induced Rac1 activation and Rac1-mediated cellular behaviors. These results define a new and conserved function for EXC-4/CLIC proteins in G_{α}-Rac signaling.

1394B The RAP-2 Small GTPase and MIG-15 MAP4 kinase promote tertiary fate in *C. elegans* VPC Patterning Razan A. Fakieh[1], Hannah Shin[1], David J. Reiner[1] 1) Institute of Bioscience and Technology, Texas A&M Health Science Center, Houston TX..

During *C. elegans* development, graded EGF signal from the anchor cell (AC) induces the six equipotent vulval precursor cells (VPCs) to assume a pattern of 3˚-3˚-2˚-1˚-2˚-3˚ cell fates. The VPC closest to the AC is induced via the Ras-Raf-MEK-ERK MAP kinase cascade to assume 1˚ fate. Presumptive 1˚ cells generate DSL ligands to induce the two neighboring cells via the Notch receptor to assume 2˚ fate. 1˚ and 2˚ developmental programs have been shown to be mutually antagonistic. Our lab showed that lower EGF dose causes Ras to switch effectors, from Raf to RalGEF-Ral, which functions to promote 2˚ fate in support of Notch. We further showed that Ral signals through GCK-2, a member of the Ste20 family of mitogen-activated protein kinase kinase kinase kinases (MAP4Ks), to trigger a p38 MAP kinase cascade to promote 2˚ fate (Shin *et al.*, 2018). 1˚ and 2˚ cells execute distinct and stereotyped division patterns to form the vulva. In contrast, 3˚ fate is typically referred to as “ground,” “uninduced,” or “passive:” 3˚ cells divide once and fuse with the surrounding epithelium. We have found that a paralog of GCK-2, MIG-15, also plays a role in VPC patterning. Upon mutation or RNAi depletion of MIG-15, we observed an increase in ectopic 1˚ as well as ectopic 2˚ cells. MIG-15 is also required for expression of a putative cell fate reporter in 3˚ cells. Thus, we hypothesize that, like 1˚- and 2˚-promoting signals, 3˚-promoting signals antagonize other vulval cell fates. Using CRISPR-Cas9, we engineered an insert of fluorescent protein and epitope tag into the 5’ end of the endogenous *mig-15* gene. We also inserted auxin inducible degron (AID), which mediates conditional degradation of tagged proteins. We will use complementary degradation

experiments and tissue-specific transgenic rescue to test whether MIG-15 functions in the VPCs to correctly pattern their fates. We will also use CRISPR to mutationally activate MIG-15, as we did previously with the paralogous GCK-2. Preliminary data suggest that RAP-2 functions similarly to MIG-15, and RAP-2 has been shown to activate MIG-15 in other systems. We hypothesize that RAP-2-MIG-15 promotes 3° fate, counter to the notion of 3˚ fate as "uninduced." Our work positions us to explore signals that promote non-specialized epithelial fate and perhaps sheds light on the relationship of cancers and surrounding stromal cells.

1396A Identifying new functions of the *lin-3 egf/ let-23 egfr* pathway through tissue-specific recombination *Silvan Spiri*[1], Louisa Mereu[1], Alex Hajnal[1] 1) Universitiy of Zurich.

Epidermal growth factor (EGF)-induced activation of EGF receptor tyrosine kinases is associated with diverse processes during animal development and adulthood. Alterations of the *egfr* signaling network and its involvement in the progression of various types of human cancers are intensely studied. *lin-3* and *let-23* are the only *C. elegans egf* and *egfr* homologs. Thus, *C. elegans* provides a simple *in vivo* model to investigate *egfr* signaling without the possible redundancy of additional *egf*ligand or receptor genes.
Our goal is to systematically identify tissue-specific functions of LIN-3 and LET-23 at different stages of development. The best-characterized function of LIN-3 is during vulval development, when the gonadal anchor cell (AC) releases LIN-3 to induce the primary (1°) cell fate in the adjacent vulval precursor cell (VPC) P6.p. However, after vulval induction during morphogenesis *lin-3* is not only expressed in the AC but also in the inner-most 1° VulF cells. *lin-3* expression in VulF is necessary to specify the uv1 cells in the ventral uterus, and possibly also other cell fates. Using an endogenous *let-23::gfp* reporter, we observed LET-23 expression in the AC from the mid-L3 stage on. Due to the essential roles *lin-3* and *let-23* play during early larval development and vulva induction, it is challenging to investigate their later functions during vulval morphogenesis. To circumvent this, we have inserted flippase reconition target (FRT) sites into the *lin-3* and *let-23* loci to generate conditional knock-out alleles through flipase-induced recombination. With this approach, we can manipulate the two essential genes in a precise spatial and temporal manner. Both FRT alleles exhibit the known vulvaless phenotypes upon tissue-specific flipase expression. Using these tools, we are currently examining the effects of deleting LIN-3 function in the VulF cells and LET-23 activity in the AC. Our findings so far show that *lin-3* and *let-23* are required for the precise alignment of the AC with the dorsal vulval lumen. Additionally, loss of *let-23* function in the AC prevents the assembly of an F-actin ring at the contact site between the AC and the VulF cells, resulting in abnormal dorsal lumen morphogenesis and an egg-laying defective phenotype in adults. We propose that LET-23 functions in the AC to stabilize F-actin assembly and form a robust connection between the uterus and the vulval epithelium.

1403B *Caenorhabditis* heterochronic genes: conservation and divergence of developmental roles *Maria Ivanova*[1], Eric Moss[1] 1) Rowan University, Graduate School of Biomedical Sciences, Stratford, NJ.

The nematode *Caenorhabditis elegans* has a genetic pathway that controls the timing of developmental events during the animal's larval stages. These are called 'heterochronic genes' for the fact that mutations in them cause alterations in the relative timing of developmental events, such as cell division patterns, differentiation, and morphogenesis. Some of these factors are conserved among animals and others are unique to nematodes. The nematode *Caenorhabditis briggsae* resembles *C. elegans* anatomically and developmentally, while the genetic investigation of *C. briggsae* shows a remarkable degree of functional divergence from *C. elegans* of some developmental programs.
We generated mutant alleles in *C. briggsae* using CRISPR-Cas9. *lin-28*, which encodes a conserved, specialized RNA binding protein, and *lin-46*, which encodes a protein-binding factor appears to exist only in nematodes of the genus *Caenorhabditis*. Loss of *lin-28* in *C. elegans* causes a "precocious" developmental phenotype, where developmental events of the second larval stage (L2) are skipped. A null allele of *C. elegans lin-46* causes a "reiterative" phenotype, where certain developmental events are repeated. In *C. elegans*, mutations in these genes supress each other since an animal mutant for both genes develops like wild type.
We were surprised to find that a null allele of *C. briggsae lin-28* does not have a heterochronic phenotype. For instance, the timing of "seam" cell divisions and differentiation, that are altered in *C. elegans*, appear normal in the *C. briggsae* mutant. The mutants do have abnormalities in the development of the egg-laying tissues, which are different from those of *C. elegans*. Soon after mutants reach adulthood they develop pathologies not observed in *C. elegans*: their movement and fertility decline, and their internal space fills with a mass of granules and vacuoles of presumably a gonadal origin.
In contrast, null alleles of *C. briggsae lin-46* show a developmental phenotype that resembles the *C. elegans* mutant. In particular, gaps in adult seam cell differentiation indicative of delayed differentiation.
We are continuing to characterize these and other alleles of heterochronic gene homologs and to examine their genetic interactions.

1414A Asymmetric Wnt signaling in *C. elegans* embryonic development and gene regulation Amanda L. Zacharias[1,2], Prativa Amom[1], Thomas M. Sesterhenn[1], Jeremy P. Crawford[1], Yannis Belloucif[1] 1) Cincinnati Children's Hospital Med Ctr, Cincinnati, OH; 2) University of Cincinnati College of Medicine, Cincinnati, OH.

Wnt signaling plays a key role in the development of the *C. elegans* embryo as well as the development of all metazoans. In *C. elegans*, the Wnt/β-catenin asymmetry pathway patterns almost all embryonic divisions after the first five, making it an ideal system to study the pathway and investigate how context controls which target genes are activated in particular cells. We previously utilized an automated lineage tracing approach to uncover enrichment of *sys-1*/β-catenin, a key Wnt pathway co-regulator, in cells that had a lineage history of consecutive Wnt signaling events. Current work in our lab aims to uncover both the mechanisms of this enrichment and its consequences for Wnt target gene regulation. We used the bipartite cGAL expression system to manipulate Wnt signaling during embryonic development in order to evaluate the current model for the Wnt/β-catenin asymmetry pathway. We mis-expressed Wnt ligand in the anterior of the early embryo to determine if nearby cells can be re-polarized. We found that ectopic Wnt expression induces abnormal division angles, similar to Wnt loss of function mutants, in both ligand expressing and non-expressing cells. This contributes to abnormal cell positions, but abnormal cell migrations also occur. We are currently investigating the effects of ectopic Wnt expression on β-catenin asymmetry and enrichment and on Wnt dependent transcription.
Another major interest of the laboratory is the structure of Wnt regulated enhancers. We are currently focused on identifying the co-regulatory transcription factors that work with *pop-1*/TCF to regulate the enhancers of *nob-1/php-3*, the homologs of *Hox12*. We have identified *ceh-13/Hox1*, *unc-62/Meis/hth*, and *elt-1/GATA* as regulators of distinct individual enhancers of *nob-1*, with additional evidence that *tbx-33/Tbx5* and *ceh-20/ceh-40/Pbx/exd* may also contribute. These results expand our understanding of the functions of Wnt signaling during development and expand the gene regulatory network of the early *C. elegans* embryo.

1417A Investigating the role of a Rac homolog in mitotic spindle orientation during asymmetric division in *Caenorhabditis elegans* embryos *Helen Lamb*[1], Malgorzata Liro[1], Lesilee S. Rose[1] 1) University of California, Davis.

Asymmetric cell division is important for generating cell type diversity during development and for tissue homeostasis. Cellular asymmetry can be generated either by internal or external cues. In many systems, pulling forces generated by a complex of dynein, LIN-5/Mud/NuMA, and the heterotrimeric G-protein $G\alpha_i$ orient the spindle along the anterior/posterior axis. In the one-cell embryo of *Caenorhabditis elegans*, the complex is asymmetrically localized by the G-protein regulator LET-99 in response to internal polarity cues. In contrast to the one-cell, the EMS cell divides asymmetrically in response to signals from the neighboring cell, P2. In this division, partially redundant Wnt and MES-1/SRC-1 pathways specify endoderm versus mesoderm fate and instruct the EMS mitotic spindle to orient along the anterior/posterior axis prior to division. We previously identified LET-99 and the dynein regulator LIN-5/Mud/NuMA as regulators of EMS spindle orientation. We have now identified CED-10, a homolog of the small G protein Rac-1, as a member of the MES-1/SRC-1 pathway. We hypothesize that MES-1/SRC-1 signaling regulates an antagonistic interaction between LET-99 and CED-10 to tune cortical branched actin levels and thereby regulate the localization of force generators. To test whether CED-10 acts upstream or downstream of SRC-1 activity, we stained for SRC-dependent phosphorylation at the P2-EMS contact. Preliminary results show that phosphorylation signal intensity is not significantly different between wild-type and *ced-10* mutant embryos. To determine whether branched actin is part of the MES-1/SRC-1 pathway, we depleted the actin nucleator Arp-2/3 by RNAi in a Wnt mutant background. Unexpectedly, we found that branched actin and Wnt signaling are required together during the earlier spindle orientation of the P1 cell, which is the precursor to both EMS and P2. We are currently generating strains to test whether P1 polarity is perturbed in *arp-2/3;wnt* embryos. We are also testing whether LET-99 affects CED-10 localization. Overall, this work will contribute to a more complete understanding of cytoskeletal regulation by cell-cell signaling during asymmetric cell division.

1482C Regulation of Hedgehog signaling and compartment-specific cell survival by membrane potential in the *Drosophila* wing disc *Maya Emmons-Bell*[1], Riku Yasutomi[1], Iswar Hariharan[1] 1) University of California, Berkeley, Berkeley, CA.

It is well known that the growth and development of multicellular organisms depends on cell-cell contacts, diffusible chemical signals, and patterns of physical force. However, relatively little attention has been paid to another core cellular parameter: the electrical potential across the cell membrane. We show that membrane potential is patterned in the *Drosophila* wing-imaginal disc. The wing disc is separated into two lineage-restricted compartments, the anterior (A) and posterior (P) compartment, which are separated by a compartment boundary, across which cells do not cross. We find that cells immediately anterior to the compartment boundary, a region of high Hedgehog (Hh) signal transduction, are more depolarized than their neighbors in the posterior compartment.

Patterned depolarization is due in part to enrichment of the epithelial sodium channel Ripped pocket (Rpk), whose expression anterior to the compartment boundary is both regulated by, and required for, normal Hh signal transduction. Hh signaling is dependent on the stabilization of Smoothened (Smo) at the plasma membrane. Increased depolarization of salivary glands and imaginal disc tissue increases the membrane abundance of Smo, while hyperpolarization dramatically diminishes it. Thus, Hh signaling and membrane depolarization are mutually reinforcing. High levels of Hh signaling increases Rpk expression, thus depolarizing cells anterior to the compartment boundary. Membrane depolarization, in turn, promotes Hh signal transduction by increasing Smo levels at the membrane. Preventing membrane depolarization in this region results in an irregular compartment boundary demonstrating the importance of this mechanism in segregating A and P cells.

We investigated the effect of manipulating membrane potential on cell survival and proliferation. When we generated clones overexpressing a depolarizing ion channel, clones were recovered preferentially in the A compartment, and eliminated in the P compartment via a mechanism reminiscent of cell competition. Conversely, clones of cells expressing RNAi against *rpk*, which are predicted to be more hyperpolarized than their neighbors, survive in the P compartment and are eliminated from the A compartment. These observations suggest that there is a different membrane potential that is optimal for cell survival in each compartment.

Thus, we show that membrane potential is an important parameter that regulates both growth and patterning in a developing tissue.

1483A *Early girl* is a novel component of the Fat signaling pathway *Jyoti Misra*[1], Kenneth Irvine[2] 1) University of Texas at Dallas; 2) Waksman Institute, Rutgers University.

During development, organs grow to achieve a consistent final size and shape, and this is achieved by precise coordination of growth and morphogenesis. The evolutionarily conserved Hippo signaling network plays a central role in organ size control, and when dysregulated can be associated with cancer and other diseases. The evolutionarily conserved protocadherins, Dachsous (Ds) and Fat constitute a signaling pathway that coordinates growth and morphogenesis by regulating the Hippo pathway and planar cell polarity (PCP) respectively. The atypical myosin, Dachs is a key downstream effector of Fat signaling that mediates both of these effects, and apical localization is critical for Dachs function. Fat regulates growth and PCP by modulating the levels and polarity of Dachs at the apical membrane. However, how Dachs localization is regulated is not well understood. A palmitoyl transferase, Approximated (App) is required for proper Dachs localization. But the exact mechanism by which it regulates Dachs is unknown. Recently, we identified a novel gene, Vamana (Vam), which encodes an SH3 domain containing adapter protein that plays a critical role in apical membrane localization of Dachs. Further, Vam functions as an adapter by physically connecting Dachs to Ds and Fat. To identify additional regulators of this signaling pathway, we conducted a genetic screen using RNAi targeted against the ubiquitin ligases encoded by the *Drosophila* genome and isolated a novel RING domain E3 ligase, Elgi, which when depleted results in tissue overgrowth and PCP defects, phenotypes reminiscent of mutations in Fat signaling. At the cellular level, depletion of Elgi results in significant increase in Dachs and Vam levels. Interestingly, expression of a dominant negative Elgi results in failure of Dachs and Vam to localize to the apical cortex and their accumulation in the cytoplasm in punctate structures. Similar accumulations are observed when Elgi and App are depleted simultaneously. Taken together, these results indicate that Elgi and app coordinately regulate the trafficking of Dachs and Vam, the downstream effectors to the apical membrane.

1494C Investigating growth regulation within synchronously developing epithelia *Sophia Friesen*[1], Iswar Hariharan[1] 1) University of California, Berkeley.

Animal development frequently requires multiple epithelia to grow in coordination with each other to produce functional multilayered organs. The

Drosophila wing disc, the precursor to the adult wing and thorax, consists of two closely associated epithelia - the disc proper (DP) and the peripodial epithelium (PE) - which grow synchronously during larval development. Studies of the disc proper have yielded valuable insights into growth regulation in epithelia, but little is known about how the two layers of the disc achieve coordinated growth. Two conserved morphogens, Hedgehog (Hh) and Decapentaplegic (Dpp/TGF-β), are crucial regulators of growth of the DP. With the goal of understanding how growth is coupled between the two epithelial layers, we have investigated the role of these two signals in the growth of the PE.

As in the DP, cells in the PE that are immediately anterior to the compartment boundary express *dpp*. In the DP, *dpp* is expressed in response to Hh, but it has previously been reported that *hh* is not expressed in the PE, raising the question of what activates *dpp* expression. Using a temperature-sensitive *hh* allele, we have shown that *dpp* expression is dependent upon Hh function. Moreover, knockdown of *smoothened* (*smo*) in the *dpp*-expressing cells severely impairs PE growth, indicating that these cells are indeed responding to activation of the Hh signaling pathway. Thus, although *hh* expression has not been visualized in the squamous cells of the PE, it is likely expressed in these cells at low levels.

Two Gal4 driver lines that allowed manipulation of gene expression in specific regions of the PE were used to knock down expression of the Dpp receptor *thickveins (tkv).* These experiments showed that, in contrast to the DP, Dpp signaling is dispensable for cell survival in the PE. Furthermore, when clones homozygous for a null allele of *tkv* were generated in the PE using mitotic recombination, mutant cells were still able to proliferate. Dpp signaling does, however, seem to be involved in PE function during morphogenesis, as peripodial *tkv* knockdown results in thorax defects consistent with impaired peripodial cell migration.

These results demonstrate that growth regulation within the PE does not operate as a simple parallel to growth in the DP, and suggests that alternative regulatory pathways may be more important in the PE. We are currently working to identify those pathways.

1527C The influence of shear forces on *Drosophila* ventral furrow formation *Amanda Goldner*[1], Konstantin Doubrovinski[1] 1) University of Texas Southwestern Medical Center, Dallas, TX.

In *Drosophila* embryos, gastrulation begins with ventral furrow formation (VFF): cells along the ventral midline constrict apically, lengthen laterally, and finally shorten back to their original length as the monolayered tissue invaginates to form the ventral furrow. The phenotype of this folding process is strongly dependent on the morphogenetic forces involved. Current understanding pinpoints apically-localized forces as the key player in VFF; however, theoretical analysis from our laboratory strongly suggests that folding is additionally driven by non-apical forces. We constructed a computational model of VFF based on physical parameters directly measured in our laboratory. Our model shows that furrow formation succeeds in the absence of basal membranes. If the level of cytoplasmic viscosity assumed in our model is decreased, lowering the amount of shear force exerted on membranes, the furrow fails to form. We hypothesize that viscous shear forces contribute to tissue invagination by pulling the ventral embryo surface inward. To test this *in vivo* we studied the furrow forming capabilities of anillin RNAi embryos, which exhibit delayed basal membrane formation. Anillin RNAi embryos are fully capable of VFF despite their lack of basal membranes. Neurotactin staining additionally reveals ventral cell lateral membranes disintegrate during late stages of VFF in anillin RNAi embryos. We plan to further analyze this phenotype using TEM and live imaging under 2-photon microscopy. We believe these shear forces should be considered one of the driving forces behind tissue folding in future models of gastrulation.

1532B Interactions between the *18-wheeler* gene and *X*-chromosome linked genes affect salivary gland development in *Drosophila melanogaster* *Jaquelyn Villalba*[1], Elizabeth Eldon[1] 1) California State University, Long Beach.

Drosophila melanogaster salivary gland development is a model to study tube morphogenesis, which serves an evolutionarily conserved role in compartmentalization of organ function. The *18-wheeler* (*18w*) gene found in *Drosophila melanogaster* directs epithelial cell migration during embryonic development and affects salivary gland morphogenesis. Homozygous *18w* mutant embryos have abnormal salivary glands. To identify genes interacting with *18w* during epithelial organ development we utilize the observation that embryos heterozygous both for *18w* and for a gene that interacts with *18w* produce defective salivary glands. We are systematically searching the *X*-chromosome for interacting genes using a collection of 93 *X*-chromosome-linked deficiencies (*Df(1)*). Together these deficiencies delete 2,288 of the 2,331 euchromatic genes on the *X*-chromosome (98.1%). To obtain embryos that are heterozygous for *18-wheeler* and an *X*-linked deficiency, males carrying an *18w* mutation and a green fluorescent protein (GFP) reporter expressed in salivary glands are mated with females heterozygous for an *X*-linked deficiency. Their other *X*-chromosome is a GFP-expressing “balancer”. Control embryos are obtained by using males that are wild type at the *18w* locus, but still carry the GFP salivary gland reporter. Embryos are collected, fixed, and subjected to immunocytochemistry to detect GFP, which is expressed in the salivary glands of the *Df(1)*; *18w* embryos. If the mutations interact, salivary gland morphogenesis will be abnormal. *Df(1)BSC719* shows an interaction, but not as predicted. When crossed to the *18w* mutant, wild type glands are observed, but when crossed to wild type *18w*, the glands show a defect in migration and development. This suggests reducing the dosage of the 18-Wheeler protein rescues defect. *Df(1)ED7374* also shows a genetic interaction. Embryos heterozygous for the both the deficiency and *18w* show abnormal salivary gland migration and morphology. Embryos heterozygous only for the deficiency have wild type glands. From these results we can conclude that *Df(1)ED7374* may contain candidate gene(s) interacting with *18-wheeler*. Current work focuses on mapping the location within *Df(1)BSC719* and *Df(1)ED7374* to identify the genes that interact with *18-wheeler* during salivary gland morphogenesis.

1545C Wound induced cell cycle regulation *James White*[1], Kimberly LaFever[1], Shane Hutson[1], Andrea Page-McCaw[1] 1) Vanderbilt University Nashville, TN.

Epithelial tissue is normally quiescent, stationary, and non-invasive, but following wounding this tissue dramatically changes its behaviors becoming proliferative, migratory, and invasive. In addition to proliferation, wounds induce other cell cycle behaviors such as G2 stalling and increased DNA content within single cells. However, the mechanisms by which these behaviors are induced and patterned are not known. To explore these questions, we induce laser wounds in the pupal notum, an epithelial monolayer that can be live-imaged throughout the repair process. Preliminary data from our lab using a cell cycle indicator fly (FUCCI) indicates that cells stall in the G2 stage of the cell cycle around wounds. This G2 stalling behavior has been previously reported to be JNK-dependent in imaginal wing discs wounded *ex vivo*. Our FUCCI data also reveals an apparent increase in nuclear volume close to the wound margin suggesting DNA content increases in this tissue. It has been reported that Yorkie, the fly homologue to Yap/Taz, and its downstream effectors drive increased DNA content through endocycling (re-replication without division) in response to wounds in

adult epithelium. To investigate DNA content increases in the Drosophila pupa we developed a novel dissection and fixation protocol allowing us access to this previously unstainable tissue. By combining *in vivo* live-imaging with our dissection and staining protocol of the pupal notum we aim to understand the wound-induced signals that pattern these cell cycle behaviors in the tissue surrounding a wound.

1551C Elucidating the Role of Uncharacterized Tin-Positive Pericardial Cells in Drosophila Heart Development *Bill Hum*[1], Marco Tamayo[1], Rolf Bodmer[1], Georg Vogler[1] 1) Sanford Burnham Prebys Medical Discovery Institute.

Drosophila is an excellent model system for studying developmental biology because of their small size, short generation time, the abundance of eggs laid, and the plethora of genetic tools for the manipulation of gene expression in time and space. In addition, many genes that cause human heart diseases are found in *Drosophila* and play similar roles in heart function and development. We are studying heart development at high spatio-temporal resolution in the fruit fly by single cell sequencing of cardiac cells and whole mount in situs by hybridization chain reaction. Specifically, we are focusing on novel genes involved in heart development in fly embryos to learn what stages of development these genes are expressed and, using mutational analysis, to what extent they are required for the formation and differentiation of cardiac tissue.

We have found new marker genes for a group of uncharacterized embryonic pericardial cells that express the cardiac master regulator Tinman (tin-positive pericardial cells, TPCs), but not Even- or Odd-skipped, the other markers for pericardial cells. These TPCs co-express a specific set of genes, including *cut*, *Wnt4*, *Nrt*, *Scb*, *CrzR*, and *DOR*. We are conducting immunofluorescent staining and imaging of fly embryos to visualize the protein and mRNA expression patterns of these marker genes in the entire embryo, the heart, and heart-associated tissues. In addition, we are characterizing these cells to learn about their cell-specific roles during heart development. For the purpose of studying these roles in heart development, we are knocking down or overexpressing them in the embryonic heart using heart-specific Gal4 driver lines and examining trans-heterozygous combinations of loss of function alleles for these genes.

By determining the gene regulatory program of the TPCs and characterizing in-depth the development and function of these pericardial cells, we hope to elucidate their role in *Drosophila* heart development.

1663A Cofactor-dependent and -independent functions of Hox reveal two distinct evolutionary lineages of insect wing tissues *Madison Moe*[1], Yoshinori Tomoyasu[1] 1) Miami University.

The acquisition of novel structures is critical in facilitating organismal adaptation. Insect wings are a classic example of such a novelty whose origin remains a widely debated mystery. Most extant insects possess two pairs of wings (on the second and third thoracic segments, T2 and T3), while T1 and the abdominal segments are wingless. Using the *Tribolium* beetle, we have shown that there are two types of tissues (pleural and tergal) in the wingless T1 segment that are serially homologous to the wing. The suppression of these wing serial homologs (WSHs) from forming wing tissue is achieved by the action of the Hox protein in this segment. Reducing Hox input in T1 allows the WSHs to merge and form an ectopic wing. These observations led us to propose that insect wings have evolved from a merger of two previously distinct tissues that are maintained in a more ancestral state, namely that insect wings have a dual evolutionary origin. Although this hypothesis could potentially unify the two currently competing wing origin hypotheses (i.e. pleural vs. tergal wing origin debate), it remains unclear how the two WSHs individually contributed to the evolution of the insect wing.
We reasoned that serial homology among WSHs can be further subdivided based on distinct modes of Hox action, allowing us to dissect the contribution of each WSH. We see an example of this distinction in the differentiation of the T3 wing in *Drosophila* (the haltere). The Hox gene acting in this segment (*Ubx*) controls the identity of the entire haltere, while the proximal region requires the action of the Hox cofactor complex, Homothorax/Extradenticle (Hth/Exd). This suggests that there are tissues which are Hox cofactor-dependent or -independent within the wing-related tissues including the WSHs. Here we investigated the Hox cofactor-dependence of the WSHs in *Tribolium* via RNAi for the cofactor genes. In T1 where the Hox gene *Scr* suppresses wing identity, we found that the pleural WSH is cofactor-dependent, while the tergal WSH is cofactor-independent in maintaining its ancestral state. In T3, the entire wing requires Ubx input, yet the proximal region also requires input from Hth/Exd. These results suggest that the pleural tissues contribute to the formation of proximal wing structures, while the more distal portion of the wing is formed from the tergal contribution. Our analysis will contribute in revealing the complex interactions that orchestrated the evolution of the insect wing.

1686C The MEK-ERK signaling pathway promotes maintenance of cardiac chamber identity *Yao Yao*[1], Deborah Yelon[1] 1) University of California, San Diego, CA.

The vertebrate heart is comprised of two types of chambers, ventricles and atria, which exhibit unique structural and contractile properties due to chamber-specific differences in gene expression. Effective cardiac function depends upon the distinct characteristics of ventricular and atrial cardiomyocytes. Intriguingly, despite the early specification of ventricular and atrial lineages, chamber identity needs to be actively reinforced even after myocardial differentiation is underway. Our prior studies in zebrafish have found that sustained FGF signaling is required for ventricular cardiomyocytes to maintain their ventricle-specific features: upon inhibition of FGF signaling, these cells extinguish expression of the ventricular gene *vmhc* and initiate ectopic expression of the atrial gene *amhc*. However, the genes that mediate ventricular maintenance downstream of FGF signaling remain unclear, especially since there are multiple effectors that are known to respond to FGF receptor activation. Here, we show that MEK-ERK signaling plays an important role in promoting ventricular identity maintenance. Inhibition of the MEK1/2-ERK1/2 pathway following cardiomyocyte differentiation results in ectopic *amhc* expression in the ventricle, as well as reduced *vmhc* expression in ventricular cells, similar to the effects of inhibition of the FGF pathway during the same timeframe. In contrast, inhibition of either the JNK or PI3K pathways fails to induce ectopic *amhc* expression within the ventricle. We also demonstrate that different portions of the ventricle exhibit differential degrees of plasticity: for example, upon MEK-ERK pathway inhibition, cells in the inner curvature of the ventricle readily initiate ectopic *amhc* expression, whereas cells in the outer curvature are more resistant. Together, our data suggest a model in which the MEK-ERK pathway acts downstream of FGF signaling to enforce the maintenance of ventricular chamber identity, yet not all ventricular cells require the same levels of MEK-ERK signaling. Our ongoing studies aim to identify the specific targets of MEK-ERK signaling in this context, as well as the factors that confer differential requirements for MEK-ERK signaling in distinct regions of the ventricle.

1687A Identification of two distinct pro-epicardial populations during development *Nathalia Holtzman*[1, 2], Sana Khan [1, 2] 1) Queens College; CUNY; 2) The Graduate Center; CUNY.

The epicardium is the outer most layer of the heart and plays crucial roles in cardiac development and cadiac wound healing. The epicardium arises from precursor cells in the proepicardial organ (PEO) that forms around the base of the cardiac inflow tract. The location and initial symmetry is conserved across species despite morphological differences. Three cellular mechanisms of proepicardial migration have been proposed: 1) The floating cyst model - PEO cells released into the pericardial cavity are directed by fluid movements to migrate onto the myocardium; 2) Villi transfer - cardiac contractions may mediate multicellular PEO villi contact to the myocardium; and 3) Tissue bridge-mediated transfer - PEO cells migrate along a bridge to contact the myocardium. Using zebrafish as a model organism, we explored the spatio-temporal differences in mRNA and transgenic reporter protein expression of conserved PEO genes *wilm's tumor 1 (wt1)* and *transcription factor 21 (tcf21)*. Our data suggest two distinct PEO populations: atrium-specific (A PEO) and ventricle-specific (V PEO). *Wt1* is expressed widely in PEO cells from 30 hours post fertilization (hpf), whereas *tcf21* is expressed in a subset of PEO cells around the inflow tract, in V PEO cells and not expressed in A PEO until 96 hpf. In addition, we detected PEO villi in close proximity to the ventricular myocardial surface. To demonstrate the cardiac contractility requirement for villi-mediated PEO migration, cardiac contractions were inhibited using two chemicals and one genetic mechanism. Our results indicate two distinct PEO cell migration mechanisms: 1) Atrium-specific migration is independent of cardiac contractions as cells migrate directly on the atrial myocardial surface. 2) Ventricle-specific migration is dependent on cardiac contractions. Our discernment of PEO subpopulations and chamber-specific migration mechanisms establishes context that may be helpful in our understanding of PEO migration regulation.

1688B The Smarcc1 subunit of the SWI/SNF chromatin remodeling complex is required to define the dimensions of the atrioventricular canal in the embryonic zebrafish heart *Ivy Fernandes*[1], Heidi Auman[2], César Berríos-Otero[2], Sophie Colombo[2], Deborah Yelon[1] 1) Division of Biological Sciences, University of California, San Diego; 2) Developmental Genetics Program and Department of Cell Biology, New York University School of Medicine.

The atrioventricular canal (AVC) is a specialized region of the heart that forms at the junction between the atrial and ventricular chambers and ultimately gives rise to the future atrioventricular valve. The proper differentiation of this region depends upon the restricted expression of a set of genes that are first broadly expressed throughout the linear heart tube and later become confined to the AVC. Despite the functional importance of AVC development, we do not fully understand the molecular mechanisms that pattern the heart tube and define the dimensions of the AVC. Here we show that the zebrafish gene *smarcc1a*, encoding a SWI/SNF-type ATP-dependent chromatin remodeling complex subunit homologous to mammalian BAF155, is critical for the establishment of correct AVC patterning. In *smarcc1a* mutant embryos, myocardial differentiation and the initial assembly of the heart tube appear to proceed normally. Subsequently, however, the *smarcc1a* mutant heart fails to exhibit the appropriate refinement of gene expression patterns. Multiple genes expressed in the myocardium, such as *bmp4*, as well as genes expressed in the endocardium, such as *notch1b*, fail to become restricted to the AVC and instead retain the broad expression pattern seen at earlier stages. The persistence of AVC gene expression within the cardiac chambers is accompanied by the failure of cardiac chamber expansion and maturation in *smarcc1a* mutants. These defects in cardiac patterning may originate with inappropriate regulation of *tbx2b* expression: whereas *tbx2b* normally serves as an early marker of the future location of the AVC, we find ectopic expression of *tbx2b* throughout the *smarcc1a* mutant heart tube. Together, our data support a model in which Smarcc1-containing chromatin remodeling complexes repress *tbx2b* expression in the cardiac chambers, thereby establishing the proper boundaries of the AVC.

1694B Characterizing intracranial lymphatic development in zebrafish *Daniel Castranova*[1], Bakary Samasa[1], Marina Venero Galanternik[1], Hyun Min Jung[1], Brant Weinstein[1] 1) Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD.

The recent discovery of meningeal lymphatics in mammals is reshaping our understanding of fluid homeostasis and cellular waste management in the brain. The optical clarity and experimental advantages of zebrafish have made this an essential model organism for studying lymphatic development, but the existence of meningeal lymphatics has not yet been reported in this species. Using high-resolution optical imaging of the meninges in living adult transgenic animals, we show that zebrafish possess a meningeal lymphatic network comparable to that found in mammals. Using vital staining of the skull we are able to distinguish between extracranial lymphatics and intracranial lymphatics in living zebrafish. We confirm that the meningeal lymphatic network is separate from the blood vascular network by injecting fluorescent quantum dots into the blood vasculature and demonstrating that they fail to enter the lymphatic network. We also show that meningeal lymphatic vessels drain interstitial fluid from the brain by injecting fluorescent quantum dots into the brain and observing their drainage through the lymphatic vessels. Ongoing experiments are aimed at mapping the full complexity of the adult zebrafish intracranial lymphatic network. This exciting new discovery opens up new avenues for experimental analysis of meningeal lymphatic development and meningeal lymphatic function as it relates to environmental and genetic insult, aging, and disease.

1695C Evidence for Conserved Mechanisms of Neurulation in the Zebrafish Forebrain *Dominique Brooks*[1], Allyson Caldwell[1], Jonathan Werner[1], Maraki Negesse[1], Rachel Brewster[1] 1) University of Maryland Baltimore County, Baltimore, MD.

The formation of the neural tube, the developmental precursor of the central nervous system, is facilitated by the bending and folding of the neuroepithelium; this process is termed neurulation. Neural tube defects (NTD) including exencephaly and anencephaly are one of the most common neural tube birth defects, which are primarily caused by improper neural tube formation. However, the underlying cellular and molecular mechanisms that drive neurulation still remain mostly unknown. With the emergence of genome sequencing and identification of genetic variants in patients with NTDs, there is a pressing need for identification of genetic risk factors. The morphogenesis of the neural tube is facilitated by the formation of hingepoints, which are subsets of neuroepithelial cells that undergo apical constriction to form a wedge shape. Apical constriction occurs when a cell acquires a molecularly defined apical surface through apically polarized tight junction molecules, such as zona occludens (ZO1) and PARD3, in addition to the recruitment of an actomyosin contractile ring. Apical polarity and apical constriction establishment are promising areas for NTDs research, as they are necessary for proper hingepoint formation. Live imaging of hingepoint dynamics would further advance our understanding of hingepoint formation; however, it is difficult to perform in traditional model organisms. In contrast, the transparency and early accessibility of zebrafish embryos make them amenable to time lapse microscopy. However, the hallmarks of primary neurulation, specifically hingepoints, have not been so far reported in the zebrafish neuroepithelium. Nonetheless, our work reveals cellular and molecular evidence for the presence of wedge-shaped, medial dorsolateral hingepoints within the forebrain tissue of the zebrafish. Using immunolabeling and confocal microscopy, we show the apical localization of apical polarity molecules, ZO1 and PARD3, in a cluster of medial, wedge-shaped cells in the anterior neuroepithelium. Furthermore, we reveal that disruption of the apical actomyosin contractile ring, using the myosin inhibitor blebbistatin, prevents apical constriction and impairs forebrain development. These findings provide evidence for the presence of hingepoints in zebrafish and highlight

the conservation of neural tube morphogenesis across vertebrates. Our findings pave the way for future investigations on the cellular and genetic basis of NTDs using the zebrafish as a model organism.

Thursday, April 30 12:00 PM - 3:00 PM

Developmental Genetics/Quantitative Genetics 1 - Poster Q&A 1309A Regulatory kinase genetic interaction profiles differ between environmental conditions and cellular states *Siyu Sun*[1], Anastasia Baryshnikova[2], Nathan Brandt[1], David Gresham[1] 1) New York University; 2) Calico Life Science LLC.

Cell growth and quiescence in eukaryotic cells is controlled by an evolutionarily conserved network of signaling pathways. Signal transduction networks operate to modulate a wide range of cellular processes and physiological properties when cells exit proliferative growth and initiate a quiescent state. How signaling networks function to respond to diverse signals that result in cell cycle exit and establishment of a quiescent state is poorly understood. We studied the function of signaling pathways in quiescent cells using quantitative genetic interaction mapping in Saccharomyces cerevisiae (budding yeast). We performed pooled analysis of genotypes using molecular barcode sequencing to test the role of ~4,800 gene deletion mutants and ~14,400 pairwise interactions between all non-essential genes and the protein kinases TOR1, RIM15, PHO85 in three different nutrient-restricted conditions in both proliferative and quiescent cells. We detect nearly five-fold more genetic interactions in quiescent cells compared to proliferative cells. We find that both individual gene effects and genetic interaction profiles vary depending on the specific pro-quiescence signal. The master regulator of quiescence, RIM15 shows distinct genetic interaction profiles in response to different starvation signals. However, functional annotation of genetic interaction profiles of RIM15 identifies consistent genetic interactions with vacuole-related functions in response to different starvation signals. Those results suggest that RIM15 integrates diverse signals to maintain protein homeostasis in quiescent cells. Our study expands genome-wide genetic interaction profiling to additional conditions, and phenotypes, highlighting the conditional dependence of epistasis.

1311C Identifying Genetic Loci Whose Effects on Phenotype are Influenced by Changes in Genetic Background *Christoph Rau*[1], Natalia Gonzales[3], Joshua Bloom[1], Danny Park[4], Abraham Palmer[2], Aldons Lusis[1], Noah Zaitlen[1] 1) University of California, Los Angeles, CA, USA; 2) University of California, San Diego, CA, USA; 3) University of Chicago, Chicago, IL, USA; 4) University of California, San Francisco, CA, USA.

Commonly used genetic models of phenotype assume that allelic effect sizes are constant in the population and do not vary from individual to individual. Significant evidence, however, also points to the contribution of epistatic interactions, where two or more alleles interact, introducing phenotypic variability. These epistatic interactions suggest that the effects of genetic and environmental perturbations may, in fact, vary across a population. We have developed a statistical test that we call Gene by Ancestry (GxΘ), which determines whether the effect of a polymorphism on a complex phenotype changes as a function of a definable ancestral background such as one found in an admixed human or model organism population. We demonstrate through simulation that our test is capable of identifying epistatic interactions and, in fact, increases in efficacy as the number of interacting epistatic sites increases.

We apply our test to data from two model organisms. We observe significant GxΘ loci in fourteen of fifteen yeast crosses. We identify 98 significant loci in 38 of 40 observed phenotypes across these crosses. We then examine two large mouse cohorts, the Hybrid Mouse Diversity Panel and an Advanced Inter-Cross between LG/J and SM/J for evidence of GxΘ interactions, and observe 49 significant associations across 14 distinct phenotypes, as well as over 1,400 Bonferroni-corrected interaction associations in mouse gene expression. Further analysis of one of the mouse cohorts revealed evidence of rapid selection pressure on specific polymorphisms

Unlike our prior work in human populations, we observe widespread evidence of ancestry-specific SNP effects in model organism populations. We observe that the number of identifiable GxΘ effects appears to decrease as a function of phenotype and organismal complexity, with the greatest number observed in phenotypes relating to organismal survival in yeast, then gene expression in mice, physiological traits in mice and, finally, behavioral traits in mice, likely reflecting weaker effect sizes as complexity increases. These results suggest that individual polymorphism, and by extension changes to the genome brought about by technologies such as CRISPR, will be affected by genetic background, a form of complex gene-gene interaction.

1316B The Genetic Interaction Ontology (GIO) incorporating the Genetic Interactions Structured Terminology (GIST) *Christian Grove*[1], Rose Oughtred[2], Kara Dolinski[2], Mike Tyers[3,4], Paul Sternberg[1] 1) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA; 3) The Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; 4) Institute for Research in Immunology and Cancer, Université de Montréal, Montréal, Québec, Canada.

Genetic interactions have been studied for more than a century as a means to elucidate gene function. Built on prior work by the authors, a new unified genetic interaction ontology (GIO) incorporating the genetic interactions structured terminology (GIST) is proposed for inclusion into the Human Proteome Organization (HUPO) Protein Standards Initiative (PSI) Molecular Interactions (MI) controlled vocabulary (https://github.com/HUPO-PSI/psi-mi-CV). The updated, proposed ontology includes a restructuring of the upper level of the ontology, clear references to definitions and meanings described in the literature, direct incorporation of genetic interaction terms from the Biological General Repository for Interaction Datasets (BioGRID) database (https://thebiogrid.org/), relabeling of some terms, GIST synonyms for existing terms, obsoletion of outdated terms, and some new proposed terms to incorporate GIST and ensure consistent ontology structure. An important distinction is made between genetic interactions that are reported as modifications of an existing phenotype versus genetic interactions that defy expectation (i.e. deviate significantly from an inherent or mathematically determined expected double genetic perturbation phenotype), as both meanings have been used in the literature and in biological data repositories. The proposed ontology can accommodate many existing use cases for quantitative genetic interactions described in the model organism literature, but is not intended or purported to be exhaustive or comprehensive. Suggestions for edits or new terms from the genetics community are strongly encouraged.

1320C Using breed-origin-of-alleles in genomic prediction *Mario Calus*[1], Claudia Sevillano[1], Pascal Duenk[1], Yvonne Wientjes[1], Piter Bijma[1], Jeremie

Vandenplas[1] 1) Wageningen University & Research, Animal Breeding and Genomics.

Breeding is based on selecting the genetically best individuals as parents of the next generation. Traditionally, selection relied on breeding values estimated from phenotypic records of the individual itself and its relatives, connected using pedigree information. In the last decade, many livestock and plant breeding programs have adopted genomic selection, which estimates breeding values based on genome-wide marker data. Genomic prediction enables accurate selection early in life, leading to faster genetic improvement.
In pig and poultry breeding the production individuals are crosses between three or four breeding lines. Similar strategies are used in crops like maize, where the breeding lines are intentionally inbred, while in livestock breeding lines inbreeding is restricted. In all these cases, the breeding goal is to improve crossbred performance. Selection in pigs and poultry, however, takes place among individuals within a breeding line, and selection decisions are often based on purebred performance. The genetic correlation between purebred and crossbred performance is generally lower than unity, which can limit the progress in crossbred performance. This limitation can be removed by using a crossbred reference population in genomic prediction. Crossbred individuals carry alleles from several parental lines, which may affect genomic prediction.
We developed a method to derive the Breed-Origin-of-Alleles (BOA) for individuals resulting from crossing non-inbred breeding lines, based on phased genotype data and haplotype frequencies observed in the parental lines. We were able to derive BOA for 92-95% of the alleles in crossbred broilers and pigs. We hypothesized that using BOA in genomic prediction models would increase genomic prediction accuracy for crossbred performance. Empirical accuracies, however, were at best slightly improved, despite the observation that allele frequencies and estimated allelic effects for crossbred performance did differ among the parental lines. Using BOA, relationships with respect to a focal breeding line are only based on alleles from this line, while alleles from other lines are ignored. The limited benefit in accuracy when using BOA is likely because alleles from other lines contribute little to variation in relationships of the focal line. Nevertheless, the BOA methodology is potentially beneficial in genomic prediction in admixed populations in other species, especially if founder populations are very different.

1322B Genomic mate selection for clonally propagated crops: improving the chance of breeding top ranking clones by predicted variance in total genetic merit *Marnin Wolfe*[1], Jean-luc Jannink[1,2] 1) Cornell University, Ithaca, NY; 2) USDA-ARS, Ithaca, NY.

Diverse crops ranging from staples (e.g., cassava) to cash crops (e.g., cacao) are both outbred and clonally propagated. In these crops, exceptional genotypes can be immortalized and commercialized as clonal varieties, which is a distinct advantage relative to many other crops. Genomic prediction can incorporate both additive and non-additive effects in clonal crops to select candidates with high breeding value as parents for crossing and candidates with high total genetic merit as varieties for release to farmers. Further improvements over genomic truncation selection have recently been made by predicting the genetic variance and selecting crosses instead of parents. In plant breeding, prediction of progeny variance was used to accelerate inbred line development. In animal breeding, it was shown that selection of parents whose gametes are predicted to have both high mean and variability increases response to selection and the probability of observing an elite individual. In this study, we extend these developments to the breeding of clonally propagated organisms. First we predict both additive and non-additive variances in outbred crosses using genome-wide phased parental haplotypes, marker effects estimates, and recombination maps. Next, we use the predicted distribution of progeny merit to derive the probability of observing an elite clone from a cross with a given number of matings. We then use simulations to assess the accuracy of variance prediction in a scenario modelled after cassava breeding. Finally, we compare our predictions to the realized means, variances and family sizes of >200 real crosses from a cassava genomic selection program and consider where selection and field testing efforts are predicted to have been over or underspent. We discuss strategies for optimizing mate selection and clone testing efforts to serve simultaneous population improvement and variety development in clonally propagated crops.

1345A Network analysis of complex trait evolution: the shift to C4 photosynthesis *Elli Cryan*[1,2], Daniel Kliebenstein [1], Jeffrey Ross-Ibarra[2] 1) Plant Sciences, UC Davis, Davis, CA; 2) Evolution and Ecology, UC Davis, Davis, CA.

A complex trait can be represented by a network of interactions between metabolites and proteins underpinned by a network of genes that encode the proteins. Studying these genes in a network context allows complex trait evolution to be represented by both changes in network architecture and gene sequences across evolutionary time. Network architecture changes include the rewiring of links between nodes as genes are exapted to new functions and the creation of new nodes in cases of gene duplications. Additionally, network architecture properties such as centrality can give insight into the 'choice' of which gene in a family will be exapted. Established comparative measures of gene sequence evolution like Dn/Ds can be mapped onto the network to provide a more holistic view of complex trait evolution. An example of an important complex trait in the green tree of life is C4 photosynthesis, which is an adaptation that reduces the incidence of photorespiration by partitioning gas exchange and carbon capture to different cells. C4 photosynthesis is a complex trait that has evolved independently from a C3 photosynthesis ancestral state in more than seventy documented events. By comparing the gene networks of pairs of species on either side of independent C4 evolution events, we hope to better understand the process of complex trait evolution.

1357A Network-based functional prediction augments genetic association to predict candidate genes for inflammatory bowel disorder in mice Montana Kay Lara[1], Karolyn G. Lahue[2], Alisha A. Linton[1], Brigitte Lavoie[1], Qian Fang[3], Mahalia M. McGill[2], Jessica W. Crothers[3], Cory Teuscher[3], Gary M. Mawe[1], Anna L. Tyler[5], Dimitry N. Krementsov[2], J. Matthew Mahoney[1,4] 1) Department of Neurological Sciences, University of Vermont; 2) Department of Biomedical and Health Sciences, University of Vermont; 3) Department of Medicine, University of Vermont; 4) Department of Computer Science, University of Vermont; 5) The Jackson Laboratory.

Inflammatory bowel disorder (IBD) is a complex, heterogenous disease for which hundreds of candidate genes have been identified through genome wide association studies (GWAS). Likewise, mouse studies of IBD have identified multiple quantitative trait loci (QTL) controlling IBD susceptibility. However, integrating mouse and human genetic diversity to study molecular mechanisms of IBD remains a challenge, as risk alleles do not always align, and QTL mapping resolution is relatively low. Nevertheless, we hypothesize that human risk factors and their mouse orthologs are functionally related genes that act in the same biological processes. Therefore, to predict candidate genes influencing IBD susceptibility, we propose a novel network-based machine learning approach to positional and functional information from mouse models of IBD with human risk alleles from GWAS. Using support vector machine (SVM) classifiers with gene expression signatures in mouse immune cells, we prioritized functionally related genes associated with human IBD susceptibility. The wild-derived PWD/PhJ (PWD) and standard C57BL/6J (B6) strains show marked differences in susceptibility to two models of IBD induction. Using consomic mice carrying PWD chromosomes on the B6 background, we identified PWD chromosomes (Chrs) 1, 2, and 12 as novel loci that profoundly enhance IBD susceptibility. These loci augment previous reports of the *Ccc1* locus on

Chr 12 that had been identified from the Collaborative Cross strain CC011/Unc for its high penetrance of spontaneous IBD. To identify translational candidate genes in these risk loci, we trained SVM classifiers to identify subnetworks enriched with IBD GWAS genes in two tissue-specific functional networks in mice: the intestinal and hemolymphoid networks. These classifiers rank all genes in the genome by functional relatedness to IBD GWAS genes. We scored all genes on mouse Chrs 1 and 2 and the *Ccc1* locus to identify network based functional candidates genes. We further enriched the functional predictions by integrating transcriptional data comparing gene expression across five different types of B6 and PWD immune cells. Finally, we integrated functional and expression-based rankings to produce a final score. A total of 47 unique genes were prioritized as possible candidates to be pursued in follow-up studies, including *Pip4k2a*, *Lcn10*, *Lgmn*, and *Gpr65*, all of which have strong functional evidence for association to IBD. Our results demonstrate the predictive potential of network-based machine learning for candidate gene ranking across species.

1358B Digging into the adaptive response to drought in Asian rice (*Oryza sativa* L.) *Annarita Marrano*[1], Brook Moyers[1] 1) University of Massachusetts Boston.

Asian rice represents a staple food for more than 50% of the global population. In the near future, rice demand, along with the frequency of extreme climate events and water shortage, will increase, making the development of high-yielding, climate-resilient rice cultivars a priority for rice breeding. We are studying the genetic architecture of response to drought stress using a Multi-parent Advanced Generation Inter-Cross (MAGIC) population developed by inter-crossing 16 elite varieties from the *indica* and *japonica* subspecies. We examined the genetic architectures of yield, biomass, and other agronomically important traits under seedling stage and reproductive drought stress across multiple growing seasons. We find consistent genotypic effects as well as major genotype by environment interactions. Many loci exhibit dynamic reaction norms of trait effect size with respect to environment and developmental time. This plastic genetic architecture suggests that effectively meeting breeding targets in drought stress may require targeting selection to specific environments, rather than developing a "jack-of-all-trades" genotype that could be resilient across many environments. To pursue this goal, we are using these genome-wide associations to predict trait polygenetic scores in a diverse collection of more than 3,000 cultivated rice accessions. Shifts in allele frequencies at candidate loci can reflect historical and geographic patterns of selection for key traits, and identify potential donor lines. This work will expand our understanding of the adaptive response of rice cultivars to drought stress and enable the prediction and selection of high yielding individuals under restricted water availability.

1379B Population structure sinference using phenotype data *Anthony Greenberg*[1] 1) Bayesic Research.

Assigning individuals to populations is important for a variety of applications. Given the abundance of genotype data, multiple widely-used methods are available for population structure inference. Employing these methods, we have learned a great deal about evolutionary history of species. Phenotypic differences among populations are then typically studied given the genotype-based population assignments. However, it would be useful to infer population differentiation using phenotypic data alone. Studying the concordance, or lack thereof, between genotype and phenotype driven assignments can yield insights into local adaptation and identify useful sources of breeding material for crop and livestock improvement. I will describe a Bayesian Gaussian mixture model that uses (possibly replicated) measurements of multiple traits to infer population structure. I will present an R package, MuGaMix, that can perform such inference (source code will be available on GitHub by the time of presentation). Its performance will be assessed on simulated data as well a data set from domesticated rice, *Oryza sativa*.

1580B Genetic Dissection of Developmental Heterogeneity of the Drosophila Testis Stem Cell Niche *Jeeun Song*[1], Katie Conlon[1], Erika Matunis[1] 1) Johns Hopkins University School of Medicine, Baltimore, MD.

Adult stem cells require a specialised microenvironment to maintain their pluripotent state and capacity for self-renewal. The Drosophila testis is a well-established model system for the in vivo analysis of stem cell niche function. In this tissue, niche cells (hub cells) maintain two stem cell populations that respectively replenish the germline gametes and the somatic stroma. Intriguingly, the somatic mesenchymal cells that coalesce to form the niche initially arise from multiple parasegments in the Drosophila embryo. Whether the cellular components of a stem cell niche are uniform or heterogenous in nature is an open question. Little definitive work has explored the possibility of cellular heterogeneity or its developmental origins across stem cell niches. Here, we use the Split Gal4-UAS ternary system to target combinatorial patterns of homeobox gene expression during gonadogenesis in the Drosophila embryo. Preliminary results using fixed immunostaining and live imaging *in vivo* and *ex vivo* show that these constructs are capable of expressing transgenes in sub-populations of somatic gonadal precursors in the embryo. Furthermore, preliminary data indicate that this approach enables lineage tracing that persists beyond embryogenesis. Ultimately, these genetic drivers would enable the labelling, lineage tracing, and manipulation of cells according to their parasegmental origin within the *Drosophila* testis stem cell niche.

1621A Glycosylation of specific Notch EGF repeats by Ofut1 and Fringe regulates Notch signaling in *Drosophila* ASHUTOSH PANDEY[1], BETH M HARVEY[2,4], MARIO F LOPEZ[1], ATSUKO ITO[3], ROBERT S HALTIWANGER[2,3], HAMED JAFAR-NEJAD[1] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, TX; 2) Department of Biochemistry and Cell Biology, Stony Brook University, NY; 3) Complex Carbohydrate Research Center, University of Georgia, GA ; 4) Department of Cell and Developmental Biology, University of Pennsylvania, PA.

Fringe proteins differentially modulate the interactions of Notch receptors to Delta/DLL versus Serrate/Jagged ligands by GlcNAc elongation to *O*-linked fucose on Notch epidermal growth factor-like (EGF) repeats. Notch receptor possesses 22 *O*-fucosylation sites. However, the biologically-relevant sites that affect Notch functions during animal development *in vivo* in the presence or absence of Fringe are not well not known. Here, we used a variety of assays and found important roles for GlcNAc-fucose-*O* glycans on three sites i.e. EGF8, 9 and 12 in *Drosophila* Notch signaling. *O*-fucose monosaccharide on EGF12 (in the absence of Fringe) found to be essential for Delta-mediated lateral inhibition during fly embryogenesis. Wing vein development depends on GlcNAc elongation to EGF8 and 12 by Fringe, with minor contribution from EGF9. During wing margin formation, Fringe modifications of EGF8 and 12 together prevent Notch from *cis*-inhibiting Serrate. This study shows the combinatorial and context-dependent roles of O-fucose glycans on these sites in *Drosophila* Notch-ligand interactions.

1670B Genetic Architecture of Population Differences in the Sequential Hermaphroditism of *Kryptolebias marmoratus* *Eric Haag*[1], Ryan Earley[2], Troy Anlage[1], Lena Boyer[1], Alison Chafitz[1], Emily Gerard[1], Brianna Pierce[1], Daniel Markstein[1], Aidan McLoughlin[1], Matthew Muniz[1] 1) University of Maryland, College Park; 2) University of Alabama, Tuscaloosa.

The mangrove killifish, *Kryptolebias marmoratus*, possesses two unusual sex-related attributes. One is its self-fertile hermaphrodite sex, which is assumed at sexual maturity by the vast majority of fish. The other is sequential hermaphroditism, in which the initial hermaphrodite sex can tran-

sition into a dedicated male. These males lose all ovarian tissue, proliferate large testes, and assume typical male behavior and pigmentation. As a result, limited outcrossing does occur in both natural and laboratory conditions [1, 2]. Interestingly, within an effectively isogenic line individuals may or may not change sex, and lines can differ greatly in their tendency to undergo sex change when grown in a common environment [3]. For example, in the Floridian strain SOB10 about 4% of individuals become males, while the Belize Twin Cays strain BWS29 changes roughly 70% of the time. In addition, BWS29 males often develop without passing through an obvious fertile hermaphrodite phase, indicating some are so-called primary males. To investigate the genetic architecture of this difference, we are taking hybrid genetics approach. Through pair-wise crosses between BWS29 and SOB10 lines and microsatellite genotyping [1] of offspring we identified five F1 hybrids. All of these remained hermaphrodites into their third year, indicating that early, frequent sex change is fully recessive to the SOB10 condition of rare/slow change. F2 offspring were collected, and a number have shown early sex change. This suggesting that variants in a small number of loci (potentially as few as one) may govern the propensity and timing of sex change in *K. marmoratus.*

[1] Mackiewicz, M., Tatarenkov, A., Perry, A., Martin, J.R., Elder, J.F., Jr., Bechler, D.L., and Avise, J.C. (2006). Microsatellite documentation of male-mediated outcrossing between inbred laboratory strains of the self-fertilizing mangrove killifish (*Kryptolebias marmoratus*). *J Hered 97*, 508-513.
[2] Mackiewicz, M., Tatarenkov, A., Taylor, D.S., Turner, B.J., and Avise, J.C. (2006). Extensive outcrossing and androdioecy in a vertebrate species that otherwise reproduces as a self-fertilizing hermaphrodite. *Proc Natl Acad Sci U S A 103*, 9924-9928.
[3] Turner, B., Fisher, M., Taylor, D., Davis, W., and Jarrett, B. (2006). Evolution of 'maleness" and outcrossing in a population of the self-fertilizing killifish, *Kryptolebias marmoratus*. *Evol. Ecol. Res*. *8*, 1475-1486.

1672A Unique thermosensitive trajectories of urogonadal genome-wide gene expression evolved in turtles with sex chromosomes, distinct from turtles with temperature-dependent sex determination *Thea Gessler*[1], Zhiqiang Wu[2], Robert Literman[3], Nicole Valenzuela[1] 1) Iowa State University, Ames, IA; 2) Colorado State University, Fort Collins, CO; 3) University of Rhode Island, Kingston, RI.

The molecular basis of phenotypic plasticity in vertebrates remains poorly understood, but is critical for understanding the development and evolution of phenotypes. Most turtles display a classic case of phenotypic plasticity in the form of temperature-dependent sex determination (TSD) while others utilize sex chromosomes as a form of genotypic sex determination (GSD). Thus, turtles are an ideal group for addressing these issues. Here we study urogonadal development of two turtles: *Chrysemys picta* (TSD) and *Apalone spinifera* (ZZ/ZW-GSD), incubated identically, under temperatures that induce males and females in *Chrysemys*. We performed transcriptomic and the first ever global trajectory analysis of gene expression in turtles, to disentangle the effects of sex and temperature on sexual development in *Apalone*, and the genome-wide changes in gene expression that accompanied the evolution of sex chromosomes from the TSD pattern present in *Chrysemys.* Our data illuminate the molecular mechanisms underlying the evolution of development underlying sex determination, and the interactions of genome and environment. Our multivariate analysis revealed for the first time how global genome transcriptional trajectories respond to temperature in developing TSD males and females over time, and how unique and plastic gene expression trajectories evolved along with sex chromosomes in *Apalone*'s lineage, but differ from those seen in *Chrysemys*. Our findings exemplify developmental systems drift, and the retention of evolutionary potential for GSD-to-TSD reversal in a vertebrate with sex chromosomes.

1674C Characterizing bacteriophage genes that control arthropod reproduction *Dylan Shropshire*[1], Mahip Kalra[1], Seth Bordenstein[1] 1) Vanderbilt University, Nashville, TN.

Wolbachia are maternally-inherited and intracellular bacteria that infect approximately half of all arthropod species. They are currently at the forefront of vector control efforts to curb arbovirus transmission using a drive system called cytoplasmic incompatibility (CI) to replace target vector populations with vector-resistant insects. CI results in a sperm-genome modification that kills embryos unless they are infected with *Wolbachia*. Therefore, CI results in a fitness advantage for infected females that transmit the bacteria to offspring. Recent studies have determined that CI is caused by the expression of two genes from *Wolbachia's* prophage WO, *cifA* and *cifB*, in males and rescue of CI is caused by the expression of *cifA* in females. However, less is known about the specific functions of these proteins. Here, we use transgenic expression of mutagenized *cif* genes in *Drosophila melanogaster* to assess the importance of conserved protein residues for CI and rescue phenotypes. Results highlight the central role of prophage WO in shaping *Wolbachia* phenotypes that are significant to arthropod evolution and vector control.

1730B Generation and characterization of ebp mutations in zebrafish *Somayeh Hooshmand*[1], Wei-Chia Tseng[1], Ana Johnson-Escauriza [1], Chon-Hwa Tsai-Morris[1], Benjamin Feldman[1], Christopher Wassif [1], Forbes Porter [1] 1) National Institutes of Health, Eunice Kennedy Shriver, National Institute of Child Health and Human Development (NICHD), Bethesda, MD.

Conradi-Hünermann-Happle syndrome (CHH) is an inherited X-linked dominant disease caused by mutations in the Emopamil-binding protein (EBP) gene. CHH patients are characterized by skeletal and skin abnormalities, cataracts, short stature and the biochemical accumulation of 8(9)-cholestenol. EBP also known as the 3beta-hydroxysteroid-delta (8), delta (7)- isomerase functions within the cholesterol synthesis pathway and catalyzes the conversion of 8(9)-cholestenol to lathosterol. The aim of this study is to generate and characterize ebp-null mutants generated by CRISPR/Cas9-mediated gene targeting. We have 2 independent null ebp lines that are indistinguishable, a deletion of ebp^{del4} and $ebp^{del4ins21}$. Phenotypically at 7 days post fertilization (DPF), both ebp mutants and controls appeared healthy, active, equally feeding and indistinguishable in size. However, growth seems to have arrest here or shortly thereafter, by 12DPF the ebp mutants were significantly smaller in size compared to controls (3.66 and 5.99 mm respectively, n=33 min per genotype). These 12-day epb mutants demonstrated a reduction in locomotion remaining stagnant in the dishes until prodded into motion, and ultimately died between 12 and 14 days DPF. Pathological evaluation of the ebp mutants at 12 days revealed a significant reduction in ossification of the ebp mutants. Hematoxylin and eosin staining of 12 DPF larvae was notable for significant disorganization of the myotendinous junction (MTJ) and detached myofibers in the ebp mutants. Phalloidin staining of actin filaments also referred to as F-actin when imaged by confocal microscopy confirmed the H&E muscle findings. Neutral sterol analysis at 7DPF found equal amounts of cholesterol in all genotypes while levels of 8(9)-cholestenol were below the limit of detection in all genotypes. Sterol analysis at 10 and 12DPF control and mutant zebrafish noted a significant elevation in the 8(9)-cholestenol in the ebp mutant fish while still undetectable in the controls. Further analysis is needed to determine when the muscle dysmorphology begins, and what roles the accumulation of the 8(9)-cholestenol result in the muscle dysmorphology, growth retardation and ultimate death of epb zebrafish.

Thursday, April 30 12:00 PM - 3:00 PM

Developmental Genetics 2 - Poster Q&A 1393A Reciprocal Regulation Between DBL-1/BMP Signaling and Cuticle Collagen Genes in *C. elegans* Uday Madaan[1,2], Lionel Faure[3], Albar Chowdhury[1], Shahrear Ahmed[1], Tina L. Gumienny[3], *Cathy Savage-Dunn*[1,2] 1) Queens College, CUNY, Flushing, NY; 2) The Graduate Center, CUNY, New York, NY; 3) Texas Woman's University, Denton, TX.

Cell differentiation and homeostasis depend on interactions with the cellular environment. In addition to responding to their environments, cells shape them, in part by modulating the extracellular matrix (ECM). Disruption of these interactions results in pathologies such as cancer or fibrosis. Signaling by Transforming Growth Factor-β (TGF-β) family ligands is known to modulate ECM, and their signaling pathways may respond to matrix stiffness. We have established the nematode *C. elegans* as an *in vivo* model to study the interplay of TGF-β signaling and ECM. *C. elegans* DBL1, a TGF-β family member related to BMP2/4, is a determinant of body size. We previously showed that DBL1/BMP signaling determines body size through transcriptional regulation of cuticle collagen genes. The *C. elegans* cuticle is a flexible barrier composed of multiple collagen layers with a lipid-rich epicuticle. We have now obtained evidence of feedback regulation of DBL-1/BMP by collagen genes. Inactivation of DBL-1-regulated cuticle collagen genes reduces DBL-1/BMP signaling, as measured by a Smad activity reporter. Furthermore, depletion of these collagens reduces GFP::DBL-1 fluorescence, and acts unexpectedly at the level of *dbl-1* transcription. We conclude that cuticle, a type of ECM that is functionally analogous to mammalian skin, impinges on DBL1/BMP expression and signaling. The feedback regulation of DBL-1/BMP signaling by collagens is likely to be contact-independent, due to the physical separation of the cuticle from DBL-1-expressing cells in the ventral nerve cord. In summary, the DBL1/BMP pathway provides a unique *in vivo* model to study bidirectional interactions between cell signaling and the ECM in the context of the intact organism. We propose that reciprocal interactions permit robust yet environmentally-responsive control of body size.

1407C Matrix assembly and function of a *C. elegans* ZP protein *Jennifer Cohen*[1], Meera Sundaram[1] 1) University of Pennsylvania Perelman School of Medicine.

Apical extracellular matrices (aECMs) line apical surfaces, like tube lumens and the epidermis. aECMs are rich in Zona Pellucida domain (ZP) proteins, whose dysfunction is associated with human diseases. However, how ZP proteins assemble in the aECM is unclear. We found that one *C. elegans* ZP protein, LET-653, shapes several tubes, including the vulva, a large multicellular lumen whose size enables aECM visualization. We are using LET-653 to study how ZP proteins incorporate in the aECM.

To define LET-653 aECM localization, we used CRISPR to tag endogenous LET-653 with Superfolder GFP (SfGFP). LET-653::SfGFP localized transiently to a matrix layer lining the apical membrane via its ZP domain. To determine how the LET-653 ZP domain is recruited to its membrane matrix layer, we performed structure-function analysis. ZP domains consist of two subdomains, ZPn and ZPc. *In vitro* studies suggest that ZPn domains polymerize to form fibrils, but roles for ZPc domains remain unclear. Surprisingly, the LET-653 ZPc domain was both necessary and sufficient to rescue *let-653* lethal tube defects and to form a stable, but transient, aECM layer along the apical membrane. This is the first report of a ZPc domain with ZPn-independent functions.

To probe LET-653 ZPc domain regulation, we tested if it requires C-terminal cleavage by mutating the cleavage site. ZP proteins are generally cleaved at the C-terminus in a reaction that is thought to relieve inhibitory ZPn-ZPc interactions. Mutating the LET-653 cleavage site when the ZPn domain was present prevented both ZPc aECM localization and function. In contrast, when the ZPn domain was absent, mutating the cleavage site did not impact ZPc aECM localization but did prevent its function. We propose a two-step model for LET-653 ZPc domain matrix assembly: first, cleavage removes ZPn inhibition of the ZPc to enable membrane recruitment by a partner; second, the ZPc domain changes conformation to allow function. This work offers novel insights into aECM assembly of ZP proteins.

1408A Molded by Matrix: A multi-layered, pre-cuticular apical extracellular matrix shapes the *C. elegans* vulval lumen Alessandro Sparacio[1], Jennifer Cohen [1], David Hall[2], *Meera Sundaram*[1] 1) University of Pennsylvania, Philadelphia, PA; 2) Albert Einstein College of Medicine, Bronx, NY.

Apical extracellular matrices (aECMs) are lipid and glyco-protein-rich layered structures that carry out diverse yet enigmatic functions during development, one of which is to shape the lumens of biological tubes. For example, a glycocalyx shapes and protects capillaries in the vascular system, and lung surfactant helps keep narrow airways open. The structure and assembly of such aECMs are poorly understood in part due to difficulties in imaging them in most *in vivo* settings. We are using the *C. elegans* vulva tube to understand the composition, assembly and functions of a glycocalyx-like aECM that precedes cuticle secretion and shapes many *C. elegans* epithelia. This aECM contains chondroitin proteoglycans, Zona Pellucida (ZP) domain proteins, and various other glycoproteins and lipid transporters related to those in mammals. The vulva lumen is relatively large and allows extraordinary resolution of aECM structures.

Our approach is three-fold: to tag endogenous aECM components with fluorophores to visualize the matrix in live animals; to use high pressure freezing and freeze substitution for transmission electron microscopy to visualize matrix ultrastructure; and to use genetic approaches to remove specific aECM components and test effects on aECM structure and tube shape. Our results reveal a complex, dynamic vulva aECM that fills the entire lumen and contains at least three distinct gel-like layers. These layers are punctuated by a meshwork of several fibrillar components, some of which run along specific vulval cells, while others are centrally located within the lumen. We also see evidence for a variety of potentially matrix-secreting intracellular vesicles as well as extracellular vesicles within the matrix. Finally, mutant analyses suggest that the current "sponge model" for vulva lumen expansion by chondroitin proteoglycans is inadequate to explain the changes observed after depletion of chondroitin and other aECM factors. Instead, we propose that chondroitin proteoglycans have both lumen expanding and lumen constraining activities related to their roles as building blocks within a complex matrix scaffold.

1422C Interaction of the DBL-1/BMP signaling pathway with BLMP-1/BLIMP1 in *Caenorhabditis elegans* *Mohammed Farhan Lakdawala*[1], Neethu Issac[1], Tina Gumienny[1] 1) Texas Woman's University, Denton, TX.

Animals use multiple signaling pathways for cell-to-cell communication, which are essential for proper development. One signaling pathway is defined by its ligand family of bone morphogenetic proteins (BMP). In the roundworm *C. elegans*, BMP member DBL-1 has a well-defined pathway that includes conserved core components and regulators. The DBL-1 signaling pathway is involved in a spectrum of traits, including body size, brood size, male tail morphogenesis, and distal tip cell migration. How does this BMP pathway control target gene expression? We are using the *C. elegans* system, which has a toolbox of genetic and molecular resources available, to specifically address this question. Previous studies in *C. elegans* show

that transcriptional regulator BLMP-1 affects a similar array of traits as DBL-1. In mammals, both BMPs and BLIMP1 affect overlapping developmental and homeostatic processes and are implicated in cancers. However, the relationship between BMP and BLIMP1 is not clear. We discovered that DBL-1 and DBL-1 signaling are affected by loss of BLMP-1. Notably, we also found that DBL-1 negatively regulates *blmp-1* expression in a stage-specific manner. In addition, ChIP-seq data analyses of SMA-3, a DBL-1 pathway transcription factor, and BLMP-1 suggest that these two transcriptional regulators control expression of some common target genes, and may act together. In the future, we will elucidate the molecular mechanism underlying the interaction between this BMP pathway and BLIMP-1 in *C. elegans*, to gain an understanding of how BMP regulates proper growth and development of animals.

1424B Identification of genetic interactions between the DBL-1/BMP-like pathway and other body size-associated genes in *Caenorhabditis elegans* Mohammed Farhan Lakdawala[1], Bhoomi Madhu[1], Lionel Faure[1], Mehul Vora[2], Richard Padgett[3], *Tina Gumienny*[1] 1) Texas Woman's University, Denton, TX; 2) Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ; 3) Waksman Institute of Microbiology, Cancer Institute of New Jersey, Rutgers University, Piscataway, NJ.

Bone morphogenetic protein (BMP) signaling pathways are conserved in animals and are used to control many developmental and homeostatic processes, including cell size and extracellular matrix remodeling. To identify novel regulators of BMP signaling, we performed a forward genetic screen in *C. elegans*for genes involved in body size regulation, a trait under the control of BMP member DBL-1. We isolated mutations that suppress the long phenotype of *lon-2*, a gene that encodes a negative regulator that sequesters DBL-1. We effectively isolated alleles of several core components of the DBL-1 pathway, demonstrating the efficacy of the screen. We found additional alleles of previously identified but uncloned body size genes. We also identified widespread involvement of extracellular matrix proteins in the DBL-1 regulation of body size. We characterized interactions between the DBL-1 pathway and extracellular matrix genes and other genes that affect body morphology. We discovered that loss of some of these genes affects DBL-1 pathway signaling. Two prominent cuticular collagens, DPY-2 and DPY-9, have stage-specific effects on DBL-1 signaling. We propose a model in which DBL-1 controls multiple body size factors, and the DBL-1 pathway is itself affected by novel regulators.

1425C Which GEF activates Rac1/CED-10 during epidermal morphogenesis? *Denver Baptiste*[1], Thejasvi Venkatachalam[1], Karla Larios[1], Martha Soto[1] 1) Rutgers University.

A properly organized actin cytoskeleton promotes cell migrations during embryonic development. Improperly organized actin promotes cell migrations during cancer metastasis. We study *C. elegans* embryonic cell motility and cell shape changes to better understand the transformation of newly differentiated cells into viable tissues during morphogenesis. We have described the morphogenesis role of Rac1/CED-10, a GTPase that activates the nucleation promoting factor WAVE/SCAR. Rac1/CED-10 promotes the collective cell migrations of epidermal ventral enclosure, by recruiting WAVE/SCAR to activate Arp2/3, to create branched actin that powers cell migration. Guanine Nucleotide Exchange Factors (GEFs) regulate GTPase activation and synchronize the position and level of their target GTPases, like CED-10/Rac1. We identified several upstream guidance receptors, SAX-3/ROBO, UNC-40/DCC and VAB-1/EphrinB, that regulate the distribution of Rac1/CED-10 and WAVE, thereby regulating actin levels and organization. Our current goal is to uncover how these receptors activate Rac1/CED-10 by identifying GEFs that connect the receptors to Rac1/CED-10 activation. Candidate Rac1 GEFs have been identified in other organisms, and in various processes in *C. elegans*. For example, TIAM-1 is thought to enable UNC40/DCC regulation of growth cone lamellipodial and filopodial protrusion while CED-5/DOCK180 has been proposed to be the GEF for Rac1/CED-10 during corpse engulfment and axonal migrations. The function of many GEFs is still unknown. While mammals have over 80 GEF proteins, *C. elegans* is predicted to have only 19, yet most have not been characterized for embryonic morphogenesis function. As part of a systematic search for the Rac GEF during embryonic morphogenesis, we have cloned all 19 GEF candidates into RNAi vectors for *C.* elegans and beginning CRISPR tagging and deletion of promising candidates. Surprisingly, we find that CED-5 is not the GEF for Rac1/CED-10 during ventral enclosure. Loss of CED-5 results in increased F-actin at the leading edge of the migrating cells, elevated ARP-2::GFP and elevated GFP::WVE-1, all opposite to what we expect from loss of a CED-10 GEF, and opposite to what we see for loss of *ced-10*. We will present our findings, including some GEFs that have only been studied in postembryonic neuronal development, like TIAM-1, appear to regulate CED-10 during epidermal morphogenesis.

1485C *D. pachea* as a model to unravel the development of left/right asymmetry *Benedicte Lefevre*[1,2], Virginie Courtier-Orgogozo[1], Michael Lang[1] 1) CNRS, UMR 7592, Institut Jacques Monod, Paris, France; 2) Université Paris Diderot, Sorbonne Paris Cité, France.

Most animals present left-right asymmetries. However, the mechanisms underlying the evolution of such structures remain poorly understood. We use *Drosophila pachea* as a model to study the evolution of left-right asymmetry from ancestral symmetry. Males of this species have a pair of asymmetric external genital lobes, with the left lobe being about 1.5 times longer than the right lobe. Lobes are not found in closely related species and present an evolutionary novelty. Based on a immuno-fluorescent staining approach on fixed samples, we found that lobe growth occurs between 24 h and 36 h after pupa formation (APF). Cell number but not cell size increases in the left lobe compared to the right lobe, which grow to about 410 cells and 220 cells, respectively. However, no differences in cell division rates were detected between the two lobes at various time points of development. We are now preparing life-imaging of developing genitalia in transgenic *D. pachea* that express fluorescent markers in the cell membrane to monitor the dynamics of lobe growth and to test if cell number differences result from a pulse or short time period of proliferation, differential cell death or cell recruitment from the flanking tissues. Furthermore, the lobe outgrowth occurs during genitalia rotation, a conserved process in flies during which the whole male genitalia will undergo a 360° clockwise rotation. The timing characterization of this event in a mutant *D. pachea* stock that presents a variable left lobe length suggested that the rotation occurs 6 h earlier than in wild-type stocks. Thus, genitalia rotation might be important for left-right asymmetric development of genital lobes and might have been co-opted to derive a new left-right asymmetry during evolution. Altogether, lobe asymmetry likely depends on intrinsic asymmetric cellular growth processes in the lobes, but also on the particular spatio-temporal tissue context in which they develop.

1511B Characterizing the Role of Doublesex in Creating Sexual Dimorphism in the *Drosophila* Gonad *Natalie Murphy*[1], Ellen Baxter[1], Mark Van Doren[1] 1) Johns Hopkins University, Baltimore, MD.

The Doublesex (Dsx) and Mab-3 Related Transcription factor (DMRT) family proteins are paramount for sex determination in most animals, from planaria to flies, birds, mice, and humans. In *Drosophila*, the embryonic gonad is formed when a bipotent cluster of somatic gonadal precursors (SGPs) coalesces with the germ cells. Sex determination is regulated by X chromosome dosage, which activates an alternative splicing cascade that yields Dsx^F in females and Dsx^M in males. Both Dsx isoforms have the same DNA binding domain, but they regulate their targets differently to yield sexual dimorphism. Dsx, like mammalian Dmrt1, is first expressed during embryogenesis and is required for male vs. female gonad development.

We are interested in determining the cell fate decisions regulated by Dsx and the target genes it controls to make these decisions. It is known that *dsx* is expressed in early SGPs during development, but the exact timing and role of Dsx in sex-specific cell fate specification during gonad development is unknown. We generated an N-terminally tagged GFP-Dsx to characterize Dsx expression in the somatic gonad. We found that males always express Dsx, while female Dsx expression changes during development. Additionally, while we have previously characterized how SGPs contribute to testis development, little is known regarding ovarian development – how do female SGPs (and other cells that may migrate into the gonad) achieve the greater diversity of ovarian somatic cell types compared to the testis? For lineage analysis, we are using G-TRACE, a *gal4-UAS* technique that permanently labels cells and allows for analysis at desired time points. Interestingly, using *tj-gal4*, which should label all embryonic SGPs, we found an unlabeled population of somatic cells, first visible in the larval apical cap, which migrate downwards between ovarioles during pupal development. These results identify a previously unknown population of somatic cells in the ovary that are unlikely to come from SGPs, and whose origins are currently unknown. Lastly, we are using small *dsx* LOF clones to investigate which cells of the gonad require autonomous sex information, and which may be regulated through cell-cell signaling. Ultimately, we aim to elucidate the mechanisms of Dsx in regulating the sex determination of *Drosophila* gonad somatic stem cell development.

1520B Smog as a putative receptor for Fog to regulate apical constriction during *Drosophila* SG invagination *Vishakha Vishwakarma*[1], SeYeon Chung[1] 1) Louisiana State University.

A major cell shape change during epithelial tube formation is apical constriction, driven by pulsed contraction of the actomyosin cytoskeleton. During invagination of the *Drosophila* embryonic salivary gland (SG), apical constriction is clustered in the dorsal/posterior region of the SG placode. Coordinated apical constriction is critical for the proper shape of the SG. We previously showed that the Folded gastrulation (Fog) signaling pathway is upregulated in the dorsal/posterior region of SG during invagination and is regulated by the Fork head (Fkh) transcription factor. Rho-associated kinase (Rok), in response to Fog, accumulates in the apicomedial region of SG cells and further results in apicomedial myosin formation and apical constriction near the invagination pit. However, the receptor that senses and transduces the Fog signal in the SG has not yet been identified. Here, we show the Smog G protein-coupled receptor (GPCR) as a potential candidate to act as a receptor for Fog in the *Drosophila* SG. Both *smog* mRNA and protein are upregulated in the dorsal/posterior region of the SG, where Fog signaling is active. The enrichment of *smog* mRNA in the dorsal/posterior region is abolished in *fkh* mutant SGs. SG cells with *smog* knockdown showed mild apical constriction defects and dispersed Rok along the apical surface. Myosin signals, however, were not obviously affected in these SGs. When *smog* was knocked down both maternally and zygotically, the whole embryo showed morphological defects, with elongated SGs along the dorsal/ventral axis. Dispersed Rok in the apicomedial region and reduced myosin was observed in these SGs. As our recent studies showed that overexpression of Fog in SG cells leads to overaccumulation of Rok and wiggly cell junctions, we are currently testing if *smog* knockdown can suppress the Fog overexpression phenotypes. Identifying the receptor(s) for Fog will help us to improve our understanding of how the Fog signal is recognized and translated to change cytoskeletal organization.

1547B Hedgehog signaling between the wing-disc epithelium and muscle precursors revealed by single-cell analysis in Drosophila *Nicholas Everetts*[1], Melanie Worley[1], Riku Yasutomi[1], Nir Yosef[1], Iswar Hariharan[1] 1) University of California, Berkeley.

Organ development is influenced by parameters that operate at both local and global scales. Locally, single cells are responsible for biomass production and maintenance of genetic identity, yet globally, all cells must collectively generate an organ of predictable size and shape. Additionally, organs are composed of distinct cell types that need to coordinate growth and patterning for proper development. The mechanisms of how this is achieved are not fully understood. Linking single-cell decisions to organ-wide patterning likely intertwines signaling pathways, physical forces, cellular heterogeneity, and local heterotypic interactions. We address questions related to these topics with the wing-imaginal disc of *Drosophila melanogaster*, a relatively simple structure composed of both epithelial and mesodermal cells that develop into the adult wing blade, thorax, and flight muscles. We have collected single-cell RNA sequencing data from wing-disc cells and used a lab-developed deep learning model to process, harmonize, and characterize cell types and genetic patterns. Our analysis has clearly identified both disc epithelial cells and adult flight muscle precursor cells (AMPs), and we have constructed single-cell RNA atlases that define the primary domains within these two cell types. To identify methods of communication between the disc epithelium and AMPs, we queried our atlases for the expression of reciprocal receptor-ligand pairs. Specifically, we observed that the AMPs that underlie the posterior compartment of the disc epithelium transduce Hedgehog (Hh) signaling. Our analysis enabled us to identify AMP-specific Hh targets within these cells. We used immunohistochemistry and genetic perturbation experiments to show that Hh signaling is crucial for fate specification in a subset of the AMPs, especially those that generate the direct flight muscles (DFM). Remarkably, even DFM precursors that underlie the anterior compartment of the disc epithelium, which do not seem to be actively transducing the Hh signal, are affected when Hh signaling is disrupted. Thus, these cells are either receiving a second signal relayed from more posterior cells, or alternatively, they have migrated from the posterior to the anterior following specification. Our study showcases the importance of a heterotypic interface between wing disc epithelium and AMPs in conferring identity for correct adult flight muscle development.

1548C Regulation of adult flight muscle morphology in *Drosophila* by signals emanating from the wing disc epithelium Riku T. Yasutomi[1], Nicholas J. Everetts[1], Melanie I. Worley[1], Nir Yosef[1], Iswar K. Hariharan[1] 1) University of California, Berkeley, Berkeley, CA.

Drosophila flight muscles consist of morphologically and physiologically unique direct flight muscles (DFMs) and indirect flight muscles (IFMs). DFMs are composed of eight tubular muscles, which maneuver wing positioning to steer flight. IFMs exhibit fibrillar morphology and are subdivided into dorsoventral flight muscles (DVMs) and dorsal-longitudinal flight muscles (DLMs). The DVMs and DLMs contract anti-synchronously to generate the mechanical force that propels flight. Both DFMs and IFMs are derived from adult muscle precursor cells (AMPs) underlying the developing wing imaginal disc. AMPs exhibit distinct fates in third instar larva characterized by differential expression of *vestigial* and *cut* along the dorsal-ventral axis, thought to give rise to IFMs and DFMs respectively. However, little else is known about the specification of AMPs. We show that the Hedgehog signal secreted from the posterior domain of the wing disc specifies a unique subpopulation of AMPs, which upregulate the transcriptional target, *patched.* Genetic perturbations of Hedgehog signaling in AMPs cause posture defects and loss of flight in adults. Loss of Hedgehog transduction in AMPs generates adults with wild-type DLM morphology, but abnormal DVM and DFM morphology. Specifically, posterior DVM muscle fibers appear morphologically similar to anterior DVM fibers and posterior DFM morphology is disrupted. Constitutive activation of Hedgehog in AMPs generates adults with wild-type DLM morphology, loss of anterior DVMs, and disrupted anterior DFMs. In extreme cases, DVMs in these flies are transformed into DLM-like muscles. Thus, we provide evidence that Hedgehog signaling between the epithelium and myoblasts is necessary for muscle cell-type specification and adult muscle morphogenesis.

1552A Overexpression of Met or Gce in larval hemocytes is sufficient to increase lamellocyte formation in response to juvenile hormone mimic treatment *Areeba Choudhry*[1], Rebecca Spokony[1] 1) Baruch College, CUNY, New York, NY.

Ectopic juvenile hormone mimics (JHMs) have been known to induce melanotic pseudotumors in a juvenile hormone receptor (JHR), Methoprene-tolerant (Met), dependent way, for decades. This response resembles the tumor formation in *hop*$^{tum\text{-}l}$ mutants, with constitutively activated hop inducing a cellular immune response leading to the formation of lamellocytes. Lamellocytes are specialized hemocytes ordinarily only found in immune challenged larvae. *Drosophila* contain two JHRs, Met and gce. Previously, we found that two JHMs (methoprene and pyriproxyfen) induce lamellocyte formation in wildtype larvae and but fail to induce lamellocytes in *Met*$^{-}$*gce*$^{-}$ larvae. Here we tested the hypothesis that overexpression of Met or gce in hemocytes would lead to increased lamellocyte formation in the presence of methoprene. We used the hemese-Gal4 driver to turn on UAS-Met or UAS-gce in hemocytes. A lethal phase analysis was conducted; there was no difference in eclosion rate between hemese-Gal4, hemese-Gal4>Met or hemese-Gal4>Gce animals. Lamellocyte induction was compared for each genotype treated with ethanol alone or 25 ug of methoprene in ethanol per vial. The results indicated that overexpression of the JH receptors are not enough for lamellocyte formation without methoprene, as all of the ethanol treated larvae had no lamellocyte induction. Overexpression of either Met or Gce was sufficient to increase lamellocyte production in response to methoprene. Mean lamellocyte count per larvae was estimated by using a hemocyter. Mean lamellocyte count following treatment for Gal4>Met and Gal4>gce were both greater than Gal4 alone, ($p=0.0003$, $p<0.0001$, respectively). These results are consistent with the mutant results showing that lack of JHR leads to a lack of response, indicating that the lamellocyte induction is caused by the methoprene exposure. We hypothesize that the fat body also plays a role in this response. The next steps include conducting experiments to study the overexpression of UAS-Met or UAS-gce using Gal4 in the fat body.

1553B The *Imaginal disc growth factor 3* interacts with a Planar Cell Polarity component during dorsal-appendage tube formation in *Drosophila* *Claudia Espinoza*[1], Celeste Berg[1] 1) University of Washington.

The *Drosophila* Imaginal disc growth factors (Idgfs) are related to human Chitinase-Like Proteins (CLPs), which are secreted proteins that are up regulated in inflammatory diseases, infections, and cancer. In flies, Idgfs induce proliferation and cell-shape changes in cultured cells, and they are up-regulated during infection. Despite how important Idgfs are to *Drosophila* biology and CLPs are to human health, our understanding of the genetic pathways and the molecular effects of *Idgfs* and *CLPs* is poorly understood. To expand our knowledge of *Idgfs,* I used the dorsal appendages of the *Drosophila* egg to identify candidate genetic partners of one of the *Idgfs* and to characterize the effect of the interaction between the *Idgf* and the candidate gene. Previously we showed that overexpressing *Idgf3* in the *Drosophila* egg chamber gives rise to aberrant dorsal appendages about 50% of the time. I screened deficiency lines that uncover most of chromosome 3L and identified large regions that enhanced and suppressed the *Idgf3*-overexpression phenotype. Using partially overlapping deficiencies, I narrowed down six interacting regions to a few candidate genes. Using a combination of RNAi and loss-of-function or knock-out alleles, I identified a genetic interaction between *Idgf3* and *combover* (*cmb)*, a gene that interacts with the Planar Cell Polarity (PCP) pathway to regulate wing hair growth. I used confocal microscopy to show that follicle cells overexpressing *Idgf3* and missing one copy of the *cmb* gene have larger apical areas compared to cells from *cmb/+* lines, to *Idgf3*-overexpression lines, and to control lines. These results suggest that *Idgf3* interacts with *cmb* to control the apical area of cells that form the dorsal appendages of the egg chamber. I am currently testing other PCP pathway components to ask whether this observation is due to a PCP effect. If so, we hypothesize that *Idgf3* interacts with the PCP pathway to expand the apical side of the cells in a polarized manner. Alternatively, since the PCP signaling pathway regulates actin during wing hair growth, the *Idgf3—cmb* interaction might be regulating actin polymerization during dorsal appendage formation.

1696A *osr1* and *hand2* Act in Opposition to Regulate Formation of Kidney and Vessel Lineages *Jessyka Diaz*[1,2], Elliot Perens[1,2], Agathe Quesnel[1], Amjad Askary[3], J. Gage Crump[3], Deborah Yelon[1] 1) Division of Biological Sciences, UC San Diego; 2) Division of Pediatric Nephrology, UC San Diego; 3) Stem Cell Biology and Regenerative Medicine, University of Southern California.

Proper organogenesis depends upon defining the precise dimensions of organ progenitor territories. Kidney progenitors originate within the intermediate mesoderm (IM), but the pathways that set the boundaries of the IM are poorly understood. Our prior studies in zebrafish have demonstrated that the bHLH transcription factor Hand2 limits the size of the embryonic kidney by restricting IM dimensions. *hand2* is expressed laterally adjacent to the IM, and venous progenitors arise at the boundary between these two territories. *hand2* promotes the emergence of venous progenitors while also inhibiting IM formation at this interface, suggesting that Hand2 plays an important role in balancing the specification of kidney and vein lineages.

The zinc finger transcription factor Osr1 has also been implicated in both kidney and vessel development, although its precise roles during IM and venous progenitor specification are not yet clear. Through analysis of zebrafish *osr1* mutants, we find that loss of *osr1* function inhibits IM formation and leads to subsequent kidney defects. Importantly, these phenotypes are partially suppressed by *hand2* loss-of-function, indicating antagonistic influences of *osr1* and *hand2*. *hand2* and *osr1* are co-expressed in the posterior lateral mesoderm, although *osr1* expression decreases prior to the formation of the venous progenitors. Intriguingly, we find that venous progenitors arise prematurely in *osr1* mutants. In contrast, overexpression of *osr1* results in inhibition of venous progenitor formation, as well as slightly enhanced IM formation. Together, our data suggest that *osr1* acts in opposition to *hand2* to balance the formation of the IM and venous progenitor lineages: initially, *osr1* promotes IM specification while inhibiting venous progenitor specification, and later, as *osr1* expression recedes, IM specification ceases and the venous progenitors emerge.

Our ongoing work aims to identify the effector genes that execute these roles of *osr1* and mediate its interactions with *hand2* during organ territory establishment. Elucidation of these transcriptional networks is likely to provide new insights into the genetic regulation of organ formation and the origins of congenital defects.

1698C The role of Npc2 in zebrafish embryonic development *Wei-Chia Tseng*[1], Ana Johnson-Escauriza[1], Chon-Hwa Tsai-Morris[1], Benjamin Feldman[1], Christopher Wassif[1], Forbes Porter[1] 1) Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD.

Niemann-Pick disease (NPC) is a rare fatal neurodegenerative lysosomal storage disease caused by mutations of either *NPC1* or *NPC2*. NPC2 is a soluble lysosomal protein that in coordination with NPC1 is responsible for the efflux of unesterified cholesterol from the lysosome. Mutations of both genes present a similar cellular pathology, such as the accumulation of unesterified cholesterol and other lipids in the late endosome/lysosome, while reducing cholesterol bioavailability. Here we present our results of a *npc2* null zebrafish model generated by CRISPR/Cas9 gene targeting.

Zygotic *npc2*$^{m/m}$ zebrafish from the intercross of *npc2*$^{+/m}$ individuals showed significant unesterified cholesterol accumulation at larval stages. Most *npc2*$^{m/m}$ adults survived but exhibited a 15% reduction in body size compared with *npc2*$^{+/+}$ of the same age. Additionally, zygotic *npc2*$^{m/m}$ adults exhibited motor and balance defects shortly before a premature death. These findings mimic defects found in human and mice, however the phenotype at embryonic stages were milder than expected. We hypothesized that the lack of phenotype in zygotic *npc2*$^{m/m}$zebrafish was due to the presence of maternal *npc2* mRNA present in the oocyte at the time of fertilization. To address this issue, we crossed male *npc2*$^{+/m}$ to female *npc2*$^{m/m}$ zebrafish to obtain maternal-zygotic (MZ) *npc2*$^{m/m}$ zebrafish. MZ*npc2*$^{m/m}$ zebrafish exhibited significant developmental defects including absent otolith, abnormal head/brain development, curved/twisted body axis, no circulating blood cells, and usually die by 72 hpf while *npc2*$^{+/m}$ siblings developed normally. Interestingly, these defects have not been previously reported in connection with either Npc2 or intracellular cholesterol trafficking. RNAseq analysis conducted on 30 hpf *npc2*$^{+/m}$ and MZ*npc2*$^{m/m}$ embryos revealed a significant reduction in *notch3* expression as well as reduction in other downstream genes in the signally pathway such as *hey1* and *her12.* Preliminary results showed that microinjection of a plasmid containing the constitutively active *notch3* ICD at the 1-cell stage could partially rescue the defects found in MZ*npc2*$^{m/m}$ embryos at 30 hpf, suggesting that Notch3 signaling might be involved some aspects of the pathology found in MZ*npc2*$^{m/m}$ zebrafish.

1699A The Hedgehog effector Netrin controls cell motility during early eye morphogenesis *Sarah Lusk*[1], Hannah Gordon[1], Kristen Kwan[1] 1) University of Utah.

Developmental defects in eye structure commonly account for visual impairment in newborns. One such defect, uveal coloboma, is caused by failed development of the optic fissure, a transient yet critical structure through which retinal ganglion cell axons exit the eye and vasculature enters. In humans, this structural defect is a significant cause of pediatric blindness worldwide. Although the genetic underpinnings of coloboma are heterogeneous, one pathway central to optic fissure development is the Hedgehog (Hh) signaling pathway: loss-of-function mutations in the Hh receptor *ptch2* lead to overactive Hh signaling, resulting in coloboma. Previously, using zebrafish multidimensional imaging and quantitative analysis of cell behaviors, we determined the basis of optic fissure formation, and pinpointed morphogenetic defects in *ptch2* mutants (Gordon and Lusk et al., 2018). Overactive Hh signaling disrupts optic fissure development in a Gli-dependent manner, through both cell- and non-cell-autonomous mechanisms. Our model is that a specific level of Hh signaling is crucial for optic fissure formation; overactive Hh signaling transcriptionally upregulates multiple factors that are directly responsible for disrupting cell movements and morphologies.

To identify the downstream transcriptional targets of Hh signaling that control optic fissure morphogenesis, I have initially taken a candidate approach and am examining the Netrin family of secreted ligands: multiple Netrin ligands are upregulated in the *ptch2* mutant at the right time and place to be influencing optic fissure development. To test if upregulation of Netrin is sufficient to cause coloboma, I have taken two gain-of-function approaches: overexpression of *netrin1a* in a spatiotemporally specific manner in wild type embryos disrupts optic fissure formation, resulting in coloboma. To determine whether Netrin is necessary for the *ptch2* mutant coloboma phenotype, I created loss-of-function alleles for each Netrin gene to test if loss of Netrin in the *ptch2* mutant background rescues the coloboma phenotype. Additionally, our model suggests that multiple Hh effectors are responsible for disrupting optic fissure development in the *ptch2* mutant, thus I am carrying out single-cell RNA-sequencing to identify additional Hh target genes, to be tested using similar gain- and loss-of-function approaches. Together, this work will uncover molecular mechanisms controlling optic fissure morphogenesis, and in turn, coloboma.

1701C Cellular analysis of forebrain morphogenesis in zebrafish shows conservation of mechanisms in vertebrates *Maraki Negesse*[1], Jonathan Werner[1], Jafira Johnson[1], Rachel Brewster[1] 1) UMBC.

Cranial neurulation is the process via which the brain primordium is shaped during early embryogenesis. Defective neurulation in the cranial region results in the most severe types of neural tube defects (NTDs), exencephaly and anencephaly. Morphogenetic studies of cranial neurulation in the mouse embryo have primarily focused on the midbrain and hindbrain. In contrast, far less is known about neurulation in the forebrain (FB). Fate maps of vertebrate embryos reveal that the prospective FB occupies the lateral edges of the eye field. The optic vesicles evaginate from the neural tube as neurulation proceeds. We explore here the cellular mechanisms that shape the prospective FB in the zebrafish embryo. Preliminary data from our laboratory indicate that FB morphogenesis in zebrafish presents hallmarks of primary neurulation in amniotes, namely medial and lateral hinge point-like structures and neural folds that converge and fuse at the dorsal midline. The medial hinge point (MHP) forms in a superficial layer of mesenchymal like cells, which subsequently undergo epithelialization and intercalate radially between the deep cells, contributing to the expansion of the optic vesicles. Molecular characterization of this MHP reveals apical enrichment of actomyosin. Blebbistatin treatment prevents MHP formation. Disruption of the Planar Cell Polarity (PCP) component Vangl2, previously implicated in MHP formation in amniotes, is required for the formation of the MHP counterpart in zebrafish. Ongoing studies aim to test whether Shroom3 is required for MHP formation. Together, these findings reveal the presence of previously overlooked structures characteristic of primary neurulation in the zebrafish, highlighting conservation of mechanisms. Overall, these studies establish the zebrafish as a model organism to screen for genetic factors that cause NTDs.

Thursday, April 30 12:00 PM - 3:00 PM

Developmental Genetics/Quantitative Genetics 2 - Poster Q&A 1324A Dissecting the Genetic Basis of Thermal Tolerance in a Multi-Parental Population of Fruit Flies *Patricka Williams-Simon*[1], Enoch Ng'oma [1], Ronel Ghidey [1], Troy Zars[1], Elizabeth King [1] 1) Division of Biological Sciences, University of Missouri, Columbia, MO.

Thermal tolerance is a complex trait that is a fundamental survival skill in many species. For example, everyday tasks such as: foraging, finding a mate, and escaping predation, are highly dependent on how well an organism can tolerate extreme temperatures. Understanding the natural variants of the genes that control this trait is of high importance if we want to better comprehend how this trait evolves in natural populations. Here, I take a quantitative genetics approach to dissect the genetic basis of thermal tolerance using Drosophila Synthetic Population Resource (DSPR). This multi-parental population consists of approximately 1,800 Recombinant Inbred Lines (RILs), which allows for high-resolution genome wide scans, and the identification of loci contributing to naturally occurring genetic variation. Using a highly sensitive apparatus known as the “heat box” we presented the flies with a constant 41°C for a total of 9 ½ mins. and recorded the time it takes for an individual to become incapacitated as our measure of thermal tolerance. We found that thermal tolerance ranges from, 24.8 sec – 361 sec, which is ~14-fold difference between the lowest to the highest tolerant RILs. We then performed a genome scan and identified several loci (QTL) affecting thermal tolerance, including one large effect

locus. We performed RNA-seq to identify differentially expressed candidate genes within these QTL that affect thermal tolerance. Future work will aim to validate the function of these genes.

1326C Loss of predictive power of polygenic risk scores in admixed populations *Barbara Bitarello*[1], Iain Mathieson[1] 1) Perelman School of Medicine, University of Pennsylvania.

Polygenic risk scores (PRS) summarize the results of GWAS into a single number that can predict quantitative phenotype or disease risk. One barrier to the use of PRS in clinical practice is that the majority of GWAS come from cohorts of European ancestry, and predictive power is lower in non-European ancestry cohorts. There are many possible reasons for this decrease; here we show that differences in allele frequencies, LD patterns, and phenotypic variance across ancestries are unlikely to be driving this pattern. Rather, our results suggest that differences in marginal effect sizes across ancestries play a major role.

We focus on PRS for height in cohorts with European (N=10,486) and admixed African and European (N=20,405) ancestry, which allows us to test for ancestry-related differences in PRS prediction while controlling for environment. We first show that that the predictive power of height PRS increases linearly with European ancestry (partial-R^2ranges from 0.02-0.12 for 0-100% European ancestry). We replicate this pattern with effect sizes re-estimated within sibling pairs, ruling out residual population structure. We also find that the pattern persists when PRS is computed using subsets of SNPs in regions of both high and low LD, and that ancestry-related differences in effect size are not correlated with local recombination rate. This suggests that differences in LD are not a major driver of low transferability. Next, we show that frequency differences of associated variants between African and European ancestry backgrounds explain only up to 11% of the observed reduction in predictive power and that there is no association between ancestry and phenotypic variance, indicating that the reduction in PRS predictive power cannot be explained by causal variants that are specific to the African ancestry background. Finally, we see a modest improvement in prediction when using a multi-PRS approach that includes ancestry-specific effect sizes in the PRS. As estimates of effect sizes for non-Europeans become more precise through the collection of larger sample sizes, this increment using a multi-PRS should be greater.

1333A Enhancing QTL detection power in multiparental populations *Frederick Boehm*[1], Clare Smith[2], Megan Proulx[1], Daniel Gatti[5], Michael Kiritsy[1], Sherry Kurtz[3], Karen Elkins[3], Gillian Beamer[4], Christopher Sassetti[1] 1) University of Massachusetts Medical School; 2) Duke University; 3) Food and Drug Administration; 4) Tufts Cummings School of Veterinary Medicine; 5) College of the Atlantic.

We present a strategy to enhance power to detect QTL in multiparental populations. We leverage position and allele effect information for individual traits in one population to increase power to detect similar associations in the same population or a second multiparental cohort. We demonstrate our approach in studies with Diversity Outbred (DO) and Collaborative Cross (CC) mice. We complement our analyses with simluation studies to assess statistical power to detect QTL. We discuss applications for this approach in high-dimensional expression QTL studies and for joint analysis of CC and DO mice.

1336A A natural variant of the essential *MMS21* gene underlies resistance to the parasitic 2-micron plasmid in *Saccharomyces cerevisiae* *Michelle Hays*[1, 2], Janet Young[1], Harmit Malik[1,3] 1) Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 2) Molecular and Cellular Biology Program, University of Washington, Seattle, WA, USA; 3) Howard Hughes Medical Institute.

Selfish genetic elements are DNA parasites that exploit their host cells for their own reproduction, thereby reducing host fitness. How can host cells evolve to defend themselves against these genetic parasites? We seek to address this question using the 2-micron plasmid, a selfish element naturally found across budding yeasts. This plasmid hijacks host cellular machinery to replicate and segregate itself, resulting in a 1- 3% fitness cost to the host. Despite this cost, most *Saccharomyces cerevisiae* isolates carry the plasmid, indicating that it is a remarkably successful, co-evolved genetic parasite of yeasts. By screening a panel of natural isolates, we identified strains that naturally do not harbor the 2-micron. We find that when the plasmid is reintroduced in the laboratory, these strains reproducibly lose the 2-micron, indicating that plasmid loss is a heritable trait. Furthermore, this plasmid loss phenotype is consistent with a genetically dominant trait, supporting the hypothesis that these strains have evolved a restriction factor targeting the 2-micron plasmid. We took a QTL mapping strategy, identifying a genomic locus underlying plasmid loss. Preliminary data suggest a natural polymorphism in an essential host gene *MMS21* is partially responsible for this parasite resistance. Our ongoing experiments explore the mechanistic basis for plasmid loss and potential host fitness tradeoffs of this natural polymorphism.
Additionally, we have developed a rapid plasmid loss assay that facilitates monitoring of plasmid stability in live cells across a population at single cell resolution. This assay is higher throughput and can better explore population heterogeneity compared to traditional plasmid loss methods. This work allows us to explore the genetics, molecular mechanism, and possible fitness consequences of naturally evolved parasite resistance.

1337B Genome-wide association study of bone strength and body weight in commercial crossbred layer chickens Martin Johnsson[1], Fernando Lopes Pinto[1], Heather McCormack[2], Bob Fleming[2], Andreas Kindmark[3], Alejandro Rodriguez Navarro[4], Ian Dunn[2], Helena Wall[1], *Dirk Jan de Koning*[1] 1) Swedish University of Agricultural Sciences, Uppsala Sweden; 2) The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Scotland, UK; 3) Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 4) Departamento de Mineralogia y Petrologia Universidad de Granada, Granada, Spain .

Osteoporosis and bone fractures are a serious problem for the welfare of laying hens, with genetics making a substantial contribution to bone strength. The genetic basis of bone strength in chickens has previously been mapped in experimental intercrosses and within pedigree lines. We performed a genome-wide association study of tibial breaking strength and body weight in 860 commercial crossbred chickens from two different companies, kept in either furnished cages or floor pens.
We genotyped 882 chickens at 57,636 single nucleotide variants, using the Illumina Infinium assay. The genotyping was performed by the SNP&SEQ Technology Platform at Uppsala University, Uppsala, Sweden. We excluded 14 individuals that high missingness, as well as 19 individuals that appeared to belong to the wrong crossbred based on a principal component plot of the genotypes.
We performed genome-wide associations studies using linear mixed models and a genomic relationship matrix as implemented in GEMMA version 0.97. We performed genome scans of analysed tibial breaking strength and body weight, separately for each housing system and jointly, combining the housing systems. Tibial breaking strength scans included body mass and crossbred, and in the case of the joint scan also housing system, as additive covariates. Body weight scans included crossbred, and in the joint scan also housing system, as additive covariates.
As expected, the two production systems produced a large difference in bone breaking strength, where floor pen-raised chickens had stronger

bones. Genome-scans either combining or independently analysing the two housing systems revealed no genome-wide significant loci for bone breaking strength. However, we validated (P < <0.01) three candidate regions from previous studies on chromosome 1, 19, and 23. There was very little correspondence between the GWAS results for the different housing systems suggesting that there is strong gene x enverinoment interaction. We detected three loci for body weight on chromosomes 4, 6 and 27, that were shared between the housing systems (either genome-wide significant or suggestive when the housing systems were analysed individually).
In summary, we found little evidence for large-effect loci for tibial breaking strength in commercial crossbred chickens, consistent with a highly polygenic architecture for bone strength in the production environment.

1338C Transcriptional effects of genetic-epigenetic interactions on local genes and distant gene networks *Lauren Kuffler*[1], Daniel Skelly[1], Anne Czechanski[1], Gary Churchill[1], Steven Munger[1], Christopher Baker[1], Laura Reinholdt[1], Gregory Carter[1] 1) Jackson Laboratory/Tufts University.

Modulation of gene expression is fundamental to development, differentiation, and maintenance of homeostasis. Modern genetics has long recognized the transcriptional effects of genetic variants in regulatory elements within a gene's "local area", as well as the contribution of stable epigenetic factors (or the "epigenotype"). The interaction between genetic and epigenetic regulatory elements are not systematically understood, and their effects on downstream gene networks are unknown. This project aims to move past isolated genotype or epigenotype influences on expression and explore the extent of genotype-by-epigenotype interaction, its effects on expression, downstream gene networks, and ultimately phenotype. We have worked towards this goal by leveraging genetic and epigenetic data from a panel of Diversity Outbred mouse embryonic stem cells, implementing computational tools to identify local effects of genotype-by-epigenotype interaction. This analysis has revealed that interactions between genetic variation and areas of open chromatin occur preferentially within topologically associating domains, in patterns explained by the structural context of these chromatin loops, thus providing a biological framework for a gene's "local area" and pointing toward best methods for profiling gene regulation and analysis of genetically diverse datasets.

1340B Local adaptation in *Populus trichocarpa* *Hari Chhetri*[1], Gancho Slavov[2], David Macaya-Sanz[1], Gerald Tuskan[3], Stephen DiFazio[1] 1) Department of Biology, West Virginia University, Morgantown, West Virginia; 2) Scion, Bay of Plenty, New Zealand; 3) Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Because of its rapid growth, hybrid vigor, broad geographic distribution, transformation potential, and the availability of tremendous genetic resources and wide phenotypic variation, *Populus* is a highly desirable species for biofuel production and other wood products. Understanding the genetic mechanisms underlying local adaptation is key for the sustainable management and domestication of forest trees like *Populus*. Here we report on the possible mechanisms underlying local adaption in *Populus trichocarpa* using whole genome re-sequencing, phenotypic and geo-climate data for 869 trees. First, we show that morphological and physiological traits are strongly correlated with the geo-climate variables of the source locations in *P. trichocarpa*. Second, using Genotype-Environment Association Analysis (GEA) and Redundancy Analysis (RDA) we identified several outlier loci that occur within and near genes related to important plant physiological functions and cuticular wax formation. A total of 32 genes were shared between RDA and GEA methods. Third, using RDA, we decomposed the among population variance of 869 trees into climate and geography. While climate and geography predictors together explained 7.6% of the total variation in the SNP matrix, climate alone explained 2.6% of the total variation. Partitioning the variance components in the response matrix of phenotypic traits into the explanatory matrices of SNPs, climate and geography, explanatory matrices altogether explained 22% of the total variation, whereas SNPs alone explained 3.9% of the total variation. These findings have important implications for developing management and conservation strategies and sustainability of forest resources in the face of climate change.

1363A Mapping mitonuclear epistasis in yeasts. *Tuc Nguyen*[1], Margaret Geertz[1], Meghan Lendhardt[1], Mark Schwartz[2], Weiwei Liu[1,3], John Wolters[1,4], Anthony Fiumera[1], Heather Fiumera[1] 1) Binghamton University; 2) Saint Louis University; 3) Ohio State University; 4) University of Wisconsin-Madison.

In emerging yeast populations, mitonuclear interactions are selected on to maintain optimal mitochondrial functions and organismal fitness. Mapping the mitonuclear genetic variants using association approaches is challenging because a strong population structure results in high linkage disequilibrium and because each strain may contain unique mitonuclear interactions. Here, we created a novel population of *Saccharomyces cerevisiae* yeasts that allowed the detection of naturally occurring nuclear alleles that contribute to mitonuclear interactions. Beginning with a genetically diverse set of strains engineered to contain a single mitochondrial haplotype, we isolated and sequenced 200 strains following seven rounds of random mating and meiosis. We then replaced the mtDNA in each strain with a second mitotype, and phenotyped both collections using high density colony arrays under a variety of conditions. To validate association approaches, we first performed a GWAS for growth differences on copper sulfate using biallelic SNP data and identified a highly significant region of chromosome VIII near CUP1, a metallothionein known to confer resistance to copper. We then built an association model to identify SNPs that were dependent or independent of mitotype and used this model to detect alleles that conferred growth differences between basal and elevated temperatures. Depending on the media, we identified 5 to 197 associated SNPs. In general, associated mitotype-independent nuclear variants were in genes involved in general metabolism, while associated mitotype-dependent variants within genes involved in stress responses. By lowering LD and fixing mtDNAs, these novel yeast strains improve the power of association approaches to detect both nuclear and mitonuclear genetic variants.

1371C Chromosome mapping of thermal divergence among *Saccharomyces* yeast species *Nilima Walunjkar*[1], Justin Fay[1] 1) Department of Biology, University of Rochester, Rochester, NY.

Identifying the genetic basis of phenotypic divergence between species is key to understanding evolution. While much has been learned from studies of intraspecific variation, these results may not be representative of interspecific divergence. For instance, divergence between species could be caused by numerous small effect changes in the same gene. A major hurdle to unearthing patterns of interspecific divergence is the sterility and inviability of hybrids, which limits genetic mapping of traits to closely related species. We overcome these barriers by using chromosome level loss of heterozygosity during mitosis to map thermotolerance in hybrids of two distantly related *Saccharomyces* yeast species. Thermotolerant *S. cerevisiae* diverged approximately 15 million years ago from the thermosensitive species, *S. uvarum*. Hybrids of these two species are mitotically stable and retain thermotolerance of the *S. cerevisiae* parent. A previous non-complementation screen failed to identify single genes of large effect that contribute to this phenotype. However, this screen excluded essential genes and did not test for multiple genes acting together to confer thermotolerance. To overcome these shortcomings, we used hybrid aneuploids and mitotic recombinants to screen the *S. cerevisiae* genome for thermo-

tolerance genes. Using a set of centromere marked *S. cerevisiae* strains, we generated whole chromosome loss of heterozygosity and measured its effects on growth at different temperatures. Our results are inconsistent with an infinitesimal model, which predicts that loss of thermotolerance is proportional to the size of the *S. cerevisiae* chromosome being lost. Instead, we find that a few *S. cerevisiae* chromosomes have a moderate effect on high temperature growth, and we see differences in these candidate chromosomes between fermentative and non-fermentative thermal profiles. Using CRISPR we show that mitotic recombination in hybrids can in principle, be used to resolve chromosome level effects to individual genes and determine the genetic architecture of this trait.

1385B A consistent estimator of kinship for admixed populatons, applied to heritability studies. Jerome Goudet[1], *Bruce Weir*[2] 1) University of Lausanne; 2) University of Washington.

Kinship, or relatedness, is a central concept in many areas of biology and genetics. In quantitative and human genetics, it is an essential element in the animal model, a mixed model used to estimate heritability and carry out Genome Wide Association Studies. Kinship captures the genetic-based dependencies among observations from pairs of individuals. We are particularly interested in good kinship estimators for understudied populations with mixed ancestries.
We have presented kinship estimators, based on genome-wide similarity, in two recent papers: Weir and Goudet, Genetics 206:2085-2103, 2017 and Goudet, Kay and Weir, Molecular Ecology 27:4121-4135, 2018. We have now applied these estimators to the set of all individuals in the 1000 Genomes data to show the consistency of our estimates, in contrast to the inconsistency of standard estimates. If our kinship matrix is written as Kas and the standard `correlation'-based matrix as Kc, then the ranking of elements of Kc depend on the study sample, whereas the ranking of elements of Ks does not have this inconsistency. It seems desirable that individual-pair kinships should have the same ranking for all study samples to which these individuals belong. We show that, when elements of Kc are the ratios of averages over SNPs of single-SNP statistics, written as Kc(0), it is the double-centered version of Kas. The biological motivation of replacing an element of a kinship matrix by the deviation of that element from its row and column means is not clear, although there are useful mathematical relationships between a matrix and its double-centered value. When elements of Kc are averages over SNPs of ratios of single-SNP statistics, written as. Kc(-1), they have larger bias than do elements of Kc(0). The differences between Kas and Kc increase with greater departures from Hardy-Weinberg equilibrium.
We then simulated quantitative traits for all 1000 Genomes individuals using various numbers of causal SNPs and various additive genetic models for trait values. We used mixed models, implemented in various software packages, including Gaston and GCTA, to estimate the heritability of each simulated trait. Using Kas gave lower bias and lower variance than did using the standard Kc(-1), although we found by theory and observation that Kas gave identical values to those with Kc(0). We establish that the expected values of heritability estimates using Kc(0) or Kas depend on the within-study inbreeding coefficient Fis rather than the total inbreeding coefficient Fit, as would be the case if kinships were known rather than were estimated relative to values in some reference population.
We advocate the use of the identity-in-state based kinship estimators we have discussed previously for studies of both kinship and of heritability.

1387A Analysis of Genetic Responses to the Antipsychotic Medicine Haloperidol with RNA-Seq Data from Diverse Mouse Recombinant Inbred Crosses (RIX) *Vasyl Zhabotynsky*[1], Wei Sun[2], James G. Xenakis[3], James J. Crowley[3], Paola Giusti[3], Patrick Sullivan[3,4,5], Fernando Pardo-Manuel de Villena[3,6], Fei Zou[1,3] 1) Department of Biostatistics, The University of North Carolina at Chapel Hill, North Carolina, USA ; 2) Biostatistics Program, Public Health Sciences Division, Fred Hutch, Washington, USA; 3) Department of Genetics, The University of North Carolina at Chapel Hill, North Carolina, USA; 4) Department of Psychiatry, The University of North Carolina at Chapel Hill, North Carolina, USA; 5) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden ; 6) Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, North Carolina, USA .

Schizophrenia is a chronic brain disorder that affects about 1% of the human population worldwide. H*aloperidol* is a typical antipsychotic medication used for treating schizophrenia. Haloperidol is known to have significant short term and long term side effects. In this project, we examined the transcriptomic changes in the brain associated with haloperidol treatment using a large number of RIX samples generated from the mouse Collaborative Cross population. Through a generalized negative binomial regression model, we jointly model additive genetic and treatment effects, as well as the genetic-by-treatment interaction. We identified 198 down versus 316 up-regulated genes (at FDR of 0.05) in RIX treated with haloperidol. Gene-set analysis of upregulated genes highlights a number of processes related to synapse part, neuron part, plasma membrane, vesicles, neuronal cell body, and neuron projection, suggesting that haloperidol may play an important role in altering neuronal morphology and/or density. Additionally, gene-set analysis of downregulated genes point to processes and functions related to cell secretion, transmembrane transporter activity, ion transport, and synaptic plasticity, suggesting that haloperidol may alter synaptic plasticity and cell signaling, via alterations in channel expression, localization, or modulation. These findings are consistent with our understanding of the molecular and cellular changes associated with chronic haloperidol exposure. We are particularly interested in connecting these transcriptional changes to extensive behavioral characterization done in these RIX, which may shine additional light on the pharmacogenetics of haloperidol treatment.

1559B Generation of mechanosensory neurons in adult *Drosophila* *Ismael Fernandez Hernandez*[1], Evan Marsh[1], Michael Bonaguidi[1] 1) UNIVERSITY OF SOUTHERN CALIFORNIA.

Sensory hair cells in the inner ear do not regenerate after exposure to excessive noise, ototoxic drugs or ageing, leading to irreversible hearing and balance disorders in over 5% of the world's population. Research on hearing and balance function restoration will remarkably advance by using scalable, genetically tractable *in vivo* platforms to analyze hair cells regeneration, from genes to circuits to behavior. We recently developed a modified lineage tracing system to report for the first-time generation of mechanosensory neurons in the Johnston's Organ (JO) of adult *Drosophila*, which are functional counterparts to mammalian hair cells. Adult-born JO neurons develop cilia, express an essential mechanotransducer gene and target brain circuitry. Mechanistically, we identified self-replication of JO neurons as an unexpected mode of neuronal plasticity. Furthermore, our system allows identification of compounds increasing the frequency of JO regeneration on intact animals. Overall, our findings introduce a unique platform to expedite the research of mechanisms and compounds mediating mechanosensory cell regeneration, with implications for hearing and balance restoration in humans.

1618A Uncovering a link between H2Av and the cell cycle during early Drosophila embryonic development *Pakinee Phromsiri*[1], Michael Welte[1] 1) The University of Rochester.

During Drosophila oogenesis, histones H2A, H2B, and H2Av (the sole H2A variant in Drosophila) are sequestered on lipid droplets (LDs); this

sequestration promotes stable storage of maternal histones for later use in the embryo. During the syncytial blastoderm stage, LD sequestration also serves a buffering role specifically for H2Av: H2Av continuously exchanges between LDs through the cytoplasm, reducing the rate of H2Av import into the nucleus. Sequestration is mediated by the LD protein Jabba. *Jabba* mutants lack H2Av buffering, overaccumulate H2Av ~two-fold in nuclei, and exhibit increased nuclear falling. We now report that *Jabba* mutants also display abnormal cell cycle regulation, specifically in nuclear cycle (NC) 13. While NC 10-12 are indistinguishable between wild-type and mutant, the mutant embryos complete NC13 1.4 min faster. To determine if nuclear overaccumulation of H2Av is responsible for this phenotype, we examined embryos expressing four copies of *H2Av*. Not only do these embryos overaccumulate nuclear H2Av, they also show a similar speed-up of NC13 as well as increased nuclear falling. We hypothesize that insufficient time to complete replication in NC13 causes DNA damage and thus nuclear falling. To test this idea, we will examine if a shortened NC13 is sufficient to induce nuclear falling. NC13 is the first embryonic cycle to be under the control of the ATR/Chk1 (Mei-41/Grp) checkpoint. In the wild-type, checkpoint activation causes a cell-cycle delay that is necessary for proper completion of NC13. We find that dosage reduction of *grp* speeds up NC13 by ~2 min, indicating that the amount of Grp is limiting; we are now examining nuclear falling in these embryos. To investigate whether *grp* and *H2Av* act in the same pathway, we are now performing genetic interactions tests. As both Grp and H2Av are downstream phosphorylation targets of the kinase Mei-41, they might compete with each other for limited Mei-41 activity; in this case, more H2Av could lead to less phosphorylated Grp, the form that slows cell cycle progression. However, this scenario is unlikely: we generated 4x *H2Av* embryos in which two of the *H2Av* genes express a non-phosphorylatable form of H2Av. These embryos still exhibit a faster cell cycle, similar to 4x *H2Av* embryos with fully wild-type genes. In a complementary approach, we will determine if H2Av overaccumulation results in global gene expression changes that might explain a faster cell cycle progression.

1622B Role of Autophagy and Epidermal Growth Factor Receptor Signaling on Growth Regulation of *Drosophila Melanogaster* Larvae Under Environmental Stress *Xianyu Hao*[1], Julian Martinez-Agosto[1] 1) University of California, Los Angeles, CA.

Organismal growth is often affected by various environmental cues and requires the integration of signals like Target of Rapamycin (Tor) to determine the final body size. However, the molecular mechanisms that regulate organismal growth under environmental stress remain unclear. A previous genetic screen of larval growth regulators identified the epidermal growth factor receptor (EGFR) as a regulator of growth. We hypothesized that EGFR, and one of the downstream targets of Tor signaling, autophagy, play a key role in controlling growth under environmental stress. Here, we disrupted *Drosophila* larval development by starvation or hypoxia treatment and manipulated the autophagy/EGFR genes in the fat body to study the mechanisms of growth restriction and metabolism. Overexpression or downregulation of autophagy activity had no detectable effect on larval growth and metabolism under environmental stress. Overexpression of an EGFR ligand, Spitz (Spi), in the fat body and the muscles resulted in restricted body size and lipid droplet aggregation resembling a similar phenotype to those under environmental stress. Alternatively, Spi downregulation in the fat body showed patchy lipid droplet accumulation across individual cells, which might indicate heterogeneity of fat cell populations. Our findings suggest that Spi could be a negative regulator of organismal growth, in addition to its established roles in promoting cell proliferation and survival. Furthermore, we propose a potential crosstalk between the muscles and the fat body in the systemic regulation of organismal growth. Our findings will shed light on how environmental stressors affect normal development, and ongoing experiments will uncover a novel role of EGFR in fat body patterning and crosstalk between different tissues that contribute to growth control.

1710C Identification of extrinsic cues promoting target-selective axon regeneration *Lauren Walker*[1], Camilo Guevara[1], Michael Granato[1] 1) University of Pennslvania, Philadelphia, PA.

The vertebrate peripheral nervous system has significant capacity for axon regeneration. Regenerating axons must extend over significant distances to reconnect with their original synaptic targets for functional recovery. However, re-establishing a complex trajectory that includes navigating multiple choice points and then selecting the appropriate target long after this circuitry was established during development, represents a unique challenge. To visualize regenerating axons as they navigate stepwise choice points, we first established the larval zebrafish pectoral fin, equivalent to tetrapod forelimbs, as a vertebrate model system in which to study this process. Each pectoral fin is innervated by four motor nerves containing dozens of axons that branch to stereotypically-innervate specific regions of two muscle layers. Using a laser system, we transect the nerves that innervate the fin and monitor axonal regeneration in real time. We have characterized the extensive challenges faced by regenerating axons including sorting at the plexus, choosing the correct muscle layer, selectively fasciculating with the appropriate axonal partners to grow into the fin, and then defasciculating at the appropriate location to reinnervate their original individual muscle fibers. By labeling single or pools of axons, we observe robust and specific regeneration of pectoral fin axons back to their original domains within 48 hours post injury, indicating the existence of local cues within the fin to guide axon regeneration. Work in several systems has focused on neuron-intrinsic factors that promote axon growth, yet less is known about the complement of extrinsic cues that guide regenerating growth cones back to their original targets. To identify local injury-dependent guidance cues in the pectoral fin, we have employed an RNAseq approach. We present results from this RNAseq analysis from denervated fins at timepoints that precede important axon guidance decisions including choosing a muscle layer, sorting at the plexus, and specific target selection. We predict that changes in gene expression may reflect regional cues important for axon growth and guidance and are testing mutants in genes that are upregulated after injury to determine their functional role in axon guidance.

1716C The glycosyltransferase *Lh3* encodes a novel regulator of optic nerve regeneration *Beth Harvey*[1], Melissa Baxter[1], Michael Granato[1] 1) University of Pennsylvania, Philadelphia, PA.

The neuronal Retinal Ganglion Cell (RGC) axons comprising the optic nerve convey visual information from the retina to the brain. Injury to RGC axons leads to irreversible vision loss due to the poor capacity of the mammalian Central Nervous System (CNS) to regenerate. Although manipulation of certain intrinsic signaling pathways can increase RGC axonal regrowth, injured axons often exhibit misguided growth along ectopic paths. Therefore, in order to restore functional optic nerve regeneration, it is critical to identify the extrinsic guidance cues that promote axonal growth along the original correct trajectories. In contrast to mammals, injured zebrafish CNS axons exhibit spontaneous regenerative capacity and provide a unique model to study axonal growth and guidance during regeneration. We recently developed a rapid and robust assay to physically transect the optic nerve and monitor optic nerve regeneration in post-developmental zebrafish (1). We observe axonal regrowth beginning as early as 24 hours post transection, and within 96 hours post transection, RGC axons show robust regrowth onto the optic tectum. Using this assay, we performed a small-scale genetic shelf screen to identify molecular mechanisms underlying spontaneous optic nerve regeneration. From this ongoing screen, we identified several genes that promote directed RGC axonal growth, including the gene encoding the collagen glycosyltransferase Lh3. In *lh3* mutants, regenerating RGC axons fail to cross the CNS midline and instead extend along ectopic trajectories, ultimately failing to reach the optic tectum.

Using a heat inducible conditional allele, we demonstrate that *lh3* functions during the process of active optic nerve regeneration. From our screen, we also found that mutants of *collagen 18a1* (*col18a1*) but not *col4a5* show optic nerve regeneration defects similar to those observed in *lh3* mutants. To further understand the early dynamics of RGC axonal regrowth, we will perform live timelapse imaging of RGC axons following optic nerve transection. We will also present results from ongoing experiments to understand the role of glia in RGC regrowth and to identify the cellular and molecular mechanism through which Lh3 promotes spontaneous optic nerve regeneration.

1. Harvey, B. M., Baxter, M., and Granato, M. (2019). Optic nerve regeneration in larval zebrafish exhibits spontaneous capacity for retinotopic but not tectum specific axon targeting. PloS one, 14(6), e0218667.

Thursday, April 30 12:00 PM - 3:00 PM

Developmental Genetics 3 - Poster Q&A 1450A SPE-51, a secreted protein with Ig-like fold, is required for sperm-egg fusion in *C. elegans* *Xue Mei*[1], Marina Druzhinina[1], Gunasekaran Singaravelu[1], Diane Shakes[1], Andrew Singson[1] 1) Waksman Institute of Microbiology.

Fertilization is central to sexual reproduction during which gametes undergo species-specific recognition, adhesion and eventually fuse to form a zygote. The molecular basis of fertilization is not well understood. In a forward genetics screen for fertility mutants in *Caenorhabditis elegans* we identified the *spe-51* gene. Mutant worms make sperm that are unable to fertilize the oocyte but otherwise are normal by all available measurements. The *spe-51* gene encodes a secreted protein that includes an immunoglobulin (Ig)-like fold and a hydrophobic sequence of amino acids. The cell autonomous mutant behavior of *spe-51* mutant sperm suggest that this sperm-secreted protein stays associated with the sperm cell surface and functions either in cis on the sperm surface or in trans with the egg cell surface to complete fertilization. This is the first example of a secreted protein required for the interactions between the sperm and egg with genetic validation for a specific function in fertilization. Our analysis of this gene could serve as a paradigm for mammalian sperm-secreted or reproductive tract-secreted proteins that coat the sperm surface and influence their survival, motility, and/or the ability to fertilize the egg.

1604B Germ cell division and encapsulation by somatic cells during *Drosophila* oogenesis require the orphan nuclear receptor *ftz-f1* *Allison Beachum*[1], Samantha McDonald[1], Elizabeth Ables[1] 1) East Carolina University.

Gamete production in mammals and insects is intimately tied to organismal physiology. However, how nutritionally-dependent physiological signals are integrated and the molecular mechanisms they control in oogenesis remains largely uncharacterized. Nuclear receptors (NRs) link physiological status to a cellular transcriptional response and are important mediators of reproduction, physiology, and tissue homeostasis. For example, mammalian NR5 family members *SF-1* and *LRH-1* are essential for gonadogenesis and sex steroid production. Two NR5 family members are encoded in the *Drosophila* genome: *Hr39*, whose role is limited to female reproductive tract development, and *ftz-f1*, whose role in oogenesis has not been explored. Given that *Hr39* is not intrinsically required for oogenesis, we hypothesized that *ftz-f1* may fill a conserved NR role in *Drosophila*. *Ftz-f1* is expressed throughout the ovary, including in germline stem cells (GSCs), germline cysts, and several populations of somatic cells. Here, we demonstrate that *ftz-f1* is essential for female fertility. Germline-specific knockdown of *ftz-f1* resulted in fewer GSCs as female flies aged. Loss of *ftz-f1* was correlated with the accumulation of cystoblasts/two cell cysts and delayed mitotic cyst divisions. Interestingly, our data demonstrate that *ftz-f1* is also necessary in somatic escort cells for GSC maintenance, cyst division, and cyst encapsulation. We also provide evidence to support the hypothesis that *ftz-f1* in escort cells cooperates with ecdysone signaling to regulate germ cell division and differentiation. Together, these results add to a growing body of literature underscoring the importance of nuclear receptors in the control of reproduction.

1605C Investigating the Predicted Enzymatic Activity of Asteroid in *Drosophila* Oogenesis and DNA Repair Corinne Croslyn*[1], Arjun Kharel*[1], Julie Merkle[1] 1) University of Evansville, Evansville, IN.

The intricate process by which gametes are formed from the germline stem cells is a fundamental question in biology. In *Drosophila*, oogenesis begins by asymmetric division of the germline stem cells, and ultimately produces a cyst of 16 cells surrounded by a layer of somatic cells. One of these 16 cells is selected as the oocyte, the future egg, while the remaining become supporting cells. A genetic screen in *Drosophila* identified mutations in several evolutionarily conserved genes that result in a failure of oocyte fate determination, leading to loss of mature eggs and fertility. Strikingly, when the germline cells are mutant for *asteroid* (*ast*), one of the genes identified in this screen, the resulting cysts contain no oocyte. Further characterization of *asteroid* mutants revealed a persistence of double-stranded DNA breaks (DSBs) during meiosis. Interestingly, the protein encoded by *ast* and its human ortholog (ASTE1) both contain XPG domains, suggesting they are nucleases involved in DNA repair. Additionally, ASTE1 is mutated in a subset of patients with colorectal cancers, although its molecular function is unknown. To study the role of Asteroid *in vivo* and *in vitro*, biochemical and genetic experiments are currently underway. These studies aim to address the necessity of *ast*'s predicted nuclease domain during *Drosophila* oogenesis, as well as to examine the enzymatic activity of Asteroid and ASTE1 *in vitro*. Preliminary observations suggest that deletion of the XPG domain in *Drosophila* does not affect oogenesis, although confirmation of this CRISPR-induced allele is underway. Additionally, the biochemical characterization of Ast protein has been challenging thus far, but these efforts are ongoing and aim to similarly assess the enzymatic activity of human ASTE1. Further investigation of *asteroid* and *ASTE1* will shed much needed light on oocyte fate determination, as well as their roles in DNA repair, and possibly cancer.

1669A A novel role for *Eip74EF* in male reproduction in promoting sperm elongation at the cost of fecundity Sharif Chebbo[1], Sarah Josway[1], John Belote[2], *Mollie Manier*[1] 1) George Washington University; 2) Syracuse University.

Spermatozoa are the most morphologically variable cell type, yet little is known about genes controlling natural variation in sperm shape. *Drosophila* fruit flies have evolved the longest sperm known, which are evolving under postcopulatory sexual selection, driven by sperm competition and cryptic female choice. Long sperm outcompete short sperm but primarily when females have long seminal receptacles (SRs), the primary sperm storage organ. Thus, selection on sperm length is mediated by SR length, and the two traits are coevolving across the *Drosophila* lineage, driven by a genetic correlation and fitness advantage of long sperm and long SR genotypes in both males and females. *Ecdysone induced protein 74EF* (*Eip74EF*) is rapidly evolving under positive selection in *Drosophila*, and it is expressed during post-meiotic stages of spermatogenesis, when spermatid elongation occurs. Partial knockdown of *Eip74EF* leads to shorter sperm but does not affect SR length, suggesting that *Eip74EF* is involved in promoting spermatid elongation but is not a genetic driver of male-female coevolution. We also found that *Eip74EF* knockdown has opposing effects on

fecundity in males and females, with an increase in fecundity for males but a decrease in females, consistent with its documented role in oocyte maturation. It is possible that knockdown males produce more sperm that are also shorter, which would explain the increase in fecundity, but this hypothesis remains to be tested. Our results document a novel function of *Eip74EF* in spermatogenesis and demonstrates that this gene influences both male and female reproductive success.

1677C Functional characterization of Ifih1 and Dhx29 during early *Xenopus laevis* development *Daron Barnard*[1], Elizabeth Devaney[1], Serena Iacovelli[1], Morgan Nelson[1] 1) Worcester State University, Worcester, MA.

Xenopus laevis oocytes are held in prophase of meiosis I and are induced to resume meiosis by progesterone, in a process known as maturation. The mechanisms involved in the maintenance of the inactive state and the resumption of meiosis require maternal mRNAs to be held in a silenced state and subsequently activated for translation, since there is no transcription of new mRNAs during this time. Early developmental events must also rely on regulated translation of maternal mRNAs, as the absence of transcription continues in the embryo until it reaches the maternal-zygotic transition (MZT). The process of cytoplasmic polyadenylation has been shown to play an important role in activating these mRNAs and control of early developmental events. During investigations into the mechanism of cytoplasmic polyadenylation, it was observed that there was a cap-specific 2'-O methylation of the second and third nucleotides at the 5' end of the mRNAs. While required, the reason for this modification is not yet understood. Interestingly, most work in this area of mRNA modification comes from the field of virology in which researchers have shown that 2'-O methylation of these nucleotides is required in the determination of which mRNAs are "self" rather than "non-self". The recognition of "non-self" and resulting translational repression can be mediated by several proteins, including the Interferon induced, with helicase C domain 1 protein (Ifih1; also known as Mda5 or Rrl-2). We have demonstrated that the mRNAs for both Ifih1 as well as its known co-sensor and interacting protein, DEAH-box helicase 29 (Dhx29*),* are found in *Xenopus laevis* oocytes, suggesting that the proteins have a role in early development. We are working to characterize the function of these proteins during oocyte maturation and early development through inhibition and over-expression studies.

1679B Kfc1 is a novel meiotic regulator required for meiosis that engages in a function specific interaction with Kar4 *Zachory Park*[1], Matthew Remillard[2], Mark Rose[1] 1) Georgetown University; 2) Princeton University.

Meiosis serves as a generator of genetic diversity that results in the formation of specialized cells called gametes that convey genetic material across time and space. In budding yeast, meiosis or sporulation also functions as an escape from extreme environmental stress. Ygl036w is a previously uncharacterized protein with no annotated function or discernable domains except for several intrinsically disordered regions. High throughput studies from our lab and others showed that Ygl036w interacts with the meiotic proteins Kar4 and Mum2, suggesting a role for Ygl036w in meiosis. We found that Ygl036w is essential for meiosis and required early before pre-meiotic S-phase. Previous separation of function screens identified alleles of Kar4 that were defective for two independent meiotic functions. The defect associated with the first function (Mei) is suppressed by over-expression of the master meiotic transcription factor *IME1*. Suppression of the second function (Spo) requires the over-expression of an additional gene, *RIM4*, encoding a translational regulator. Using the Mei- and Spo- alleles, we asked if Ygl036w engages in a function specific interaction with Kar4. We found that Kar4 mutants specifically defective for the Spo function are unable to interact with Ygl036w. Accordingly, we propose a new name for *YGL036w*, *KFC1* for *KAR4 Collaborator 1*. The necessity of *RIM4* over-expression to suppress Kar4's Spo function suggests that the Kfc1/Kar4 complex is involved in post-transcriptional regulation. Here, we further characterize the *kfc1*Δ/Δ meiotic defect at the cellular level, probe Kfc1's protein interactions, and present evidence of a role for Kfc1 in post-transcriptional regulation during meiosis.

1724B Roles of Adamts9 in germ cell migration, ovary development and ovulation in zebrafish *Jonathan Carver*[1], Nichole Carter[1], Yong Zhu[1] 1) East Carolina University, Greenville, NC.

Involvement of Adamts9 (a disintegrin and metalloprotease with thrombospondin type-1 motif, member 9) in germ cell migration has been demonstrated in C elegans and Drosophila and dramatic changes of Adamts9 expression has been shown recently during the ovulation in zebrafish and human. The roles of Adamts9 during germ cell migration and ovulation have not been determined in any vertebrates, partly due to embryonic lethality in Adamts9 knockout mice. To determine the roles of Adamts9, we generated knockout (adamts9−/−) zebrafish using CRISPR/Cas9 and characterized the effects of the mutation. Adamts9 knockout did not affect survival during the embryonic development, though knockout affected survival during the juvenile and adult zebrafish. From 1047 fish generated by crossing adamts9+/− pairs, we found significantly fewer adult adamts9−/− fish (4%) than predicted by Mendelian ratios (25%). Of the mutants found, there was a significant male bias (82%). Only 3 female mutants were identified (7%), and they had small ovaries with few stage III and IV oocytes compared to wildtype (wt) counterparts of comparable size and age. Astoundingly, the remaining mutants (11%) did not appear to have normal testis or ovaries. Instead there was a pair of transparent, ovarian-like membranous shells that filled the abdominal cavity. Histological examination confirmed that shells were largely empty with no internal structure. Surprisingly, seminiferous tubules and various spermatocytes including mature spermatozoa were observed on the periphery of these transparent shells. No female or female like knockouts were observed to release eggs, and no ovulated oocytes were observed in histological sections. Further studies found germ migration delay in adamts9-/- as germ cells were more distant to each other between 15 and 48 hpf, which may affect germ cell survival and lead to late defect in sex ratios and ovarian development. To our knowledge, this is the first report of an adamts9 global knockout model in any adult vertebrates and the first description of how gonadal sex and structure are affected-highlighting the importance of Adamts9 during germ cell migration, gonadal development, ovulation and the value of zebrafish as a model organism.

Friday, May 1 12:00 PM - 2:30 PM

Evolutionary and Population Genetics 1 - Poster Q&A 986B Evolution of boldness and exploratory behavior in giant mice from Gough Island *Jered Stratton*[1], Mark Nolte[1], Bret Payseur[1] 1) University of Wisconsin - Madison.

Organisms on islands often evolve extreme phenotypes. Novel environmental conditions such as a lack of predators can shift long-standing adaptive peaks to new optima. House mice from Gough Island are a prime example of rapid and extreme phenotypic evolution following island colonization. These mice have nearly doubled in body size and frequently predate on nesting seabirds. We hypothesized that a lack of both natural predators and human commensals stimulated the evolution of increased boldness and exploration in mice from Gough Island. To test this hypothesis, we conducted a series of behavioral tests in a controlled laboratory setting using wild-derived inbred strains of mice from Gough Island and the Eastern United States. Open field tests show Gough Island mice are more active and spend more time in open, brightly lit areas than mainland

mice. These differences are more pronounced after sexual maturity, suggesting a life-history component is involved. Nevertheless, a predator-cue test demonstrates that Gough Island mice and mainland mice have similar aversions to fox urine. Phenotypes of F1s from crosses between Gough Island mice and mainland mice suggest an additive genetic basis for behavioral evolution. These results indicate Gough Island mice have evolved a reduction in anxiety-like behaviors in potentially risky environments but have retained pathways involved in response to direct predator cues. Our findings lay the foundation for genetic dissection of behavioral evolution in a natural population of island mice.

995B Finding the genetic underpinnings of an ancient thermotolerance divergence in yeast *Faisal AlZaben*[1], Carly Weiss[2], Julie Chuong[1,3], Melanie Abrams[1], Rachel Brem[1,3] 1) UC Berkeley; 2) Stanford University; 3) Buck Institute for Research on Aging.

Saccharomyces cerevisiae and its closest relative, Saccharomyces paradoxus are two closely related species of yeast that differ with respect to thermotolerance. Weiss et. al (2018) found that S. cerevisiae alleles of eight genes confer a modest growth advantage at 39°C when they replace the native allele at the endogenous locus in an S. paradoxus background by creating an interspecific hybrid of the two species and subjecting it to transposon mutagenesis. While each S. cerevisiae allele has a modest effect on its own, the effects of the alleles in combination are unknown. We suspect that the effect exerted by these alleles may depend on the genomic context, interactions between these very alleles, or evolutionary contingencies. Several potential models may explain the phenotype of the S. paradoxus containing all eight of the S. cerevisiae alleles that are implicated in thermotolerance. This project aims to examine the phenotype of this “Mega-Swap”, as well as to identify the potential genetic contingencies that these thermotolerance allele depend on, since previous work provides evidence that their benefit is temperature-specific.

996C A limited impact of recessive deleterious variants on signals of adaptive introgression in human populations *Xinjun Zhang*[1], Bernard Kim[2], Kirk Lohmueller[1], Emilia Huerta-Sanchez[3] 1) University of California, Los Angeles, CA; 2) Stanford University, Palo Alto, CA; 3) Brown University, Providence, RI.

Several gene regions have been previously identified to be candidates of adaptive introgression (AI) that facilitated human adaptation to specific environments. However, simulation-based studies have suggested that other population genetics processes without beneficial mutations, such as the heterosis effect created by different sets of recessive deleterious variants in each population coming together, can also lead to an increase in introgressed ancestry and patterns of variation which resemble adaptive introgression. The extent to which the presence of deleterious variants impact the accuracy and the power of current methods to detect AI has not been fully assessed for human populations. Here, we used extensive simulations to show that recessive deleterious mutations can increase the false positive rates for AI tests compared to models without deleterious variants. We further examined candidates of AI in modern humans identified from previous studies and show that although most signals remain robust when deleterious variants are considered, we found that two AI candidate genes, *HYAL2* and *HLA,* are particularly susceptible to high false positive rates due to deleterious variation. We show that high rates of false positives are primarily associated with high exon density and low recombination rates, which can be accentuated by the rapid population growth in recent human evolution. Although the combination of such parameters is rare in the human genome, caution is still warranted in other species with different genomic structure and demographic histories. Lastly, based on our findings, we present a novel machine learning-based method to detect genome-wide AI that accounts for the effect of deleterious variants. We demonstrate the power of our new approach on simulated data as well as human genetic variation data. We confirm the signals of a number of previously reported AI candidate genes, and find additional candidate loci.

1012A The genome of the Devil Worm, a subterrestrial nematode, reveals an evolutionary strategy for adaptation to heat *John Bracht*[1], Deborah Weinstein[1], Sarah Allen[1], Maggie Lau[2], Mariana Erasmus[3], Kathryn Asalone[1], Kathryn Walters-Conte[1], Gintaras Deikus[4], Robert Sebra[4], Gaetan Borgonie[5], Esta van Heerden[3], Tullis Onstott[2] 1) Department of Biology, American University, Washington DC 20016, USA; 2) Department of Geosciences, Princeton University, Princeton, NJ 08544, USA; 3) UFS/TIA Saense Platform, Department of Microbial, Biochemical, and Food Biotechnology, University of the Free State, Bloemfontein 9301, South Africa; 4) Department of Genetics and Genomic Sciences and Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA; 5) Extreme Life Isyensya, Gentbrugge, 9050 Belgium.

The 'Devil Worm', *Halicephalobus mephisto,* is a nematode originally discovered inhabiting a deep terrestrial aquifer 1.3 km underground. *H. mephisto* can thrive under conditions of abiotic stress including heat and minimal oxygen, where it feeds on a community of both chemolithotrophic and heterotrophic prokaryotes in an unusual ecosystem isolated from the surface biosphere. Here we report the comprehensive genome and transcriptome of this organism, identifying a signature of adaptation: an expanded repertoire of 70 kilodalton heat-shock proteins (Hsp70) and avrRpt2 induced gene 1 (AIG1) proteins. The expanded Hsp70 genes are transcriptionally induced upon growth under heat stress, and we find that positive selection is detectable in several members of this family. We further show that AIG1 may have been acquired by horizontal gene transfer (HGT) from a rhizobial fungus. Over one-third of the genes of *H. mephisto* are novel, highlighting the divergence of this nematode from other sequenced organisms. This work sheds light on the genomic basis of heat tolerance in a complete subterrestrial eukaryotic genome. We also show that heat-adapted bivalves convergently share the expanded Hsp70 and AIG1 gene families, suggesting that the pattern identified in the Devil Worm may be a general evolutionary strategy for adaptation to heat.

1014C Simulating the impact of Neandertal introgression on the distribution of fitness effects of human genetic variation *Sara Carioscia*[1], Rajiv McCoy[1] 1) Johns Hopkins University, Baltimore, MD.

Understanding how natural selection has shaped the distribution of fitness effects of standing genetic variation is a major goal of evolutionary genetics. Hominin admixture offers a useful model, given the hypothesized differences in population size between modern and archaic humans, as well as the consequent differences in the strength of genetic drift and efficacy of purifying selection in each of these populations. Specifically, studies have suggested that Neandertal populations accumulated a load of weakly deleterious mutations due to prolonged demographic bottlenecks. We thus investigated how introgression from archaic hominins contributed to the distribution of fitness effects of contemporary human genetic variation.

Population genetic simulations allowed us to examine potential effects of introgression and investigate its dependence on various evolutionary parameters, such as rates of admixture and fitness effects of new mutations. We modeled human evolutionary history using the forward genetic simulation software package SLiM, incorporating negative selection. We built upon published models of human demographic history to include divergence ~550 Kya of a small Neandertal population (Ne = 1,000) from a large modern human population (Ne = 10,000) and introgression ~55 Kya of Neandertals into a Eurasian population. Fitness effects of deleterious mutations were drawn from a gamma distribution.

We contrasted the distributions of fitness effects of mutations that introgressed from the Neandertal and arose in modern human populations, con-

trolling for allele frequency. We found that Neandertal-originating mutations were modestly enriched for deleterious effects ($p < 0.001$) compared to a background of modern human variation of similar age, reflecting the impact of genetic drift in the small Neandertal population. In contrast, rare, recent mutations originating in modern humans were enriched for deleterious effects ($p < 0.001$) compared to frequency matched archaic variation. These findings together demonstrate that rare, recent mutations make an outsize contribution to deleterious fitness effects of segregating human variation. This corroborates recent work suggesting that rare and even singleton mutations explain a large proportion of the heritability of complex traits and disease. This conclusion also raises the concern that some trait associations previously attributed to archaic admixture could instead be driven by recent and rare modern human mutations that segregate in linkage disequilibrium with archaic haplotypes. Fine-mapping and functional studies offer avenues for testing this hypothesis. Together, our findings provide a theoretical foundation to guide ongoing studies of the functional and fitness impacts of archaic introgression and comparisons with mutations of recent modern human origin.

1034B Sex-specific phenotypic effects and evolutionary history of an ancient deletion polymorphism of the human growth hormone receptor *Marie Saitou*[1], Skyler Resendez[1], Laura Parisi[1], Shigeki Nakagome[2], Yoko Satta[3], Gunes-Ekin Atilla-Gokcumen[1], Xiuqian Mu[1], Omer Gokcumen[1] 1) University at Buffalo; 2) Trinity College Dublin, Ireland; 3) Sokendai, Japan.

The deletion of the third exon of the growth hormone receptor (GHRd3) is one of the most common genomic structural variants in the human genome. This deletion has been linked to response to growth hormone, placenta size, birth weight, growth after birth, time of menarche, adult height, and longevity. However, its evolutionary history and the exact mechanisms through which it affects phenotypes remain unresolved. While the analysis of thousands of genomes suggests that this deletion was nearly fixed in the ancestral population of anatomically modern humans and Neanderthals, it underwent a paradoxical adaptive reduction in frequency approximately 30 thousand years ago, a demographic signature that roughly corresponds with the emergence of multiple modern human behaviors and a concurrent population expansion. Using a mouse line engineered to contain the deletion, pleiotropic and sex-specific effects on organismal growth, the expression levels of hundreds of genes, and serum lipid composition were documented, potentially involving the nutrient-dependent mTORC1 pathway. These growth and metabolic effects are consistent with a model in which the allele frequency of GHRd3 varies throughout human evolution as a response to fluctuations in resource availability. The last distinctive prehistoric shift in allele frequency might be related to newly developed technological buffers against the effects of oscillating resource levels.

1048A A likelihood approach for uncovering selective sweep signatures from haplotype data *Alexandre Harris*[1], Michael DeGiorgio[2] 1) Pennsylvania State University, University Park, PA; 2) Florida Atlantic University, Boca Raton, FL.

Selective sweeps are frequent and varied signatures in the genomes of natural populations, and detecting them is consequently important in understanding mechanisms of adaptation by natural selection. Following a selective sweep, haplotypic diversity surrounding the site under selection decreases, and this deviation from the background pattern of variation can be applied to identify sweeps. Multiple methods exist to locate selective sweeps in the genome from haplotype data, but none leverage the power of a model-based approach to make their inference. Here, we propose a likelihood ratio test statistic *T* to probe whole genome polymorphism datasets for selective sweep signatures. Our framework uses a simple but powerful model of haplotype frequency spectrum distortion to find sweeps and additionally make an inference on the number of presently sweeping haplotypes in a population. We found that the *T* statistic is suitable for detecting both hard and soft sweeps across a variety of demographic models, selection strengths, and ages of the beneficial allele. Accordingly, we applied the *T* statistic to variant calls from European and sub-Saharan African human populations, yielding primarily literature-supported candidates, including *LCT*, *RSPH3*, and *ZNF211* in CEU, *SYT1*, *RGS18*, and *NNT* in YRI, and *HLA* genes in both populations. We also searched for sweep signatures in *Drosophila melanogaster*, finding expected candidates at *Ace*, *Uhg1*, and *Pimet*. Finally, we provide open-source software to compute the *T* statistic and the inferred number of presently sweeping haplotypes from whole-genome data.

1054A Geographic variation for tissue-specific developmental plasticity in natural Drosophila melanogaster populations *Katherine Rickleton*[1], Sophia Davis[1], Nossin Khan[1], Hayley Sheehy[1], Ryan Hatmaker[1], David Begun[1], Didem Sarikaya[1] 1) University of California Davis.

Phenotypic plasticity plays a significant role in adaptation, especially in animals that are exposed to heterogeneous habitats. Exposure to different environmental conditions during development can yield varying morphologies through a process called developmental plasticity. Previous studies in Drosophila have shown that temperature and nutrition during development affect body size, but each organ responds to environmental conditions in a unique manner. However, whether there are population differences in organ-specific developmental plasticity remains unclear. To investigate whether local adaptation to temperate and tropical environments affects the evolution of developmental plasticity, we estimated the effect of temperature and nutrition on wing and femur size in Drosophila melanogaster populations from Maine and Panama City. Population lines were reared at either 21C or 25C on standard lab food or diluted nutrient poor food. While it is generally hypothesized that temperate populations display higher level of plasticity, body size plasticity index was higher for the temperate population only under starved conditions at 25C. Other developmental conditions yielded varying levels of plasticity. Similar to previous studies, we found that wings were more sensitive than femurs to changes in temperature. While wing size was consistently larger in the temperate population than the tropical population, femur lengths were similar for both populations under several conditions. Therefore, wing size appears to evolve faster than femur size in these populations. These patterns suggest that developmental plasticity of body size evolves in an organ-specific manner in natural D. melanogaster populations.

1082B Stronger and higher proportion of beneficial amino acid changing mutations in humans compared to mice and Drosophila *Ying Zhen*[1,2], Christian Huber[2], Robert Davies[3], Kirk Lohmueller[2] 1) Westlake University; 2) University of California, Los Angeles, CA; 3) Hospital for Sick Children, Toronto, Canada.

Quantifying and comparing the amount of adaptive evolution among different species is key to understanding evolutionary processes. Previous studies have shown differences in adaptive evolution across species; however, their specific causes remain elusive. Here, we use improved modeling of weakly deleterious mutations and the demographic history of the outgroup species and estimate that at least 20% of nonsynonymous substitutions between humans and an outgroup species were fixed by positive selection. This estimate is much higher than previous estimates, which did not correct for the population size of the outgroup species. Next, we directly estimate the proportion and selection coefficients (p^+ and s^+, respectively) of newly arising beneficial nonsynonymous mutations in humans, mice, and Drosophila by examining patterns of polymorphism and divergence. We develop a novel composite likelihood framework to test whether these parameters differ across species. Overall, we reject a model with the same p^+ and s^+ of beneficial mutations across species, and estimate that humans have a higher p^+s^+ compared to Drosophila and mice. We

demonstrate that this result cannot be attributed to biased gene conversion or hypermutable CpG sites. In summary, we find the proportion of beneficial mutations to be higher in humans than in Drosophila or mice, suggesting that organismal complexity, which increases the number of steps required in adaptive walks, may be a key predictor of the amount of adaptive evolution within a species.

1084A Evolutionary Constraints and the Distribution of Beneficial Mutational Effects in *Saccharomyces* Vineyard Adaptation *Emery Longan*[1], Justin Fay[1] 1) University of Rochester.

Evolutionary constraints can limit adaptation by natural selection. The distribution of mutational effects (DME) can impose meaningful constraints on adaptive evolution if available beneficial mutations are rare, of small effect, or very costly. Differences between closely related species in their capacity to adapt to a novel environment may therefore be attributable to differences in the beneficial mutations accessible to them including their number, effect size, and/or associated pleiotropic effects. In vineyards, *S. cerevisiae* has evolved copper and sulfite resistance whereas its sister species, *S. paradoxus*, has not. To test whether adaptation to the vineyard environment can be explained by differences in the DME, we mutagenized copper and sulfite sensitive isolates of both species and recovered mutants displaying elevated resistance to these stressors. These mutants were then subjected to high-throughput, high-resolution phenotyping on media containing the respective chemicals and in permissive conditions. We found that mutations conferring resistance, both spontaneous and induced, occur at comparable rates between the two species. Contrary to expectations, *S. paradoxus* mutants had a systematically larger effect on copper resistance than *S. cerevisiae* mutants, but they also tended to incur a significantly greater cost in permissive conditions. Further, of the 1298 mutants that were phenotyped, none displayed copper resistance on the order of domesticated strains that have tandem duplications of *CUP1*, indicating that evolutionary constraints likely hinder both species with respect to acquisition of high copper resistance. Overall, these experiments offer the insight that sister species may have similar rates of beneficial mutations when faced with a novel stressor, but the magnitude of their effect and their pleiotropic costs may be more labile components of the DME.

1086C A meta-analysis suggests different adaptive mechanisms between clinal and seasonal adaptation in *D. melanogaster* *Yang Yu*[1], Alan Bergland[1] 1) University of Virginia.

Populations of short-lived organisms, such as *Drosophila melanogaster*, can respond to spatial and temporal environmental heterogeneity through local adaptation. Although some complex fitness-related traits, as well as many alleles, have been shown to vary across clines and seasons in similar ways in *D. melanogaster* populations, we still lack an understanding of the adaptive mechanisms between clinal and seasonal adaptation. Due to the complex environmental differences between clinal and seasonal conditions, and previous evidence showing that there is only limited overlap between clinal and seasonal SNPs, we hypothesize that, the adaptive mechanisms between clinal and seasonal adaptation should be different. To test our hypothesis, we performed a meta-analysis using diverse gene expression profiles, which provides a systematic link between phenotypic and genetic variations, and allele frequency information of expression quantitative trait loci (eQTL) in multiple clinal and seasonal populations. Our analysis reveals many different features between clinal and seasonal adaptation at both genomic and tissue levels. Furthermore, our results suggest that, at the genetic level, clinal adaptation is in line with the omnigenic model while seasonal adaptation is more likely to follow the infinitesimal model. Such distinction of the adaptive models highlights the importance of studying clinal and seasonal adaptation under biological contexts in different ways, and might advance our understanding of how functional genetic variations are maintained across space and time.

1087A Continuous backslopping cycles result in genome evolution in Trappist beer yeasts. *Andrea Del Cortona*[1,2,3], Jonathan Gordon[1,2,3], Brigida Gallone[1,2,3], Stijn Mertens[1,2,3], Jan Steensels[1,2,3], Kevin Verstrepen[1,2,3] 1) VIB–KU Leuven Center for Microbiology, Leuven, Belgium; 2) CMPG Laboratory of Genetics and Genomics, Department M2S, KU Leuven, Leuven, Belgium; 3) Leuven Institute for Beer Research, Gaston Geenslaan 1, 3001 Leuven, Belgium.

For thousands of years, humans have exploited the ability of baker yeast *Saccharomyces cerevisiae* to convert sugars into ethanol for brewing alcoholic beverages. Ancient brewers were already aware that using leftover yeast to start the next fermentation (backslopping) would result in more consistent quality of the fermentation. Continuous backslopping cycles until the end of 1800s resulted in the domestication of the baker yeast and its adaptation to the brewing environment. With the isolation of pure yeast strains and the introduction of frozen yeast stocks, the evolution of the yeast within the brewing environment was interrupted. In modern brewing the fermentation is started from a frozen stock of yeast and the cycles of backslopping are limited so that fermentation performances and quality of the brew are systematically maintained. The genetic alterations associated with continuous backslopping have not yet been characterized. However, understanding how backslopping shapes yeast genomes and brewing performances would allow to tailor superior yeast strains and result in economic benefits for the brewers. We have investigated the evolution of two distinct Trappist yeasts used for up to three years of continuous backslopping in the brewery. Yeast populations and individual clones were sampled from the yeast slurries during the initial brew and after each year of continuous backslopping. Populations genomics analyses were performed with cutting-edge sequencing technologies, and all samples were phenotyped. Our results indicated that the initial populations were heterogeneous and experienced an initial selective pressure during the first year of backslopping. Afterwards, we observed large chromosomal rearrangements in the yeast genome and the emerging of a late more heterogeneous population due to the accumulation of a high number of widespread mutations. Altogether, our results provide key insights on the evolution of yeast genome in the brewing environment and provided the foundation for breeding superior industrial yeasts.

1090A Application and development of the Z_α suite of statistics for identifying regions of the genome under selection *Clare Horscroft*[1,3], Reuben Pengelly[1,3], Timothy Sluckin[2,3], Andrew Collins[1,3] 1) Faculty of Medicine, University of Southampton, UK; 2) Mathematical Sciences, University of Southampton, UK; 3) Institute for Life Sciences, University of Southampton, UK.

The search for regions of the genome that are experiencing selective pressure is of ongoing interest to researchers as this can answer questions about our history, our present and our future. Knowledge of how we have evolved in response to selective pressures, such as new diets, environmental changes and exposure to pathogens, is not just of interest to evolutionary biologists but also to medical researchers, such as those in the fields of immunology, disease susceptibility and drug metabolism.

The methods presented here use statistics based on fluctuations in the correlations between alleles, i.e. linkage disequilibrium (LD). As a beneficial mutation sweeps to fixation in a population, nearby alleles will sweep with it, causing detectable patterns in the LD between alleles. The Z_α suite of statistics presented here exploit these correlations to identify regions of the genome that may be under selective pressure. A novel addition to these statistics is the option to adjust for expected LD by supplying an LD profile. The LD profile is a lookup table providing the expected LD given

the genetic distance between two loci. LD fluctuations are ubiquitous and caused naturally by factors such as variable recombination rates and genetic drift. Therefore, the ability to adjust for expected background LD given the genetic distance between polymorphisms increases the power of the statistics. A further benefit of these statistics is the ability to combine them, by adding or multiplying them for example. This has the potential to uncover further information about a sweep, for instance Z_α/Z_β could be used to ascertain the progression of a sweep. The statistics presented here are now available in an efficient, easy to use and open-source R package.

The methods are applied to real and simulated data. The simulation study demonstrates the proof of concept for the statistics and shows the effectiveness of including the LD profile adjustment. The statistics are then applied to real genomic data with some potential regions under selective pressure highlighted. Further rigorous testing and development of these statistics under a range of scenarios will be undertaken, with the aim of improving results, reliability and reproducibility. These statistics will be applied to further studies to find evidence for natural selection in other populations and species.

1200C Machine Learning with Digital Signal Processing for Classification of Mouse Genotypes *Kathleen Hill*[1], Gurjit Randhawa[2], Nicolas Boehler[1], Hailie Pavanel[1], Ali Coyle[1], Pok Wan[1], Lila Kari[3] 1) Biology Department, Western University, London Ontario; 2) Computer Science Department, Western University, London Ontario; 3) Computer Science Department, Waterloo University, Waterloo Ontario.

The mouse provides an excellent model for testing the accuracy of a new approach to the classification of genetic backgrounds based on signatures of single nucleotide polymorphic [SNP] genotypes. There exists a broad spectrum of known breeding schemes for mice that results in an extensive diversity of genetic backgrounds. High-resolution SNP genotyping arrays such as the Mouse Diversity Genotyping Array provide genotype data for hundreds of thousands of loci and publicly available array data exist for close to 2,000 mice from wild-derived, outbred, recombinant inbred and classical laboratory mouse genetic backgrounds. A combination of supervised Machine Learning with Digital Signal Processing [ML-DSP] offers an alignment-free software tool for ultrafast, accurate, and scalable genome classification thus far successfully tested at all taxonomic levels but not previously applied within a species for SNP genotype data. We tested this new application of ML-DSP by classifying 743 mouse SNP genotypes across the different categories of genetic backgrounds and achieved 96% as a maximum classification accuracy score using a linear discriminant classifier. We tested several one-dimensional numerical representations of the SNP genotypes for the mouse genome in addition to the two-dimensional Chaos Game Representation. The 'AB only" [heterozygous genotypes = 1, homozygous and no call SNP genotypes = 0] representation gave the highest accuracy in the classification of the mouse genetic backgrounds. ML-DSP is superior in classification accuracy, speed, and scalability to larger datasets and will prove relevant in classifying new, unknown and wild-caught mouse genetic backgrounds. This successful proof-of-concept is also relevant toward application in the classification of human haplotypes based on SNP genotype data and with as yet to be tested applications in studies of inherited disease, environmental mutagenesis, neurodegeneration and cancer.

1201A Reduced hair quantity is accompanied by convergent evolutionary rate acceleration in relevant genes and conserved noncoding elements across the mammalian phylogeny *Amanda Kowalczyk*[1, 2], Nathan Clark[3], Maria Chikina[1] 1) University of Pittsburgh, Pittsburgh, PA; 2) Carnegie Mellon University, Pittsburgh, PA; 3) University of Utah, Salt Lake City, UT.

Elucidating genotype-phenotype relationships is a key question in biology. One way to answer this question is a computational method called RERconverge that performs a genome-wide scan to find associations between evolutionary rates of genetic elements and convergently-evolving traits. As similar traits evolve independently in different species, similar selective pressure shifts in genetic sequence can accompany and drive phenotypic changes. RERconverge detects these selective pressure shifts as quantified by shifts in evolutionary rate.

RERconverge was used to identify the genetic basis of reduced hair quantity in mammals using sequence for protein-coding genes and approximately 350,000 noncoding regions. Several mammals, including whales, elephants, and the naked mole-rat, have reduction in body hair, and many genes and regulatory elements associated with hair growth are known. RERconverge successfully identified such elements as having significantly enriched genome-wide ranking, including genes and noncoding regions associated with keratinization, cornification, and hair-related mouse knockout phenotypes. A skin- and hair-associated microRNA, MIR205, was likewise found to have a significant enrichment of quickly-evolving noncoding elements in its vicinity. RERconverge also identifies genes and non-coding elements associated with changes in skin, many of which may be complementary to hair loss, that represent potential confounding phenotypes.

One clear confounding phenotype with the hairless phenotype is marine habitat. Since marine mammals also represent a group with similar distinct skin changes, correcting for the marine confounder is one way to separate genetic elements associated with skin versus hair. We developed a likelihood-based strategy to identify model support for the evolution of genetic elements associated with the marine phenotype versus the reduced hair phenotype. The strategy, for example, identified stronger support for olfaction-related genes in the marine model versus the hair model and stronger support for hair-associated keratin genes in the hair model versus the marine model. We can therefore dissect fine-grain differences in phenotypes that help us more accurately predict genetic elements, both coding and non-coding, associated with reduced hair. These findings represent good candidate genes and regulatory elements to test experimentally for association with hair growth.

1210A SR drive and the evolutionary history of the Y chromosome in *Drosophila simulans.* *Cécile Courret*[1,2], David Ogereau[2], Ching-Ho Chang[1], Amanda Larracuente[1], Catherine Montchamp-Moreau[2] 1) University of Rochester, Rochester, NY, US; 2) EGCE, CNRS de Gif sur Yvette, France.

The evolutionary history of the Y chromosome in *Drosophila simulans*, a species of Afrotropical origin, is closely linked to that of X-linked meiotic drivers. Here we go further in the study of Y chromosome variation in this species, by taking advantage of the recent assembly of its Y chromosome. We sequenced 7 iso-Y lines, their Y chromosomes came from different locations in Africa. Among them, 3 lines carry a sensitive Y chromosome and 4 lines carry a Y chromosome which is able to resist the drivers. First, we confirm the very low nucleotide diversity among Y chromosomes in this species, which could be considered as a signature of recurrent genetic conflicts. While we identified an haplotype composed by 886 SNPs, with fixed differences between the 3 sensitives and the 4 resistant Y chromosomes. Secondly, the molecular polymorphism allows us to distinguish three distinct groups: the 3 sensitives Y chromosomes (93.7% of identity), the 3 resistant Y chromosome from Est Africa (94.45% of identity) and the resistant Y chromosome from Seychelles. The similarity between the 3 resistant Y chromosomes from African population suggest that they have a recent common ancestor, idem for the 3 sensitives Y chromosome. We also confirm the ancestry of the resistant lineage by examining Y-linked sequences in the sister species of *D. simulans*. Finally, we found that, the resistant Y chromosome from the Seychelles Island, seems to have a deferent evolu-

tionary history from the resistant Y chromosomes from the African continent. We highlight the potential of this new approach to go further in the understanding of the evolutionary history of the Y chromosome.

Friday, May 1 12:00 PM - 2:30 PM

Evolutionary and Population Genetics 2 - Poster Q&A 979A Drivers of Female Sperm Storage Organ Evolution in *Drosophila* *Cameron Himes*[1], Tiffini Smith[1], Mollie Manier[1] 1) The George Washington University, NW Washington, DC.

Seminal receptacles are the primary sperm-storage organ in most *Drosophila*. Evolution of long sperm in D. melanogaster is driven by sperm competition within long seminal receptacles (SR), such that long sperm outcompete short sperm, but only in long SRs. This male-female interaction means that SR length is a mechanism of cryptic female choice, and longer SRs are more selective for long sperm. Sperm and SRs are also coevolving both across species within *Drosophila* and within species, likely driven by a genetic correlation and fitness benefits for both sexes. Consistent with Fisherian runaway sexual selection of male traits and female preferences, it is assumed that SR length drives evolution of sperm length, but nothing is known about what drives the evolution of SR length. The strength of sexual selection can be approximated using female remating rate, or the average time a female waits between her first and second mating.
Flies were obtained from the Drosophila Species Stock Center or collected from the wild (*D. hydei)* and reared on media according to species specifications. Virgins males and females were collected and maintained in 10 mL vials until sexually maturest. For each four-hour mating trial, 5 females per trial were aspirated without anesthesia into individual vials and allowed to acclimate to fresh food overnight. One or two males, depending on species, was aspirated into a female's vial, and time to mating and copulation duration were recorded. When copulation completed, the male was replaced with a new male. The time to remating and number of matings in 7 days (or 1 day for rapidly remating species) was recorded. SR length and sperm length were also noted for each species, based on new measurements or from the literature.
There was no significant correlation between remating rate and SR length ($F_{1,16} = 1.673$, $P = 0.2143$), suggesting that sexual selection is not driving the evolution of SR length on a macroevolutionary scale. However, there is a genetic correlation between SR length and sperm length, and long sperm and SR genotypes tend to increase fitness for both sexes. These factors may be enough to drive sperm-SR coevolution without selection specifically acting on SR length.

981C How functional diversity and the role of a gene affect its evolutionary trajectory: large-scale population simulations of gene regulatory networks *Anastasia Teterina*[1,2], Peter Ralph[1,3], Patrick Phillips[1] 1) Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA; 2) Severtsov Institute of Ecology and Evolution RAS, Moscow, Russia; 3) Department of Mathematics, University of Oregon, Eugene, OR, USA.

Understanding the genetic architecture underlying complex phenotypes is one of the most challenging aims of quantitative and evolutionary biology. Both adaptive and non-adaptive forces can shape the structure of the gene regulatory networks that generate phenotypes. Some critical properties of gene regulatory networks, such as robustness against mutations and genetic variability under drift and selection, have been studied using evolutionary simulations (Wagner 1996; Kioukis & Pavlidis 2018). A necessary next step is to more directly connect the evolutionary dynamics of the networks themselves with signals that we can observe in empirical biological studies. We have developed a simulation framework in SLiM3 (Haller & Messer 2016, 2019) that includes selection on gene expression levels in evolvable, dynamic gene regulatory networks encoded in realistically-sized genomes. The simulations were carried out on large populations and with an environmental factor that initializes the network iterations. Using the genomic data of simulated individuals, we can estimate diversity, conduct molecular biological experiments such as estimation of the effects of knockout or overexpression of the gene on phenotypes, and describe the structure and heterogeneity of evolved networks. Across many simulations, we examined the relationship between genetic diversity, quantitative genetic measures, network centrality statistics, and phenotypic effects of molecular manipulations on the genes. We found that the amount of functional variation of a gene is more informative in determining whether a variant of the gene will sweep in process of adaptation than other measures such as the sensitivity of phenotype to up/downregulation of the expression level of the gene. To dig into the microevolution of the genotype-phenotype map, we explored how the evolutionary trajectories of genes depend on their roles in the gene regulatory network, and evaluated our ability to predict distributions of fitness effects and the course of evolution based on network structure.

998B Weird gene in a weird mammal: A highly divergent pancreatic duodenal homeobox 1 (*Pdx1*) gene in the fat sand rat *Yichen (Serena) Dai*[1], Peter Holland[1] 1) University of Oxford.

Various forces leading to strong GC skew in local genomic regions cause conflict between increasing GC levels and alteration of conserved amino acids. In most cases, natural selection will purge any deleterious alleles that arise. However, in the gerbil subfamily of rodents, several conserved genes serving key functions have undergone radical alteration in association with strong GC skew. We present an extreme example concerning the highly conserved homeobox gene *Pdx1*, a key gene in initiation of pancreatic organogenesis in embryonic development. In the fat sand rat *Psammomys obesus* and close relatives, we observe a highly divergent *Pdx1* gene associated with high GC content. In this study, we investigate the antagonistic interplay between very rare amino acid changes driven by GC skew and the force of natural selection. Using ectopic protein expression in cell culture, pulse-chase labelling, *in vitro* mutagenesis and drug treatment, we compare properties of mouse and sand rat PDX1 proteins. We find that amino acid changes driven by GC skew resulted in altered protein stability, with a significantly longer protein half-life for sand rat PDX1. We show that both sand rat and mouse PDX1 are degraded through the ubiquitin proteasome pathway. However, *in vitro* mutagenesis reveals that GC skew has caused loss of a key ubiquitination site conserved through vertebrate evolution, and we suggest that sand rat PDX1 may have evolved a new ubiquitination site to compensate. Our results give molecular insight into the conflict between natural selection and genetic changes driven by strong GC skew.

1004B Transposable element accumulation reduces fitness in maize *Michelle Stitzer*[1,2], Sarah Anderson[3], Edward Buckler[4], John Doebley[5], Sherry Flint-Garcia[6], Katherine Guill[6], James Holland[7], Asher Hudson[1], Michael McMullen[6], Sarah Odell[1], Nathan Springer[3], Jeffrey Ross-Ibarra[1,8] 1) Center for Population Biology and Department of Evolution and Ecology, University of California, Davis; 2) Institute for Genomic Diversity, Cornell University; 3) Department of Plant and Microbial Biology, University of Minnesota; 4) US Department of Agriculture–Agricultural Research Service, Cornell University, Ithaca; 5) Department of Genetics, University of Wisconsin; 6) US Department of Agriculture–Agricultural Research Service, Columbia, Missouri; Division of Plant Sciences, University of Missouri; 7) US Department of Agriculture–Agricultural Research Service, North Carolina State University;

Department of Crop Science, North Carolina State University; 8) Genome Center, University of California, Davis.

Transposable elements (TEs) are mobile DNA sequences found in all eukaryotic genomes. Their abundance scales linearly with genome size, and in maize, they make up the majority of DNA in the genome. The abundance and position of individual TE copies differs extensively between maize individuals, but it is unclear the degree to which these differences in TE content affect plant phenotypes and fitness. In organisms with smaller genomes, like yeast and Drosophila, experimental evidence for the deleterious impact of TEs on fitness is unequivocal. Yet, for larger genomes with many more TEs, it is unclear whether these same fitness costs exist. Here, we use recombinant inbred lines from two maize biparental mapping populations phenotyped in 4 to 14 environments to measure the impact of TE copy number and polymorphism on fitness related traits. From whole genome assemblies of parental lines, we project TE copy number to genotyped recombinant inbred lines (RILs), and associate this TE copy number to phenotypes. After correcting for parental ancestry, we find that TE copy number is negatively associated with fitness-related traits like seed number and plant height. There is no correlation for traits not directly tied to fitness, such as leaf width. Notably, movement of TEs during the generations of self-fertilization required to generate these populations may contribute to the deleterious cost of TEs, as bursts of new transposition are evident in whole genome resequencing of advanced generation RILs. TE copy number is also associated with changes in expression of host genes, most often dysregulation outside of parental values. Our findings suggest that even though the maize genome has evolved to tolerate its TE passengers, TEs still act as parasites with dramatic consequences.

1005C Rapid "mix-n-match" evolution of a housekeeping protein in response to bacterial antagonism *EmilyClare Baker*[1], Kristin Kohler[1], Mattew Barber[1] 1) University of Oregon.

Proteins that interact with pathogens are encoded by genes that are among the most rapidly evolving in animal genomes. The evolution of proteins dedicated to host defense has been well characterized, but pathogens often target proteins specifically involved in 'housekeeping' functions to manipulate host cells. How such housekeeping genes can evolve to circumvent pathogen antagonism without sacrificing endogenous functions remains unclear, particularly when pathogens target essential functional interfaces.

The vertebrate CEACAM family of cell surface proteins regulate a wide range of processes including cell-cell adhesion, growth, and signaling. For several members of this family, misexpression is a strong marker of carcinogenesis. This subset of CEACAMs notably overlaps with those antagonized by bacterial surface proteins termed 'adhesins,' which exploit CEACAMs in order to attach to and manipulate host cells. We have found that CEACAMs bound by adhesins show evidence of accelerated evolution in the N-domain which mediates interactions with both bacterial adhesins and other host proteins. Using a biochemical approach, we further determined that divergence between primate CEACAM1 N-domains controls recognition by the human pathogenic bacterium *Helicobacter pylori*. We also found that bonobo CEACAM1 differs substantially from its closest primate relatives both in protein sequence and in not binding *H. pylori*. Swapping in single rapidly evolving residues and motifs from bonobo into human CEACAM1 is sufficient to abolish *H. pylori* binding in a strain specific manner. Further sequence analysis has uncovered evidence of repeated gene conversion between CEACAM family members bound by bacterial adhesins. Bonobo CEACAM1 itself appears to have resulted from multiple gene conversion events along its protein binding surface. These results suggest gene shuffling of paralogous N-domains domains may provide a mechanism by which CEACAM proteins can rapidly evolve without catastrophically altering essential cellular processes. Together this work illuminates how 'housekeeping' proteins targeted by pathogens at functional interfaces are able to evolve in response to pathogen exploitation.

1010B Evolution of Protein-Protein Interaction Disruptive Variants in Human Populations *Mitchell Lokey*[1], Robert Fragoza[2,3], Haiyuan Yu[2,3], Philipp W. Messer[1,2], Andrew G. Clark[1,2] 1) Department of Molecular Biology & Genetics, Cornell University, Ithaca NY; 2) Department of Computational Biology, Cornell University, Ithaca NY; 3) Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca NY.

Protein-Protein Interactions (PPIs) are vital to nearly all cellular functions. Many studies in humans have mapped and annotated large networks of PPIs, otherwise known as the interactome. Perturbations that lead to a loss of PPIs have been shown to underlie disease, and in one study two-thirds of the disease-associated SNPs that were tested resulted in PPI disruption. However, only one study to date has made a concerted effort to assess the functional impact of a large number of naturally segregating coding variants on PPI networks. In their study, Fragoza et al. showed that, although PPI disruptive variants are associated with cancers and Mendelian diseases, a substantial number of PPI disruptive variants are segregating at unexpectedly common allele frequencies in the gnomAD dataset. In an effort to better understand the fitness effects of these interactome perturbations, we evaluated evolutionary signatures of PPI disruptive variants. We examined PPI network topology, interacting residue location, interface and sequence conservation, and population differentiation among PPI disruptive variants. We further used forward genetic simulations to assess the expected population variation and allele frequency spectrum for deleterious PPI disruptive variants under varying levels of selection and dominance with the latest models of human demography. Our results suggest that PPI disruptive variants segregating at intermediate frequencies are unlikely to be ubiquitously deleterious.

1011C Inferring parameters of selective sweeps through supervised learning *Ian Vasconcellos Caldas*[1], Andrew Clark[1,2], Philipp Messer[1] 1) Department of Computational Biology, Cornell University, Ithaca, NY; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

A selective sweep occurs when a positively selected allele rises in frequency in the population. In nature, sweep parameters are seldom known: how strong was the selection driving a given sweep? Is the selected allele associated with a single linked haplotype (hard sweep) or multiple haplotypes (soft sweep)? If the sweep is soft, did it originate from recurrent *de novo* mutations or from standing genetic variation? Here, we present a method based on supervised machine learning to answer these questions from the characteristic patterns of molecular polymorphism observed around the sweep locus. Our approach simulates training data with SLiM 3, a fast and flexible framework that allows us to train on a wide spectrum of evolutionary scenarios, with simulation parameters based on the real population of interest. Our dataset consists of summary statistics describing patterns of nucleotide diversity, haplotype structure, and linkage disequilibrium, calculated for varying genomic window sizes. We show that convolutional neural networks, which can make use of the full correlation structure of summary statistics and window sizes, perform better than linear and decision tree-based models. If selection is moderately strong, our method infers sweep parameters at more than 90% accuracy. Using sample DNA sequence data, we show that it recovers known parameters of hard and soft selective sweeps associated with the evolution of drug resistance in the malaria parasite *Plasmodium falciparum* and human immunodeficiency viruses (HIV). Overall, our study demonstrates the use of machine learning for parameter inference under complex evolutionary scenarios, and highlights the power of simulation to generate realistic datasets that go beyond the highly idealized models often used in population genetics. We demonstrate that evolutionary parameters of selective sweeps are identifiable from present-day genotyping samples, opening the door to a deeper understanding of adaptation.

1028B Inversion polymorphisms suppress recombination across megabase-scale genomic blocks between locally adapted populations of the Atlantic silverside *Maria Akopyan*[1], Arne Jacobs[1], Anna Tigano[1], Aryn Wilder[1], Hannes Baumann[2], Nina Therkildsen[1] 1) Cornell University, Ithaca, NY; 2) Department of Marine Sciences, University of Connecticut, Groton, CT.

Chromosomal inversions are known to play an important role in facilitating adaptation in populations connected by gene flow. By reducing recombination in heterokaryotypes, alternative chromosomal arrangements can capture multiple loci involved in adaptation to contrasting environments. Here, we investigate patterns of structural variability between populations of the Atlantic silverside (*Menidia menidia*), a marine fish distributed along a steep thermal cline and in which a suite of locally adapted traits has been well documented. Silversides from Georgia and New York, which exhibit local adaptation in growth rates and vertebral number, have a striking pattern of low genome-wide differentiation, punctuated with regions of extreme divergence that span megabases, suggesting the evolution of chromosomal inversions. We conduct comparative linkage mapping and quantitative trait loci (QTL) mapping to characterize the genomic basis and architecture underlying adaptation. We show that loci underlying adaptive traits map to genomic islands of differentiation between populations. Further, we identify eight large inversions on six chromosomes ranging in size from 0.7 to 11.9 megabases, spanning over 4% of the genome. Inverted regions between populations coincide precisely with patterns of genomic divergence and show how recombination is almost entirely suppressed in these regions in inter-population hybrids. We provide the first insights into the magnitude of chromosomal variation in Atlantic silversides and demonstrate the presence and impact of inversion polymorphisms between locally adapted populations. Our findings help clarify the role of inversions in facilitating adaptation in highly connected populations facing divergent selection across a climatic gradient – a selection regime that is ubiquitous in nature and being exacerbated by climate change.

1029C Genome-wide search for genes influenced by sexual selection in primates *Brianna Ports*[1], Michael Jensen-Seaman[1] 1) Duquesne University.

The intensity and type of sexual selection varies according to mating system. In polygynandrous species multiple males mate with an individual female during a single estrus cycle, often in rapid succession. In this system, much of the male-male competition occurs after copulation where males with characteristics increasing fertilization achieve greater reproductive success and pass those characteristics onto their offspring. This varies distinctly from polygynous and monogamous species where females mate with only one male and sperm competition is diminished. Many primate traits are attributed to variation in mating system such as sexual dimorphism in body size, relative testes size, coloration, sperm motility or viability, and changes in the female reproductive tract. The wide range of mating behaviors that are present between closely related primate species serves as an excellent model to examine the molecular mechanisms that drive these complex mating systems or are evolving in response to them. Here we focus on detecting genes which have evolved in response to similar selective pressures induced by differences in levels of sperm competition. To investigate these differences, we determined the primary mating system for 27 primate species and collected 19,975 aligned orthologous protein sequences from public databases. Using maximum likelihood, we estimated branch lengths representing the rate of amino acid substitutions for each protein. A linear regression model was then used to compute the relative evolutionary rate of each protein compared to genome-wide average branch lengths. Using this methodology, we detected genes which are evolving exceptionally rapidly or slowly in polygynandrous species, but not in monogamous or polygynous species. Further downstream likelihood analyses are being used to identify the strength and direction of selection on each rapidly evolving gene in order to distinguish between changes driven by positive selection or relaxed constraint.

1068C The mutation rate may change the order of adaptive mutations *Alexandre Soares*[1], Lucas Wardil[1], Louis Bernard Klaczko[2], Ronald Dickman[1,3] 1) Departamento de Física, Universidade Federal de Minas Gerais; 2) Departmento de Genética, Evolução e Bioagentes, Universidade Estadual de Campinas; 3) National Institute of Science and Technology for Complex Systems.

The fate and the predictability of an adaptive process are two of the paramount questions in evolutionary research today. It is assumed that it depends largely on the distribution of fitness effects (DFE) of *de novo* mutations and the mutation rates.

The typical DFE reflects a greater chance for less fit mutations, and a rarer incidence of more adaptive ones. This sets the tension between selection and mutation rate as a very common stage for the adaptive process. Under this circumstance, the mutation rate may define the prevailing fixing allele for a single-step adaptive process. As a natural generalization with far-reaching consequences, we ask if the mutation rate could change the order in which fixing alleles succeed during adaptation.

We performed simulations of the Wright-Fisher process of haploid populations, with sizes ranging from $N = 10^3$ to 10^9, with a genome composed by at least $L = 3$ distinct adaptive loci with $K = 2$ alleles each and one-way mutations from the wild type to the adaptive allele. Only one mutation is allowed per individual per generation. The selection coefficients of these alleles were multiples of a basic value s, and the fitness multiplicative. Also, the greater the selection coefficient of the new allele, the smaller was its mutation rate.

We found out that the probability of each path taken by the population and how it is traversed depends largely on the mutation rate. In the strong selection-weak mutation rate regime, a single genotype exists at a given time, and the population follows together a single path during a single adaptive process, which greatly varies from process to process. The most common path is the one where the allele with greatest mutation rate and lowest fitness fixes first, and the one with the lowest mutation rate and greatest fitness fixes last.

As the mutation rate is increased, the population may take more paths simultaneously, making the outcome of a single adaptive process less predictable. At a given point the most frequent sequence of adaptation inverts: the most adaptive allele fixes first and the less fit allele in the end. Remarkably, although the outcome of a single process becomes harder to anticipate, the outcome of the ensemble of adaptive processes becomes more predictable. For very extreme mutation rates, the path in which the alelles fix in order of increasing fitness becomes again the prevailing path, and the evolution of many adaptive processes becomes less predictable again.

1069A Dominance hierarchy and the genetic load linked to self-incompatibility alleles in *Arabidopsis* Audrey Leveve[1], Eleonore Durand[1], Xavier Vekemans[1], Vincent Castric[1] 1) CNRS-Université de Lille.

Self-incompatibility (SI) is a genetic system preventing inbreeding in hermaphroditic plant species. In Brassicaceae, the sporophytic SI system is controlled by a genomic region, the S-locus, and is a classical case of a particular type of balancing selection (negative frequency-dependent selection). SI enforces heterozygosity and promotes allelic diversity at the SI genes themselves as well as in the linked genomic region surrounding the S-locus,

and is therefore expected to result in the effective sheltering of a load of recessive deleterious mutations. However, direct experimental evidence for this load have remained scarce. In addition, the dominance hierarchy between SI alleles is expected to have a strong impact on the sheltering of deleterious mutations, with a more important load expected in association with dominant than with recessive S-alleles. Here, we used phased and unphased resequencing data from natural populations of two self-incompatible species *A.halleri* and *A.lyrata* to quantify the number of segregating mutations linked to SI alleles and evaluated their potential deleterious effect. We found strong evidence that dominant SI alleles are indeed hosting a larger number of predicted deleterious mutations with a stronger effect in *A. halleri,* but found no effect in *A. lyrata*, possibly due to different demographic histories. In line with theory, the sheltering effect also seems to be limited to a very small number of highly linked genes.

1078A The absence of the copulatory plug disrupts pregnancy in mice *Michael Lough-Stevens*[1], Caleb Ghione[1], Adelaide Hobbs[1], Matthew Urness[1], Colleen Sweeney[1], Matthew Dean[1] 1) University of Southern California.

Understanding the biological reasons for implantation failure is important because mammalian pregnancies may fail even when there are no known clinical abnormalities. A significant proportion of pregnancy failures may be preventable with a deeper understanding of the molecular pathways required to initiate and maintain pregnancy. Recent reviews of mammalian pregnancy suggest that the female's body orchestrates a complex set of molecular pathways to regulate the successful fertilization, implantation, and gestation of offspring. However, pregnancy is not controlled solely by females. Studies suggest that females often receive cues from their male partners, both behavioral and physical, to help regulate the initiation and persistence of pregnancy.
We used a mouse model to study pregnancy failure caused by a reproductive defect in the ejaculate of male mice: the copulatory plug, a solid mass formed from male seminal fluid in the female mouse's reproductive tract after ejaculation.
Previous research in our lab shows that female mice become pregnant ~83% of the time when paired with normal males, but if the male cannot form a copulatory plug, their pregnancy success rate falls to ~40%.
We investigated 1) whether implantation rates go down when females do not receive a plug and 2) whether progesterone, which is essential for the implantation of fertilizing eggs in mammals and is upregulated at the time of implantation, is affected by the presence or absence of a plug.

Friday, May 1 12:00 PM - 2:30 PM

Evolutionary and Population Genetics 3 - Poster Q&A 1104C Identifying gene regulatory interactions associated with hybrid male sterility in *Drosophila pseudoobscura* *Alwyn Go*[1], Alberto Civetta[1] 1) Department of Biology, The University of Winnipeg, Winnipeg, MB, Canada.

Speciation occurs with the formation of reproductive barriers that isolate individuals preventing the exchange of genetic information. A common form of reproductive isolation between species capable of interbreeding is hybrid sterility. Genomic incompatibilities between the divergent genomes of different species can contribute to a reduction in hybrid fitness. These incompatibilities can continue to accumulate after the establishment of reproductive isolation, therefore, young divergent taxa with incomplete reproductive isolation are important in understanding the genetics leading to speciation. *Drosophila pseudoobscura pseudoobscura* and *D. p. bogotana* are representative of the early stages of speciation. The subspecies pair shows incomplete reproductive isolation through unidirectional hybrid male sterility (HMS) wherein hybrid males with *D. p. pseudoobscura* mothers are fertile while those with *D. p. bogotana* mothers are sterile. HMS is due to incompatibilities between the *D. p. bogotana* X chromosome and *D. p. pseudoobscura* autosomes. A gene within the X chromosome, *Overdrive* (*Ovd*), was found to have a major contribution to HMS. *Ovd* codes for a protein with a DNA-binding domain making it a possible *trans*-regulatory element. Despite the identification of *Ovd*, interaction networks involving this gene remain unexplored. Here, we took advantage of the fact that *Ovd* is tightly linked to the *sepia* eye colour mutation in *D. p. pseudoobscura*. This allows the use of backcross to replace the sterile *D. p. bogotana* allele for *Ovd* with the fertile *D. p. pseudoobscura* allele. After 28 generations, the backcross produces non-*sepia* and *sepia* hybrid males. Both hybrid males are nearly identical to the F1 sterile male hybrids except for the introgressed *D. p. pseudoobscura Ovd* allele. Using RNA-sequencing, we have identified a subset of genes whose expression is differentially regulated by the state of the *Ovd* alleles. Interestingly, these genes are enriched for particular functions and display more protein-protein interactions than randomly expected. Our preliminary data suggests pathways involved in the manifestation of HMS during the early stages of speciation.

1106B Mechanisms of hybrid male sterility in reciprocal crosses between malaria mosquito species Jiangtao Liang[1], Michael Hodge[1], *Igor Sharakhov*[1] 1) Virginia Tech.

Hybrid male sterility (HMS) contributes to speciation by restricting gene flow between related taxa. Detailed cytological characterizations of reproductive organs in hybrid males is important for identifying phenotypes that can help guide searches of speciation genes. To investigate possible cellular and molecular causes of HMS, we performed crosses between closely related species of the *Anopheles gambiae* complex: *An. merus* with *An. gambiae* or *An. coluzzii*. We demonstrate that HMS in African malaria mosquitoes involves two defects in the reciprocal crosses: a premeiotic arrest of germline stem cells in degenerate testes and a failure of the reductional meiotic division of primary spermatocytes in normal-like testes. The premeiotic arrest in degenerate testes of hybrids is accompanied by a strong suppression of meiotic and postmeiotic genes and by small size of male accessory glands (MAG). Compared with pure species and hybrid males with normal-like testes, F1 males with degenerate reproductive organs display a shorter copulation time with females and they fail to produce mating plugs to induce female oviposition and monogamous behavior. This is despite the fact that degenerate MAGs in F1 males still express 20E hormone pathway genes. Unlike pure species, sex chromosomes in normal-like testes of F1 hybrids are largely unpaired during meiotic prophase I and all chromosomes show various degrees of insufficient condensation. Instead of entering reductional division in meiosis I, primary spermatocytes prematurely undergo an equational mitotic division producing nonmotile diploid sperm. Regulation of heterochromatin and/or genes located on autosomes is an important criterion for identifying putative X-linked HMS genes in mosquitoes. The common characteristics of known HMS genes identified in other organisms include DNA binding function, rapid evolution, and testis-biased expression. Our study identified few genes that meet these criteria and qPCR analysis showed significant downregulation of these genes in normal-like testes of F1 male hybrids. These results demonstrate that different sets of genes play roles in HMS in reciprocal inter-species crosses in the *An. gambiae* complex. Thus, our study identified cellular and molecular errors in interspecies hybrids that arise during the early stages of postzygotic isolation.

1110C Hybrid incompatibility driven by nuclear-mitochondrial sexual conflicts *Manisha Munasinghe*[1], Andrew Clark[1] 1) Cornell University.

Cellular function requires the coordinated transcription, translation, and assembly of mitochondrial proteins encoded by both the mitochondrial

and nuclear genomes. Sexual asymmetry in mitochondrial genome transmission favors mutations that are advantageous in females even if they are deleterious in males. Coined Mother's Curse, this phenomenon induces a selective pressure for nuclear variants that compensate for this reduction in male fitness, generating a specific subset of mitochondrial-nuclear interactions. Previous work has demonstrated not only the existence of these interactions but also their potential for generating hybrid incompatibility between populations. While it is easy to see that Mother's Curse mtDNA variants and nuclear compensators could act as Dobzhansky-Muller loci, it is not clear how readily it would give rise to hybrid incompatibilities. Here, we apply computer simulations using SLiM2 to expand analytical theory to investigate the consequences of sexually antagonistic mitochondrial-nuclear interactions in a subdivided population. These simulations show the range of conditions under which nuclear-mitochondrial interactions can drive population isolation and hybrid incompatibility. Results have clear relevance to observations of relatively weak consequences of interspecific mtDNA swaps, in the face of strong interpopulation intraspecific incompatibilities.

1112B The Effect of Introgression on the Joint Distribution of Gene Tree Topologies at Two Linked Loci *Michael Miyagi*[1], Andrew Blumberg[2], John Wakeley[1] 1) Harvard University, Cambridge, MA; 2) The University of Texas at Austin, Austin, TX.

Recombination rates, population sizes, and speciation times are known to affect gene-tree concordance between linked loci. By extending the Markov model of Slatkin and Pollack, we assess how two-locus concordance probabilities depend additionally on the strength and timing of introgression. In particular, we describe the joint distribution of tree topologies at two linked loci in three species when there is a pulse of introgression, and explore how the relationships between demographic parameters and summaries like concordance are affected by the presence of gene flow. We show that the probability that two gene trees are concordant with each other is higher for introgressed loci than for those that follow the species tree, but the total concordance probability may be non-monotonic or decreasing with the introgression fraction.

1128C Predicting the Genomic Resolution of Bulk Segregant Analysis *Runxi Shen*[1], Philipp Messer[1] 1) Cornell University, Ithaca, NY.

Bulk segregant analysis (BSA) is a genetic mapping technique for identifying the molecular basis of phenotypic traits. The underlying principle of this method is to genotype pools of individuals with contrasting phenotypes, which are then compared to detect alleles with diverged genotype frequencies between the pools. BSA has already been successfully applied for quantitative trait mapping in organisms ranging from yeast to crops. However, these studies have typically suffered from rather low genomic resolution, and we still lack a detailed understanding of how this resolution is affected by experimental parameters. Here, we use coalescence theory to derive an analytical theory for the expected mapping resolution of BSA. We first show that in an idealized population without genetic drift, the expected mapping resolution is inversely proportional to the recombination rate, generations of interbreeding, and the number of samples genotyped, as intuitively expected. In a finite population, coalescence events in the genealogy of the samples will reduce the number of informative recombination events, and thus the achievable mapping resolution. This is incorporated in our theory by introducing an effective population size parameter, which specifies the probability of coalescence in the interbreeding population. We also show that the mapping resolution predicted by our theory is in excellent accordance with detailed numerical simulations. Our framework will allow researchers to assess the expected power of a given BSA experiment, and to test how experimental setup can be tuned for optimizing mapping resolution.

1137C The Evolution of Starvation Resistance in Relation to Nutrient Availability *Jordyn Moaton*[1], Zeke Elkins[1], Elizabeth King[1] 1) University of Missouri - Columbia.

An animal's ability to withstand prolonged periods of food deprivation is called starvation resistance. Starvation resistance (SR) is a phenotypic trait of great environmental significance. In environments where there are shortages of food, those who can resist starvation for longer periods of time to thrive compared to other organisms. The purpose of this project is to study the underlying mechanisms in starvation resistance using *Drosophila melanogaster* as a model. We used a large- scale experimental evolution design, placing fruit flies on 3 different selection treatments, constant high, fluctuating, and deteriorating nutrients availability, for over 50 generations each with 12 replicates. For the first treatment, constant high availability (CHA), flies are given a high sugar diet their entire lifespan. In the second treatment, fluctuating availability (FA), flies on this regime are fed a standard diet then a low yeast diet then back to a standard diet to the end of their lifespan. In the final treatment, deteriorating availability (DA), flies are given a standard diet then are fed a low yeast diet to the end of their lifespan. Flies 12 days po, post oviposition, from each nutrient regime were placed on a maintenance diet, and then transferred to vials containing only nutrition less agar. These vials were checked approximately every twelve hours, beginning at 8:30am and 8:30pm daily. The number of flies confirmed dead in each period was then recorded until all flies were confirmed dead over the span of roughly two weeks. Flies on the fluctuating availability treatment are expected to exhibit higher levels of SR due to evolved higher rates of lipid and carbohydrate storage than the DA and CHA treatments. We link these phenotypic changes to changes at the genetic level in these lines. These results have implications for understanding the conditions that might select for higher or lower starvation resistance and the underlying genetic mechanisms determining those changes.

1150A Increased oxidative damage to DNA in the lab environment cannot explain why the *C. elegans* mutation spectrum is different in the lab and in nature. *Moein Rajaei*[1], Ayush S. Saxena[1], Michael Snyder[1], Robyn E. Tanny[2], Erik C. Andersen[2], Joanna Joyner-Matos[3], Charles F. Baer[1] 1) University of Florida, Gainesville, FL USA; 2) Northwestern University, Evanston, IL USA; 3) Eastern Washington University, Cheney, WA USA.

The rate and spectrum of mutation are of fundamental importance in evolutionary biology. Mutation accumulation (MA) experiments are the usual (and always the most efficient) way to estimate the properties of spontaneous mutation divorced from the influence of natural selection. It is now apparent that the base-substitution spectrum of mutations accumulated in lab MA populations of *C. elegans* differ significantly and consistently from the standing site-frequency spectrum (SFS) in nature, with a greater proportion of transversions in the lab, especially C:G→A:T transversions. There are two possible reasons: (1) natural selection skews the SFS away from the mutational spectrum, or (2) the spectrum of lab-accumulated mutations differs from that in nature.

One possible explanation for the discrepancy is that conditions in the lab result in increased oxidative damage to DNA relative to that in nature, perhaps associated with differences in metabolism (e.g., food *ad libitum*). To test that hypothesis, we performed an MA experiment with a mutant strain of *C. elegans*, *mev-1*, that is known to experience elevated oxidative stress, resulting from a defective complex II of the mitochondrial electron transport chain. If oxidative stress is a cause of the difference, we expect an even greater skew toward C:G→A:T transversions, which are a signature of oxidative damage to DNA.

Whole-genome sequencing of 24 *mev-1* MA lines that had accumulated mutations for >100 generations revealed a rate and spectrum of base-substitution mutations that are essentially identical to other *C. elegans* MA lines. Thus, there is no evidence that the discrepancy between

the lab-accumulated and natural spectra is the result of increased oxidative damage under lab conditions. Oxidative stress is known to increase the somatic mutation rate; apparently the germline is uniquely protected against oxidative damage.

1178B The evolution of short inverted repeats *Einat Hazkani-Covo*[1] 1) Open University of Israel.

Inverted repeats (IRs) are sequences with internal symmetry that form non-canonical DNA structures and can induce genome instability. Diverged IRs frequently undergo template switches, in which one arm of the repeat serves as a template for synthesis of the second arm resulting in erasing of variation between arms. In contrast to other mechanisms that resulted in the correction of IR arms during evolution, the evolutionary impact of template switching was previously neglected. If template switching occurs in genomes, then we expect it to contribute to the correction of imperfect IRs to perfect ones, resulting in the conservation of IRs through evolution. In addition, Template switching is a non-conservative mutation mechanism that introduces multi nucleotide mutations at once. Thus, it has the potential to introduce functional changes into genomes. Our analysis shows that short IRs are conserved during the evolution of Escherichia coli and Saccharomyces cerevisiae, supporting the model of IR arm corrections by template switching. The evolution of short IRs also introduces multiple changes to proteins.

1181B Evolution of transposable element composition and piRNA regulation across the Drosophila phylogeny Jullien M. Flynn[1], Andrew G. Clark[1] 1) Cornell University, Ithaca, NY.

Transposable elements (TEs) are self-replicating elements that parasitize the genomes of eukaryotes. In attempt to manage the genomic consequences of TEs, animals have evolved silencing mechanisms – one of the most prevalent being piwi-interacting RNAs (piRNAs). The piRNA response for a given TE is expected to be related to its evolutionary history of activity. However, if TEs and the host response undergo coevolution, we might see the response to similar TE situations play out differently among species. Across the Drosophila clade, TE compositions are diverse and include TEs that have been evolving similarly over millions of years as well as TEs that have diverged trajectories. Here, we employ RepeatModeler2 to annotate transposable elements in high-quality long-read genome assemblies of six Drosophila species spanning 50 million years of evolution. We combine these annotations with RNA-seq and piRNA data from male and female gonads to dissect patterns of TE landscape and piRNA regulation. We hypothesize that piRNA regulation is not only dependent on current and historical TE activity and varies greatly among TE families and species that have similar history of these TEs. Notably, DINE-1 helitron sequences are highly regulated by piRNAs both in species that contain diverged DINE-1-derived satellites and species that have recently transposing DINE-1 elements. R1 has been occupying the rDNA clusters of arthropods for millions of years and shows evidence of recent activity across all species, yet some species have negligible piRNA regulation against it while in other species this element is the most strongly regulated. We demonstrate that the piRNA response is robust across species for some TE families, but varies greatly in others. We hypothesize that these patterns might be driven by co-evolution between the parasitic elements and their hosts.

1187B Non-coding elements accelerated in subterranean mammals drive expression in zebrafish retina *Jiaxuan Yang*[1,2,3], Ana Gabriel[2], Raghavendran Partha[1], Elysia Saputra[1], Leah Byrne[2], Jeffrey Gross[2], Nathan Clark[1,4] 1) Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA, USA; 2) Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA, USA; 3) School of Medicine, Tsinghua University, Beijing, China; 4) Department of Human Genetics, University of Utah, Salt Lake City, UT, USA.

Underground mammals usually have small, regressed eyes in response to their dim-light environment. Similarly, the genetic elements contributing to eye function have also deteriorated in these nearly blind species, and as such these elements are nonfunctional in these species and now evolve at an accelerated rate. To detect such accelerated regions, our lab developed RERconverge, an R package that tests for association between relative evolutionary rates of genes and the evolution of a specific trait across a phylogeny. Using this method, we demonstrated that ocular genes and enhancers of subterranean mammals accumulated mutations at higher rates than their relatives living aboveground, due to relaxed evolutionary constraint.
We also scanned 340,000 conserved non-coding elements across genome and scored their evolutionary rates in both subterranean and aboveground mammals and identified a list of mole-accelerated, non-coding elements. Highly accelerated regions had a highly significant overlap with open-chromatin regions in mouse retina, which makes us more confident that these elements may associate with ocular function and thereby reveal previously unrecognized ocular genetic elements.
We selected the top 10 mole-accelerated elements and tested if they drive expression in the retina in a zebrafish embryo model. We built Tol2 transgenic constructs containing each non-coding element, followed by a minimal promoter and EGFP. By introducing these constructs into zebrafish embryos, we verified that a high proportion of top-ranked mole-accelerated non-coding elements could drive EGFP expression in zebrafish retina. Among these elements, we found one element close to the *Sox2* gene drives expression in both notochord and retina. *Sox2* is an important developmental gene in the central nervous system and proved to be associated with optic nerve hypoplasia and syndromic microphthalmia. We are following these regions up by scanning mutations in sequencing data of patients with eye diseases of unknown etiology.
This study showed great functional conservation of regulatory elements among different species. It proved that it is feasible to discover new elements important for eye development and function using comparative genomics and evolution. Furthermore, we believe that it would provide a new insight into ocular disease study.

1222A A burst of lineage-specific genetic innovation in *Drosophila* actin-related proteins for testis-specific function *Courtney Schroeder*[1], Sarah Tomlin[1], John Valenzuela[1], Isabel Mejia Natividad[3], Glen Hocky[4], Harmit Malik[1,2] 1) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute, Seattle, WA; 3) University of Puget Sound, Tacoma, WA; 4) Department of Chemistry, New York University, NY.

Many cytoskeletal proteins perform fundamental biological processes and are evolutionarily ancient. For example, the superfamily of actin-related proteins (Arps) specialized early in eukaryotic evolution for diverse cellular roles in the cytoplasm and the nucleus. Despite its strict conservation across eukaryotes, we find that the Arp superfamily has undergone dramatic lineage-specific diversification in *Drosophila*. Our phylogenomic analyses reveal four independent Arp gene duplications that occurred in the common ancestor of the *obscura* group of *Drosophila* and have been mostly preserved in this lineage. All four *obscura*-specific Arp paralogs are predominantly expressed in the male germline and have evolved under positive selection. We focus our analyses on the divergent *Arp2D* paralog, which arose via a retroduplication event from *Arp2*, a component of the Arp2/3 complex that polymerizes branched actin networks. Computational modeling analyses suggests that Arp2D can replace Arp2 in the Arp2/3 complex and bind actin monomers. Together with the signature of positive selection, our findings suggest that Arp2D may augment Arp2's functions in the male germline. Indeed, we find that Arp2D is expressed during and following male meiosis, where it localizes to distinct locations such as

actin cones—specialized cytoskeletal structures that separate bundled spermatids into individual mature sperm. Interestingly, we have also found a second Arp2 duplicate in the *montium* group that arose at least 8 million years ago. We hypothesize that this unprecedented and recurrent burst of genetic innovation in the Arp superfamily may have specialized for the unique male meiotic cytoskeletal machinery.

1224C Phylogenetic relationships of 200+ wild isolates of the ectomycorrhizal fungus *Cenococcum geophilum* from soils under Populus trichocarpa in the Pacific Northwest, USA. Jessica M. Velez[1], Reese M. Morris[2], Jessy Labbe[2], Rytas Vilgalys[3], Scott Emrich[1], Todd Pierson[1], Christopher W. Schadt[2] 1) University of Tennessee Knoxville, Tennessee; 2) Oak Ridge National Laboratory, Tennessee; 3) Duke University, North Carolina.

The ubiquitous ectomycorrhizal fungus *Cenococcum geophilum* has been proposed as a model organism for fungal genetics and ecological studies due to several factors, including its wide-spread association with both gymno- and angiosperm plant species, straightforward *in vitro* cultivation, and species hardiness in response to a number of soil stressors. However, *Cenococcum geophilum* is genetically complex due to an asexual nature in combination with extreme phylogenetic divergence within populations of morphologically identical members, often considered a species complex. Alternately, *C. geophilum* may represent a highly outcrossed species which would suggest cryptic sexual states and frequent recombination. In order to study these potential designations, a new isolate collection of 229 *C. geophilum* isolates was collected from soils under *Populus trichocarpa* and described from 123 collection sites spanning a ~283 mile north-south transect in Washington State and Oregon, USA (PNW). This study represents the first comprehensive study of the genetic relationships of *C. geophilum* isolates associated with an angiosperm. We performed maximum likelihood analyses to assess divergence within the PNW isolate collection using both the internal transcribed spacer 1 (ITS1) region and the glyceraldehyde phosphate-3-hydrogenase (GAPDH) gene, as well as phylogenetic analyses of 790 isolates found worldwide using publicly available data from the United States, Europe, Japan, and other countries. Fifteen unique clades with >80% bootstrap support were found in the PNW collection based on the GAPDH gene, and 13 distinct PNW clades were found using the ITS and GAPDH multigene concatenated phylogeny. Several clades include isolates from sampled sites ranging from 50 to >200 miles apart. In the global *C. geophilum* collection, 34 clades were found based on the GAPDH gene, and 25 based on the ITS and GAPDH phylogeny, with several clades containing either intra- or inter-continental isolates. Higher resolution genotype-by-sequencing was completed on 187 PNW isolates as well as the fully sequenced 1.58 *C. geophilum* strain using double digest restriction-associated DNA sequencing (ddRAD) and compared to both the single gene and multigene concatenated phylogenies. The results from these analyses are highly suggestive of divergence within multiple cryptic species, with at least three distinct phylogenetic groups implicated in the PNW isolate collection.

Friday, May 1 12:00 PM - 2:30 PM

Evolutionary and Population Genetics 4 - Poster Q&A 1129A Replaying the tape of hybrid speciation in the laboratory reveals major consequences for whole-genome duplication *Guillaume Charron*[1,2,3], Souhir Marsit[1,2,3,4], Mathieu Hénault[1,2,4], Hélène Martin[1,2,3,4], Christian Landry[1,2,3,4] 1) Institut de Biologie Integrative et des Systemes; 2) Regroupement Quebecois de Recherche sur la Fonction, l'Ingenierie et les Applications des Proteines (PROTEO); 3) Departement de Biologie, Universite Laval; 4) Departement de biochimie, microbiologie et bio-informatique, Universite Laval.

Hybridization is an important driver of genetic diversification and, in some cases, new species formation. However, hybrid species often suffer from infertility due to incompatibilities between parental species resulting from genome divergence. In obligate sexual species, fertility can be recovered through backcrosses with either parental species, often leading the hybrids to merge back into introgressed species, hindering hybrid speciation. Some eukaryotes have access to alternate reproductive strategies such as mitotic division or other mean of asexual reproduction. In these species, hybrids may recover fertility by other means than backcrossing, thus enabling hybrid speciation. We used the wild yeast *Saccharomyces paradoxus* and its sister species *Saccharomyces cerevisiae* to study the evolution of fertility following in hybrids of increasing parental divergence. We evolved 700 independent hybrids under strictly asexual propagation for more than 700 generations and under sever population bottleneck in order to allow the widest breadth of possible outcomes. We show that, most often, the fertility of mitotically propagated hybrids varied over time like a neutrally evolving quantitative trait showing incremental changes in no preferential overall direction. However, some evolved lines experienced spectacular recoveries in their fertility. Further analyses revealed ploidy changes indicating whole-genome duplication events few hundreds of mitotic generation after hybridization. Ploidy instability therefore confers to hybrids a path to fertility recovery and possibly hybrid speciation.

1134C Using yeast to explore the combinatorial fitness landscape of co-evolving human and viral proteins *Michael Chambers*[1, 2], Thomas Dever[3], Meru Sadhu[1] 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) Dept. Microbiology & Immunology, Georgetown University, Washington, DC; 3) National Institute of Childhood Disease and Human Development, NIH, Bethesda, MD.

Here we propose a method that incorporates deep mutational scanning to explore an evolutionary arms race between the human innate immune system and poxviruses. Protein kinase R (PRK) is a component of the innate immune system that detects the presence of double-stranded RNA (dsRNA), indicating the presence of a foreign entity within the cell, such as a virus. Once activated by dsRNA, PKR phosphorylates the translation initiation factor subunit eIF2α, ultimately halting translation within the cell and preventing viral replication. As a counter defense, poxviruses encode a PKR antagonist denoted as K3L which inhibits the phosphorylation and activation of eIF2α to allow the virus to replicate. Both PKR and K3L are under diversifying selection pressure as PKR must discriminate against K3L while K3L must overcome this discrimination to replicate. The interaction of these two proteins under diversifying selection drives an evolutionary arms race in which superior variants of PKR and K3L are continually pursued. We propose a novel approach to generate single-residue variant libraries of both PKR and K3L and simultaneously assess their function with a yeast growth readout. This approach will generate a variant interaction matrix covering a large combinatorial space in a single experiment, highlighting points of constraint and providing a glimpse into the evolutionary landscape in which PKR and K3L are bound.

1139B Mutation limitation and the genetic mechanisms of adaptation *Thomas LaBar*[1], Andrew Murray[1] 1) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA.

Comparative studies of evolving populations have revealed that multiple genetic mechanisms can underlie adaptation. However, it is unknown why different populations adapt using different genetic mechanisms. While the stochasticity of mutations is one likely explanation, it is also possible that certain population-level characteristics could alter the mechanisms of adaptation in different populations. Here, we will present results from an evolution experiment that addresses the role of mutation supply in altering the genetic mechanisms of adaptation. We evolved a mutant yeast strain with a defect in transcriptional activation of glycolysis at two different effective population sizes in order to understand how different genetic

alterations across mutation supplies would restore this phenotype. As expected, small populations adapted slower than large populations. Small and large populations initially fixed mutations in distinct genes: large populations mostly acquired mutations in the Mediator transcriptional coactivator complex, while small populations acquired mutations across a range of other transcriptional regulatory complexes. Small populations acquired many nonsense mutations, while large populations mostly acquired missense mutations, suggesting a role for mutation supply in driving these patterns of genetic architecture across population sizes. We expect the results from this study will provide insight into both the role of mutation limitation in shaping the genetic basis of adaptation and the evolutionary origins of novel transcriptional capabilities.

1145B Using *in-vitro* evolution to explore genome dynamics and antifungal resistance of diploid and tetraploid *Candida albicans* *Ognenka Avramovska*[1], Meleah Hickman[1] 1) Emory University.

As a common fungal pathogen that undergoes non-meiotic ploidy transitions between haploid, diploid and tetraploid states, *Candida albicans* is versatile model to study the interaction between ploidy and the emergence of drug resistance. In our previous work, we compared mutational rates and types in diploid and tetraploid *C. albicans* following exposure to two different classes of antifungal drugs. Overall, we found that antifungals disproportionately elevated genome instability (loss-of-heterozygosity) and ploidy instability in tetraploid *C. albicans* compared to diploids. For example, we measured higher overall rates of LOH and significant deviations from tetraploidy after only short-term exposure to these antifungals. Tetraploid yeasts have also been shown to adapt more rapidly than either haploids or diploids during *in-vitro* evolution in carbon-limiting conditions and generate far more aneuploidy and copy number variation during the evolution. Thus, we hypothesize that during long-term exposures to drugs, starting *C. albicans* tetraploid lines will acquire resistance more rapidly than the diploids lines and exhibit a variety of resistance-associated mutations, as tetraploid genomes are more genetically labile and can access a greater evolutionary landscape. To test these hypotheses, we evolve diploid and tetraploid lines *in-vitro* in the presence of antifungals. To determine how ploidy impacts the mechanisms or mutations by which *C. albicans* acquires resistance, we measure the ploidy of the resistant isolates using flow cytometry and the resistance-associated mutations through whole-genome sequencing. Regardless of the results of this study, we are poised to gain significant insights into the evolution of antifungal drug resistance of a widely pervasive fungal pathogen.

1148B The Dynamics of CNV Evolution in Fluctuating Environments *Farah Abdul-Rahman*[1], Angela Hickey[1], Charles Miller[1], David Gresham[1] 1) New York University.

Microbes live in dynamic environments which can pose ongoing challenges for survival. Unlike static conditions in which a selective pressure is continuous, fluctuating conditions cycle between selection and relaxation resulting in complex evolutionary dynamics. Copy number variants (CNVs) are a class of mutation in which a genomic locus varies in copy number. CNVs are widespread across all domains of life and have been implicated in diseases such as cancer. Despite the frequent occurrence of fluctuations in the natural environment, it is not well understood how they influence the dynamics of CNV generation and selection, the diversity of CNVs in a population, and their molecular structures.

Mutations that are beneficial in one condition may have fitness tradeoffs in others, which could cause their frequency in a population to oscillate when the environment periodically fluctuates. Alternatively, if the fitness tradeoff is low or neutral, mutations may stabilize in the population despite encountering cycles of selection and relaxation. To understand the dynamics of CNVs in fluctuating environments, we track their frequency in budding yeast populations over hundreds of generations, while alternating between two conditions limited for nitrogen in the form of either glutamine or proline. Previously, we have found that a glutamine transporter (GAP1) and proline transporter (PUT4) undergo gene duplication in chemostats limited for their respective nitrogen source. However, it remains unclear what the evolutionary outcome is when populations fluctuate between the two conditions. We developed dual-fluorescence CNV reporters for the two transporters enabling inexpensive and rapid detection of duplications or deletions at the two loci simultaneously. We find that while the dynamics of CNV generation and selection in static conditions is highly repeatable in early stages of evolution, fluctuating environments show greater heterogeneity in population structure between replicate experiments and a lower frequency of CNVs at both loci.

Capturing the real-time dynamics of CNV generation and selection will shed light on how periodic fluctuations in the environment have shaped the natural world.

1171A A Catalog of Genome Content Variation in *Arabidopsis thaliana* *Christopher Fiscus*[1], Daniel Koenig[1] 1) University of California, Riverside, Riverside, CA.

The dominant component of many eukaryotic genomes is rapidly evolving intergenic sequence. These sequences are integral to genome structure and regulating gene expression. However, intergenic sequence tends to be repetitive, is often absent from genome assemblies, and is mostly ignored in comparative genomic studies. Here we describe variation in genome content between 1142 *Arabidopsis thaliana* accessions using K-mer abundance profiles generated from short sequencing reads. We identify thousands of sequences that vary in copy number between accessions that can be mapped to specific genomic locations. Furthermore, we identify loci linked to copy number variation of multiple repeats in trans suggesting that these loci regulate the process of repeat mutation. Finally, we identify associations between sequence copy number and phenotypes such as flowering time, gaining insight into how copy number influences trait variation and ultimately the life history of an organism.

1207A Massively parallel discovery of splice-altering mutations in the evolution of modern and archaic humans *Stephen Rong*[1], Christopher Neil[1], William Fairbrother[1] 1) Brown University, Providence, RI.

Mutations that affect post-transcriptional splicing regulation are known to be a main contributor to both Mendelian and complex disease phenotypes. Comparisons between modern and archaic human genomes have revealed hundreds of thousands of genetic variants that distinguish us from our most recent extinct relatives, and have also shown conclusively that modern and archaic populations have exchanged genetic material multiple times. However, the functional impact on splicing regulation of these modern and archaic human lineage-specific and archaically introgressed genetic variants is still poorly understood. Here, we describe an extension of our previously published massively parallel splicing assay (MaPSy) protocol[1] to experimentally identify splice-altering mutations in the 3' and 5' ends of exons with application to modern, archaic, and introgressed variants. MaPSy utilizes oligonucleotide synthesis and high throughput minigene experiments in transfected HEK 293T cells with next generation sequencing to identify differences in exon inclusion between mutant and wildtype alleles. We applied MaPSy to study over 6,000 variants to discover hundreds of modern and archaic lineage-specific splice-altering substitutions and splice-altering variants in linkage disequilibrium with

archaically introgressed haplotypes, including splice-altering variants in *TLR1*, *SVEP1*, *FHL2*, *WDR88*, and *FAP* genes on adaptively introgressed haplotypes. In contrast to past approaches for studying splice altering variants, such as splicing quantitative trait loci (sQTLs) and overlap with functional annotations, massively parallel reporter assays can be applied equally to common, fixed, rare, and reconstructed variants, are not affected by allele frequency or linkage disequilibrium, and can identify functional variants at single-base resolution. Our findings provide a complementary approach to sQTL and functional annotation overlap for the study of the impact of genetic variants on splicing regulation in human evolution.
[1]Soemedi et al., Nature Genetics (2017)

1212C Diving mammals lose Paraoxonase 1 function in multiple different ways *Wynn Meyer*[1,2], Jerrica Jamison[1,3], Charlotte Cournoyer[4], Irene Kaplow[5], Alexis Michaels[1,6], Jiaxuan Yang[1,7], Rebecca Richter[8], Clement Furlong[8], Nathan Clark[1,9] 1) University of Pittsburgh, Pittsburgh, PA; 2) Office of Data Science Strategy, NIH, Bethesda, MD; 3) University of Toronto, Scarborough, ON, Canada; 4) University of Florida, Gainesville, FL; 5) Carnegie Mellon University, Pittsburgh, PA; 6) Duquesne University, Pittsburgh, PA; 7) Tsinghua University, Beijing, China; 8) University of Washington, Seattle, WA; 9) University of Utah, Salt Lake City, UT.

Multiple clades of mammals have adapted to semi-aquatic lifestyles, with accompanying changes in anatomy, physiology, and behavior. In addition to changes that help these animals navigate and survive in aquatic environments, these lineages also share an unexpected feature: the functional loss of the enzyme Paraoxonase 1 (PON1), a lipid antioxidant that additionally protects against neurotoxicity from the oxon byproducts of organophosphate pesticides. We here demonstrate, using both predicted protein sequence data and direct biochemical testing for the PON1 enzyme, that at least three distinct lineages of semi-aquatic mammals have lost PON1 function. In particular, within pinnipeds (seals, sea lions, and walruses), two separate lineages appear to have lost PON1 function independently, and one such loss most likely involved changes to regulatory elements, rather than to the coding sequence. In this case, we highlight several candidate regions that may underlie the loss, based on analysis of sequence evolution across carnivores and chromatin accessibility in dog liver. The observed repeated functional losses of PON1 may imply a benefit to the loss of this enzyme in a semi-aquatic environment. Within the pinniped clade, we tested for an association between loss of function and two potential environmental contributors -- diet and diving ability -- while correcting for phylogenetic relationships. We find no support for an association between the shift to a semi-aquatic diet and PON1 functional loss. There is a weak trend between longer predicted dive capacity and loss of PON1 function, but this is not significant within our small sample (10 species). In all, our research shows a more complete picture of the extent of PON1 functional loss in semi-aquatic species than was previously understood. It also suggests that certain clades of pinnipeds and lutrids (sea otters) should be monitored for any effects of organophosphate pesticide runoff in their habitats.

1230C Saccharomyces eubayanus population genomics: wild diversity and contributions to domesticated hybrids *Quinn Langdon*[1,4], David Peris[1,2], EmilyClare Baker[1], Dana Opulente[1], Juan Eizaguirre[3], Kelly Buh[1], Kayla Sylvester[1], Martin Jarzyna[1], Diego Libkind[3], Chris Todd Hittinger[1] 1) University of Wisconsin- Madison; 2) Institute of Agrochemistry and Food Technology (IATA), CSIC, Valencia, Spain; 3) Universidad Nacional del Comahue; 4) Stanford University.

How evolution drives diversification within and between species and the consequences of hybridization between independently evolving species are fundamental question in biology. The genus *Saccharomyces* is becoming an ideal model to address questions of evolutionary genomics. In this one genus, differing evolutionary trajectories, i.e. domestication versus wild diversity, can be studied; as well as the dynamics and interaction of variation within and between species. Presented here is a deep characterization of the landscape of diversity within one species, *Saccharomyces eubayanus*. The Patagonian region of South America is a hotspot of diversity for this species, where five subpopulations exists in some sympatry; but a subset of this diversity is globally distributed. Isolates from North America are dominated by an admixed lineage that showed almost 50-50 contributions from two Patagonian populations and, surprisingly, showed the exact same patterns of local ancestry across their genomes. These strains come from four distant locations in North America and show that all individuals originated from a single hybridization event and that this admixed lineage then successfully invaded regions across North America, where *S. eubayanus* is scarce. The even rarer subpopulation found only in the Holarctic ecozone is the wild progenitor of *Saccharomyces cerevisiae* × *S. eubayanus* hybrids that are used for cold-temperature lager-brewing, yet no pure *S. eubayanus* have yet been found in Europe the origin of lager-brewing. The two lager-brewing lineages have maintained variation still segregating in the wild, indicating complex origins of these lineages. Other *Saccharomyces* hybrids are frequently isolated from fermentation environments, including strains with up to four contributing species. Both wild diversity and pre-domestication have led to evolutionary innovations that have influenced the persistence of these hybrids in industrial settings, notably cold-tolerance from a wild parent and flavor-profile from a domesticated parent. This work enlightens our understanding of yeast diversity in the wild and the evolutionary consequences of hybridization and domestication, with parallels to hybridization in other organisms across the tree of life.

1231A A map of genetic variation from 781 soybean genomes *Soon-Chun Jeong*[1], Myung-Shin Kim[1], Roberto Lozano[2], Ji Hong Kim[1], Dong Nyuk Bae[1], Man Soo Choi[3], Namhee Jeong[3], Soo-Kwon Soo-Kwon[3], Michael Gore[2], Jung-Kyung Moon[4] 1) Korea Research Institute of Bioscience and Biotechnology; 2) Cornell University; 3) National Institute of Crop Science; 4) National Academy of Agricultural Sciences.

Soybean is an economically and environmentally important major crop worldwide. It is a predominant plant protein and oil source of both food and feed and has capacity to fix atmospheric nitrogen by intimate symbioses with microorganisms. Here we present a fine genome-wide variation map in 781 accessions including 418 domesticated (*Glycine max*) and 345 wild (*Glycine soja*) soybeans and 18 of their natural hybrids. We identified 31 million single nucleotide polymorphisms and 5.7 million small indels that contribute to within- and between-population variation. We describe a comprehensive characterization of the geographic and functional differentiation of rare and common genetic variants with insights into the domestication history of soybean and detection of domestication-selective sweeps. We show that this resource enables us to increase marker density of existing data sets for improving the resolution of association studies.

1234A Assortative mating and rapid adaptation shape genetic variation in admixed Cape Verdeans *Katharine Korunes*[1], Sandra Beleza[2], Amy Goldberg[1] 1) Duke University, Durham, NC; 2) University of Leicester, Leicester, UK.

Recently admixed populations offer unique opportunities to study rapid evolution. Many standard population genetic methods do not work on the scale of tens of generations. Instead, we can leverage patterns in genetic ancestry in admixed populations to understand short-term evolution in general. On very short timescales, changes in allele frequencies may be difficult to observe and biased by factors like nonrandom mating. In contrast, changes in ancestry patterns in admixed populations can be observed within tens of generations. Using genome-wide SNP data from 564 individuals spanning 7 islands of Cape Verde, we infer the demographic and selective history of the last ~20 generations since founding. Cape Verdeans

are admixed descendants of Portuguese colonizers and West African slaves who settled the islands during the 1400s. First, applying a model-based framework, we infer a positive correlation of inferred ancestry between mating pairs (r^2 = 0.25-0.66). The strength of this relationship varies between islands, and this variation is consistent with historical patterns such as consanguinity on islands with stronger genetic evidence of assortative mating. Next, we show that runs-of-homozygosity (ROH) reflect the contributions of the source populations and patterns of assortative mating. Perhaps surprisingly, the admixed population has lower levels of overall ROH than African source populations. Breaking up ROH into 3 classes by length, we find that short and medium length ROH are often broken up by mismatched local ancestry decreasing genome-wide ROH levels. However, long ROH are actually enriched in the Cape Verdeans. These excess long ROH likely reflect small population sizes and mating preferences post admixture. Finally, we use patterns of local ancestry to test for selection. We identify a long African ancestry tract surrounding the Duffy-negative allele, which is protective against a malaria parasite. Next, we compared Santiago, the only island with endemic malaria, to the other Cape Verdean islands without malaria. Accounting for difference in admixture history, we find an excess of the protective allele on the island of Santiago, but not on other Cape Verdean islands. Together, these results provide insight into the population history of Cape Verde and show how admixed populations provide powerful test cases for understanding evolutionary processes within tens of generations.

1238B Inferring the Demographic History of Inbred Species from Genome-Wide SNP Frequency Data Paul Blischak[1], Michael Barker[1], *Ryan Gutenkunst*[1] 1) University of Arizona, Tucson, AZ.

Demographic inference using the site frequency spectrum (SFS) is a common way to understand historical events affecting genetic variation. However, most methods for estimating demography from the SFS assume random mating within populations, precluding these types of analyses in inbred populations. To address this issue, we developed a model for the expected SFS that includes inbreeding by parameterizing individual genotypes using beta-binomial distributions and taking their convolution to calculate the probability of sampling biallelic variants with different population frequencies. Using simulations, we evaluated the model's ability to co-estimate demography and inbreeding using one- and two-population models across a range of inbreeding levels. We also applied our method to two empirical examples, American pumas (Puma concolor) and domesticated cabbage (Brassica oleracea L.), inferring models both with and without inbreeding to compare parameter estimates and model fit. Our simulations showed that we were able to accurately co-estimate demographic parameters and inbreeding even for highly inbred populations (F=0.9). In contrast, failing to include inbreeding generally resulted in inaccurate parameter estimates in simulated data and led to poor model fit in our empirical analyses. These results show that inbreeding can have a strong effect on demographic inference, a pattern that was especially noticeable for parameters involving changes in population size. Given the importance of these estimates for informing practices in conservation, agriculture, and elsewhere, our method provides an important advancement for accurately estimating the demographic histories of these species.

1284C Investigating the Heterogeneity of Altitudinal Pigmentation in African Drosophila melanogaster Populations *Sarah Petrosky*[1], John Pool[2], Mark Rebeiz[1] 1) Department of Biological Sciencies, University of Pittsburgh, Pittsburgh PA; 2) Department of Genetics, University of Wisconsin-Madison, Madison WI.

The connection between genotype and phenotype is rarely straightforward, especially when considering traits that have recently evolved by natural selection in a population. Most traits are polygenic, with each gene having a subtle effect on phenotype. These causative alleles may not sweep through the entire population: instead of clear “winning” and “losing” alleles, we may find a heterogeneous patchwork of different genes that, when combined, generate similar phenotypes. Fitness-affecting alleles may also experience epistatic effects which mask their influence on phenotype in different backgrounds, generating “cryptic genetic variation”. To explore how genetic heterogeneity and epistasis complicate our picture of evolution in populations, I investigated African populations of *Drosophila melanogaster.* In these populations, cuticle melanization is broadly correlated with altitude: highland flies are dark relative to lowland populations which display ancestral light coloration. This recurring clinal pattern suggests that highland pigmentation has evolved repeatedly under natural selection. Genetic mapping uncovered that this trait is genetically heterogeneous, as different causative loci underlie melanism of lines from the same population. To validate the candidate genes predicted by genetic mapping and implicate these candidates as causal alleles underlying the dark highland phenotype, I utilized *in situ* hybridization and reporter assays. *in situ* hybridization uncovered differential expression of pigment-producing enzymes such as *ebony, tan,* and *yellow* between highland and lowland flies, as well as between different isofemale lines from the same highland or lowland population. These results support the hypothesis that highland pigmentation is genetically heterogeneous. Reporter assays demonstrated differences in GFP expression of the upstream regulatory element of *yellow*, suggesting that genetic changes to this regulatory region may contribute to the highland phenotype. Future work will investigate other candidate genes, their regulatory regions, and potential epistatic interactions that affect the final phenotype.

1291A No evidence for transgenerational immune priming in *Drosophila melanogaster* *Radhika Ravikumar*[1], Brian Lazzaro[1,2] 1) Cornell University; 2) Cornell Institute of Host-Microbe Interactions and Disease.

Most living creatures are under constant and repeated exposure to pathogens, leading to them being perpetually under selection to evolve faster/more efficient ways to fight-off infections. Hence, it makes sense to expect that repeated exposure to the same pathogen would lead to evolution of memory-based immunity. It is canonically believed that this form of immunity is prevalent only among vertebrates and can generate memory-based protection against pathogens both within and across generations. However, among non-vertebrates, evidence for both intra and trans-generational priming is controversial, with studies reporting the presence of this phenotype in some, but not all, systems. In this study, we test for the presence of priming mechanisms in the fruit fly, *Drosophila melanogaster*, using 10 different bacterial pathogens. We chose to focus exclusively on trans-generational priming and tested for the same using both natural and opportunistic pathogens of *Drosophila* spanning a range of virulence from 10% to 100%. We infected mothers via septic injury and looked for evidence of trans-generational priming among offspring. We collected four sets of offspring for each bacterial treatment: offspring from mothers that survived infections, those that succumbed to infections, injury controls and unhandled controls. Subsequently, we measured offsprings’ ability to survive infection and tested whether offsrpring from infected mothers performed better in this assay. Additionally, we also compared offspring from mothers that survived infections to those from mothers that succumbed to infections to test whether mothers’ ability to survive infections enabled a greater amount of priming among offspring. We also performed similar analysis on pathogen burden data collected from each set of offspring to test whether they differed in their ability to curtail pathogen growth. Despite the extensive nature of our experimental design and the large number of pathogens used in our study, we found that offspring survival and pathogen loads were statistically equivalent regardless of their mothers’ infection status. Thus, we find no evidence for transgenerational priming in *Drosophila melanogaster*.

1293C Great ape mutation spectra vary across the phylogeny and the genome due to distinct mutational processes that evolve at different rates *Michael Goldberg*[1], Kelley Harris[1,2] 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Computational Biology Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

All heritable variation begins with mistakes in the transmission of DNA from parents to children. Different DNA motifs can have different mutation rates, and these rates can evolve over time: the spectrum of mutability of three-base-pair motifs has evolved rapidly during great ape diversification, contradicting the fixed molecular clock model. This evolution implies that multiple unknown modifiers of DNA replication fidelity have arisen and fixed on each branch of the ape phylogeny. Such mutator alleles might directly modify DNA replication or repair, or might instead act indirectly by modifying traits like reproduction or chromatin structure. Certain mechanisms of action are expected to create mutations in specific regions of the genome, meaning that the spatial distribution of lineage-specific mutations is informative about their causality. To harness this source of information, we measured mutation spectra of several functional compartments (such as late-replicating regions) whose attributes are known or suspected to affect their mutation rates. Using genetic diversity from 88 great apes, we find that most functional compartments are imprinted by localized mutational signatures but that these signatures explain very little of the mutational divergence between species. Rather, compartment-specific signatures layer with species-specific signatures to create mutational portraits that reflect both lineage and function. In particular, we identify a mutation signature enriched in endogenous retroviruses that seems to co-segregate with the experimentally-measured intensity of the hydroxymethylation of retrovirus-derived DNA. In addition, we find differences in the relative mutability of different local conformations of DNA, reflecting variance in the activity of DNA binding proteins that contribute to mutagenesis. Our results suggest that *cis*-acting mutational modifiers are highly conserved between species and rapid mutation spectrum evolution is driven primarily by *trans*-acting modifiers.

1294A Inferring the properties of mutational effects on fitness using high-throughput phenotyping *Yevgeniy Plavskin*[1,2], Maria S DeBiase[3], Yuan O Zhu[4,5], David W Hall[6], Dmitri A Petrov[4,5], Roland F Schwarz[3], Daniel Tranchina[1,7], Mark L Siegal[1,2] 1) Department of Biology, New York University, New York, NY, USA; 2) Center for Genomics and Systems Biology, New York University, New York, NY, USA; 3) Berlin Institute for Medical Systems Biology, Max Delbruck Center for Molecular Medicine, Berlin, Germany; 4) Department of Biology, Stanford University, Stanford, CA, USA; 5) Department of Genetics, Stanford University, Stanford, CA, USA; 6) Department of Genetics, University of Georgia, Athens, GA, USA; 7) Courant Institute for Mathematical Sciences, New York University, New York, NY, USA.

Spontaneous mutations are the source of novel genetic variation in populations, providing the raw material on which selection acts. To shed light on the spectrum of mutations that constitute the starting point of selection and their typical effects on fitness, we estimated the distribution of effect sizes of spontaneous mutations affecting growth in the budding yeast *Saccharomyces cerevisiae*. We studied mutational effects in a collection of >80 mutation accumulation strains to capture a snapshot of the effects of mutations that have not yet been filtered by selection. Previous work has demonstrated that these strains contain ~300 mutations, primarily single-nucleotide mutations. By combining genotype information with high-precision microcolony-based growth rate measurements from each strain, we are able to infer estimates for the mean mutational effect size, the shape of the mutational effect distribution, and the proportions of mutations with a neutral, positive, and negative effect on growth rate. We find that mutational effects on growth rate are overwhelmingly negative and highly skewed towards very small effect sizes. Interestingly, our modeling suggests that a single distribution of mutational effects does not account well for the observed distribution of growth phenotypes. Rather, growth rate effects of mutations fall into two categories: single-nucleotide substitutions, which on average have larger effects, and frequent, smaller-effect mutations of unknown origin. The estimated high frequency of mutations in the latter category, combined with the fact that they were undetected by conventional sequencing analysis, point to microsatellite mutations as likely candidates underlying the non-single nucleotide mutational effects. We are performing follow-up experiments to directly assay the effects of *de novo* microsatellite mutations on growth. Our work reveals the spectrum of effect sizes of the mutations on which evolution acts, and points to a likely key role of microsatellite mutations in shaping natural diversity.

1297A Investigating the role of odorant binding proteins in the *Drosophila* post-mating response *Nora Brown*[1], Benjamin Gordon[1], Geoffrey Findlay[1, 2], Andrew Clark[1], Mariana Wolfner[1] 1) Cornell University, Ithaca, NY; 2) College of the Holy Cross, Worcester, MA.

In many species, males transfer sperm as well as a suite of non-sperm components in the ejaculate to females during mating. A subset of these non-sperm molecules are known as seminal fluid proteins, and in *Drosophila melanogaster*, they are essential mediators of fertility and can have profound and lasting effects on female behavior and physiology post-mating. Additionally, the genes encoding seminal fluid proteins include some of the fastest evolving genes in the genome, which is thought to be due to sperm competition and sexual conflict. The composition of seminal fluid proteins in *D. melanogaster* is complex, and includes many members of large gene families. One such gene family is the odorant binding protein (Obp) family, of which several members are present in the seminal fluid. Previous work in *Drosophila* and other insects has shown that some Obps are highly expressed in the antennae, and can mediate behavioral responses to odorants and pheromones through the action of odorant receptors in olfactory receptor neurons. However, the function of these "reproductive" Obps is entirely uncharacterized. Our results using RNAi knockdown and genetic knockout suggest a role for one of these Obps, *Obp56g,* in mediating proper sperm transfer, movement, or storage in mated females. In addition to characterizing the reproductive defects of males lacking *Obp56g*, we are constructing genetic strains to test for functional redundancy among the multiple Obps present in the seminal fluid. Analysis of expression patterns and signatures of selection for these Obps across the *Drosophila* phylogeny shed light on whether Obps are "co-opted" for reproductive function over evolutionary time. Together, this work enhances our understanding of the molecular mechanisms of fertilization and suggests a novel role for odorant binding protein genes in reproduction.

Friday, May 1 12:00 PM - 2:30 PM

Evolutionary and Population Genetics 5 - Poster Q&A 1164C Population Genomics of the *Ogataea polymorpha* Species Complex Sara J. Hanson[1], Paul Fuchs[1], Georgie Nahass[1], Kenneth H. Wolfe[2] 1) Colorado College; 2) Conway Institute, University College Dublin, Dublin, Ireland.

The methylotrophic yeast *Ogataea polymorpha* has long been a useful system for recombinant protein production, as well as a model system for methanol metabolism, peroxisome biogenesis, thermotolerance, and nitrate assimilation. More recently, it has become an important model for the evolution of mating-type switching. *O. polymorpha* performs mating-type switching by inverting a 19-kilobase region of the genome to move the mating-type (*MAT*) genes between expressed and transcriptionally repressed regions. This mechanism of mating-type switching has evolved independently multiple times in the *Ogataea* clade. Here, we present a population genomics analysis of 50 strains within the *Ogataea polymorpha* species complex from the USDA-NRRL and Phaff yeast culture collections, including representatives from the species *O. polymorpha*, *O. parapoly-*

morpha, *O. haglerorum*, and *O. angusta*. In addition to examining the population structure and genetic variation within and between these species, we also examine the structure and evolution of the *MAT* region across strains to better understand how flip/flop mating-type switching has impacted the genome.

1172B Rampant gene duplication on Y chromosomes facilitates Y-linked gene evolution in the *Drosophila simulans* clade *Ching-Ho Chang*[1], Lauren Gregory[1], Colin Meiklejohn[2], Amanda Larracuente[1] 1) Department of Biology, University of Rochester, New York, USA; 2) School of Biological Sciences, University of Nebraska, Lincoln, USA.

Y chromosomes are typically heterochromatic and highly repetitive. While Y chromosomes generally do not recombine and thus lose most of their gene content, they can acquire genes from other regions of the genome. Some of these newly acquired genes expand in copy numbers after moving to the Y chromosome (e.g., ampliconic genes) and may evolve new functions and/or involve in intragenomic conflicts. Evolutionary analyses of ampliconic gene families are lacking because of the divergence of ampliconic genes between species and the paucity of sequence information for highly repetitive Y chromosomes. Here we study Y-linked ampliconic gene evolution in *Drosophila melanogaster* and three species in *D. simulans* clade (~15 Mb of Y assemblies with N50 ranging from 400 kb to 1 Mb) by generating high-quality assemblies. Although these species only diverged between 0.2 to 3 million years, their Y chromosomes are functionally diverged and cause hybrid male sterility. Consistent with other studies, we detect high rates of duplication and gene conversion on Y chromosomes. Our comparison of 21 Y-linked pseudogenes indicates that Y chromosomes are enriched for large deletions (49/130 deletions are larger than 10 bp). This pattern suggests that Y chromosomes prefer microhomology-mediated end joining (MMEJ) over canonical non-homologous end-joining. MMEJ occasionally generates tandem duplications and chromosome translocations, consistent with our observation of rampant structural and sequence divergence on Y chromosomes between species. We propose that the high Y-linked duplication rate fuels the birth of ampliconic genes, one of the most striking convergent features of Y chromosomes across species. We next asked how copy number shapes Y-linked gene evolution. Our phylogenic analyses suggest that two ampliconic Y-linked gene families are under strong positive selection ($\omega > 1.3$) and may have evolved new functions. High gene conversion rates can further fuel both purifying and positive selection of Y-linked ampliconic genes. Finally, we detect signatures of potential arms races between X and Y-linked ampliconic genes— the X-linked homologs of a Y-linked ampliconic gene family also amplified and evolves rapidly in the simulans clade. Our study reveals that distinct mutation patterns shared among Y chromosomes lead to gene acquisition, amplification, and diversification. These patterns may drive the parallel evolution of the Y chromosome organization across species.

1174A RepeatProfiler: a pipeline for visualization and comparative analysis of repetitive DNA profiles Sherif Negm[1], Anya Greenberg[1], Amanda Larracuente [1], John Sproul[1] 1) University of Rochester.

Comparative study of DNA repeats in model organism is beginning to highlight the role of repetitive DNA in many processes that drive genome evolution and phenotypic change. Because repetitive regions are much more dynamic than single-copy DNA, repetitive sequences can reveal signal of evolutionary history over short time scales that may not be evident in sequences from slower-evolving genomic regions. Many existing tools for studying repeats are directed toward organisms with existing genomic resources, including genome assemblies and repeat libraries. However, signals in repeat variation may prove especially valuable in disentangling evolutionary histories in diverse non-model groups, for which genomic resources are limited. Here we introduce RepeatProfiler, a tool for comparative study of repeats using low-coverage short-read sequence data. RepeatProfiler automates the generation and visualization of repetitive DNA coverage depth profiles and facilitates comparison of profile shape across samples for one or more repeats of interest. In addition, RepeatProfiler facilitates comparison of profiles through phylogenetic analysis by extracting signal from variants across profiles and outputting an alignment file that summarizes those variants, which can then be analyzed as molecular morphological characters. We validate RepeatProfiler with data sets from various species (e.g beetles, fruit flies, tomatoes), and highlight the potential of repetitive DNA profiles for informing evolutionary studies over short time scales.

1232B Coalescent inference of mutation spectrum histories from sample frequency spectra *William DeWitt*[1,2], Kameron Decker-Harris[1], Kelley Harris[1,2] 1) University of Washington, Seattle; 2) Fred Hutchinson Cancer Research Center, Seattle.

Models in evolutionary genetics typically assume that mutation rate is constant over time and between populations and closely related species. However, recent work casts doubt on this assumption in human and ape populations, and reveals that mutation is a complex and dynamic process. Whether arising from variation in replication fidelity, life history, or environmental exposures, mutation rate evolution can be accompanied by changes to the *mutation spectrum*: the mutation rate in different local nucleotide contexts. We extend theoretical tools based on Kingman's coalescent to accommodate a richly parameterized mutation process, varying in time and in spectrum. We infer human mutation spectrum histories from patterns of modern genomic diversity, allowing us to reconstruct trajectories of mutation spectrum divergence between populations, track a transient mutation spectrum perturbation through multiple populations, and characterize how mutation spectrum histories are structured by local nucleotide context. Mutation rate and effective population size together determine the strength of genetic drift shaping genomic variation. We introduce fast nonparametric joint inference of mutation spectrum history and demographic history from unphased genomes.

1255A Genetic diversity's twin aspects: what sequence (or expression, etc), and how many of each sequence? New tricks for combining these. *William Sherwin*[1] 1) UNSW-Sydney.

Genetic diversity is crucial to evolution and conservation management, yet it is surprisingly difficult to find a combined measure for two important aspects:

- frequency of variants

and

- functional differences between the variants, such as sequence or expression.

Incorporating these two aspects into diversity measures has met a number of stumbling-blocks, especially when considering two or more levels of diversity, such as diversity within and between populations. Firstly, sometimes diversity between populations can appear to be negative, which makes no intuitive sense. Secondly, some common measures are open to misinterpretation, such as AMOVA that partitions total genetic variation into the proportion at each level (eg, within and between population). It is often not recognised that these proportions are interdependent, eg: if two populations share no sequences at all, the proportion of variation that is between-populations must go down if the variation with each population increases, although the populations still share no sequences. This problem is also found in some frequency-only diversity measures such as Gst and Fst. Solutions to these problems are based on three related summaries of gene diversity: (0) presence/absence of variants (1) Shannon infor-

mation, and (2) heterozygosity/nucleotide diversity etc (Sherwin et al '17). It has recently been shown that functional diversity (such as sequence or expression) can be incorporated into each of these three measures, without producing counterintuitive results (Chao et al 2019).
Sherwin W et al. 2017. Trends Ecol. Evol. 32:948
Chao A et al. 2019. Ecol. Monog. doi.org/10.1002/ecm.1343

1256B Polymorphic house fly male-determining proto-Y chromosomes affect male behaviors in ways that are consistent with their distributions in natural populations Pablo Delclos[1], Kiran Adhikari[1], Anna Matuk[1], Rebecca Presley[1], Alexander Oderhowho[1], Tomi Hassan[1], Jessica Tran[1], *Richard Meisel*[1] 1) University of Houston.

Sex determination is the process by which a genetic signal or environmental cue initiates the development of sexually dimorphic phenotypes. The master regulators of sex determination frequently differ across species, and they can even be variable within species that have polygenic sex determination. Understanding the selection pressures that act on polygenic sex determination within species is informative of the factors that drive the evolution of sex determination across species. The house fly, *Musca domestica*, is a well-suited model for studying polygenic sex determination because multiple male and female determining loci segregate as polymorphisms in natural populations. Notably, two different "proto-Y" chromosomes that each carry a male determiner are found in opposing north-south clines on multiple continents. We hypothesize that temperature differences across the clines favor a cold-adapted male-determining proto-Y chromosome in the north, and a different warm-adapted proto-Y chromosome in the south. We are testing this hypothesis by asking if temperature-dependent physiology and behavior differ across males carrying different proto-Y chromosomes. Here, we will show that temperature preference differs between males carrying either the northern or southern proto-Y chromosomes, with preferences in the direction consistent with their latitudinal distribution. Moreover, developmental temperature (i.e., acclimation) affects preference, such that cold acclimated flies prefer warm temperatures, and warm acclimated flies prefer cold temperatures. At extreme cold and warm temperatures, the acclimation effect dominates, and both genotypes respond to acclimation with similar temperature preference. Only in the middle of the acclimation temperature range do we observe a genotype effect on temperature preference. Therefore, temperature preference in house fly depends on both acclimation and genotype, and the genotype effect is consistent with the natural distribution of proto-Y chromosomes. We additionally find that male mating behaviors also depend on genotype and acclimation temperature. We hypothesize that these connections between polygenic sex determination and temperature-dependent behaviors is the result of genetic variants on the different proto-Y chromosomes that affect these phenotypes. This result provides the first evidence, to our knowledge, of a link between genetic variation in sex determination and an ecologically relevant behavior.

1258A Genetic basis of de novo appearance of carotenoid ornamentation in bare-parts of canaries *Malgorzata Gazda*[1,2], Matthew Toomey[3], Pedro Araujo[1], Ricardo Lopes[1], Afonso Afonso[1], Connie Myers[4], Kyla Serres[4], Philip Kiser[5], Geoffrey Hill[6], Joseph Corbo[4], Miguel Carneiro[1,2] 1) CIBIO; 2) Universty of Porto; 3) University of Tulsa; 4) Washington University School of Medicine; 5) University of California; 6) Auburn University.

Unlike wild and domestic canaries (*Serinus canaria*), or any of the three dozen species of finches in genus *Serinus*, the domestic urucum breed of canaries exhibits bright red bills and legs. This novel bare-part coloration offers a unique opportunity to understand how leg and bill coloration evolve in birds. To identify the causative locus, we resequenced the genome of urucum canaries and performed a range of analyses to search for genotype-to-phenotype associations across the genome. We identified a nonsynonymous mutation in the gene *BCO2* (beta-carotene oxygenase 2, also known as *BCDO2*), an enzyme involved in the cleavage and breakdown of full-length carotenoids into short apocarotenoids. Protein structural models and *in vitro* functional assays indicate that the urucum mutation abrogates the carotenoid cleavage activity of BCO2. Consistent with the predicted loss of carotenoid cleavage activity, urucum canaries had increased levels of full-length carotenoid pigments in bill tissue and a significant reduction in levels of carotenoid cleavage products (apocarotenoids) in retinal tissue compared to other breeds of canaries. We hypothesize that carotenoid-based bare-part coloration might be readily gained, modified, or lost through simple switches in the enzymatic activity or regulation of *BCO2* and this gene may be an important mediator in the evolution of bare-part coloration among bird species.

1262B Exploring genomic signatures of environmental adaptation in Ethiopian sheep *Pam Wiener*[1], Christelle Robert[1], Mazdak Salavati[1], Deepali Vasoya[1], Abulgasim Ahbara[2], Joram Mwacharo[4], Juliane Friedrich[1], James Prendergast[1], David Wragg[1], Mick Watson[1], David Hume[5], Olivier Hanotte[2,3], Emily Clark[1] 1) Roslin Institute, University of Edinburgh, Midlothian, United Kingdom; 2) University of Nottingham, Nottingham, United Kingdom; 3) International Livestock Research Institute, Addis Ababa, Ethiopia; 4) International Center for Agricultural Research in the Dry Areas, Addis Ababa, Ethiopia; 5) Mater Research Institute, Brisbane, Australia.

Over the last 20 years, there has been a great interest in using population genetic-based methods for detecting signatures of selection in the genomes of livestock species. Such studies have identified numerous regions of the genome, many of which have been associated with human-imposed selection related to domestication, breed development and breeding for production traits. However, livestock are obviously also subject to natural selection and in many cases, this will be more valuable to document and understand. Dissection of the genetic basis of environmental adaptation in livestock may contribute to various outputs, e.g. prioritisation of native breeds for conservation, reduction of negative impacts of agricultural intensification by optimal matching of genotype to environment and informing breeding and farming management decisions.

This study focused on detection of environmental adaptation in Ethiopian sheep. Ethiopia is particularly interesting to study because of the wide range of environmental characteristics across the country and the diverse genetic backgrounds in livestock species, for which it is known to have served as a gateway of introduction into Africa. Samples of sheep were collected from 12 locations across a range of agro-ecological zones. Whole-genome sequencing was used to generate single nucleotide polymorphism (SNP) data across the sheep genome. Two approaches were used to test for genomic associations with environmental adaptation using location-based climate and environmental measures as proxies for adaptation phenotypes. The first involved using BayPass software to test for associations between SNPs and 20 bioclimatic variables. The second approach applied the Population Branch Statistic (PBS) to a subset of populations to specifically examine high-altitude adaptation. Functional classification was evaluated for genes encompassing SNPs with strong environmental associations. The results of these analyses suggest that rainfall has a greater influence than temperature or altitude on adaptation of these populations and that the underlying genes are enriched for expression in blood compared to other tissues.

1265B Ecological drivers of CRISPR immune systems *Wei Xiao*[1], Jake Weissman[1], Philip Johnson[1] 1) University of Maryland, College Park, MD.

CRISPR-Cas is the only known adaptive immune system of prokaryotes. It is a powerful defense system against mobile genetic elements such as

bacteriophages. While CRISPR-Cas systems can be found throughout the prokaryotic tree of life, they are distributed unevenly across taxa and environments. Since adaptive immunity is more useful in environments where pathogens persist or reoccur, ecological drivers of uneven CRISPR distribution likely involve the density or diversity of the host/pathogen community. We directly tested hypotheses connecting CRISPR incidence with prokaryotic density/diversity by analyzing 16S rRNA and metagenomic data from publicly available environmental sequencing projects. In terms of density, we found that CRISPR systems are significantly favored in lower abundance (less dense) taxa and disfavored in higher abundance taxa, at least in saltwater environments. When we extended this work to compare taxonomic diversity between samples, we found CRISPR system incidence significantly correlated with diversity in human oral environments. Together, these observations confirm that, at least in certain types of environments, the prokaryotic ecological context indeed plays a key role in selecting for CRISPR immunity, potentially due to correlations with pathogen dynamics.

1270A Genotype pinning in a periodic environment *Judith Miller*[1], Camille Hankel[2] 1) Georgetown University; 2) Harvard University.

The spatial distribution of genotypes in an invasive species arises from the interplay of ecological, evolutionary and abiotic forces; it both reflects and influences the success, speed and ultimate extent of a biological invasion. Understanding the joint spatial distribution of population density and genotypes during invasions, and the forces that create these distributions, is a central goal of invasion biology. Invasion with adaptive evolution can lead to patial sorting of genotypes and/or range pinning (invasion halting not due to environmental discontinuities). In particular, these phenomena can occur when purely ecological or neutral models would not predict limits to spread. However, we do not yet understand when, and especially why, such patterns arise.

In this theoretical study, we identify new conditions that lead to pinning of a genotype during colonization of new territory by an asexual species. This occurs in the joint absence of Allee effects, demographic or environmental stochasticity, and nonperiodic environmental gradients. In other words, periodic environmental variation plus selection can halt the advance of a genotype in the absence of conditions previously shown to facilitate range pinning.

These results are derived from numerical simulation of a deterministic competition model. The model specifies a patchy environment that varies in a spatially periodic fashion, with repeatedly alternating isolated patches of two types of habitat. The species comprises two genotypes whose fitness differs on at least one habitat type. Our findings do not duplicate the observation that competition can stabilize range boundaries between two established species (competitive exclusion). Rather, they clarify the range dynamics that arise from the simultaneous arrival of propagules of two types in a spatially periodic environment. They suggest avenues for the development and analysis of more complex models (especially of sexual species and with temporal environmental variation) that can guide efforts to manage both invasive species and species undergoing climate-induced range shifts.

1272C The role of seminal fluid gene expression in sperm competition and speciation in *Drosophila* *Bahar Patlar*[1], Lauren Fulham[1], Alberto Civetta[1] 1) University of Winnipeg.

Sperm competition is a form of post-mating male-male competition that can drive the rapid evolution of the male ejaculate. The rapid diversification of the male ejaculate can be an important driver of speciation. Conspecific sperm precedence (CSP) is a form of reproductive isolation mediated by sperm competition, where ejaculates from different species compete leading to the advantage in fertilization of the male belonging to the same species as the female. So far, the selective causes and ejaculate traits responsible for CSP are poorly understood. Seminal fluid proteins (SFPs) that are transferred during mating along with sperm have diverse functions conferring male advantage in sperm competition and can be one major factor affecting CSP among species. Previous studies of SFPs of the common model organism *Drosophila melanogaster* have identified some of those proteins as having a function in sperm competition with only a couple of SFPs found to influence CSP. Therefore, in this project, we aim to expand the functional characterization of SFP in sperm competition and ultimately in speciation. To do so, we first focus our effort to scan through about two dozen SFP previously identified as candidate genes influencing sperm competition by using an RNAi knockdown approach and we further test their role in CSP. Considering that expression of SFPs is highly plastic depending on the environment, we additionally hypothesize that variation in expression of these genes can be linked to CSP and thus the occurrence of reproductive isolation depending on the environment (i.e. ecological speciation), as well as the genetic background of males (i.e. epistasis). Therefore, we survey variation in expression of SFPs under different environmental conditions using the *Drosophila Genetic Reference Panel* (*DGRP*) inbred lines and test their function in sperm competition and CSP. Overall, we aim to shed light on the role of SFPs in sperm competition and CSP and their potential contribution to speciation.

1275C Using a genetically tractable system to study the chemical arms-race between monarch butterflies and milkweeds *Derrick Yip*[1], Marianthi Karageorgi[1], Anurag Agrawal[2,3], Simon Groen[1,4], Elizabeth Ordeman[1], Noah Whiteman[1] 1) Department of Integrative Biology, University of California, Berkeley, CA, USA; 2) Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA; 3) Department of Entomology, Cornell University, Ithaca, NY, USA; 4) Department of Biology, Center for Genomics and Systems Biology, New York University, New York, NY, USA..

A long standing hypothesis in evolution is that chemical arms-races drive coevolution of toxic plants and their herbivores. A well-known example is the milkweed-monarch system. Butterflies of the milkweed butterfly lineage (Danaini) have become specialists of toxic cardenolideproducing milkweeds by evolving increasing levels of resistance to cardenolides through amino acid substitutions in their ATPα (α subunit of the Na+/K+ ATPase, the physiological target of cardenolides). We previously generated four Drosophila melanogaster knock-in lines carrying different resistance combinations of mutations in their ATPα (LAN, LSN, VSN, VSH) as observed outside and within the monarch butterfly lineage. These knock-in fly lines possess increasing levels of resistance (in the increasing order of LAN, LSN, VSN, VSH) to the cardenolide ouabain. Yet, it still remains unknown whether the lines will possess an identical order of increasing resistance when presented cardenolide mixtures of increased toxicity, as found in milkweeds. We first performed in vitro physiological assays with mutant Na+/K+ ATPases in the knock-in fly lines, comparing resistance levels of the four knock-in lines using isolated cardenolides with known parameters affecting their toxicity (polarity, structure, and concentration). Then, we performed bioassays to examine how fly media containing tissue from ten milkweed species ranging from high to low toxicity affected survival and growth rate of the knock-in fly lines. Our experiments reveal which ATPα substitutions confer the highest level of resistance to the cardenolides found in milkweeds and provide more insight into the chemical arms-race between monarch butterflies and milkweed plants.

1283B Differential targeting of the apical extracellular matrix is associated with extreme cell shapes accompanying morphological diversification

in *Drosophila* genitalia *Ben Vincent*[1], Mark Rebeiz[1] 1) University of Pittsburgh.

A major challenge in evolutionary and developmental biology is to map the genetic causes of morphological differences between species and determine how these genetic variants exert their effects on developing tissues. Despite many successful case studies that focus on traits like pigmentation, trichome loss or courting behavior, it has proven challenging to dissect the genetic basis of interspecific differences in the size and shape of multicellular 3-dimensional organs. We used high-resolution imaging to investigate the cellular basis of morphological diversification in the posterior lobe, a genital structure specific to the *Drosophila melanogaster* clade that varies in size and shape between *Drosophila simulans* and *Drosophila mauritiana*, two species that can generate viable hybrid offspring. By tracking the development of the pupal epithelium that forms the posterior lobe, we found that the increased area of the *Drosophila simulans* lobe compared to *Drosophila mauritiana* results from a larger number of cells committed to the posterior lobe fate, as well as a subsequent tapering process that occurs in *Drosophila mauritiana*. Cells in both species adopt extreme apical dimensions, with some cells in *Drosophila mauritiana* reaching an apical surface area of 2-3 square microns. We used lectin staining to correlate some of these differences in epithelial remodeling with the apical extracellular matrix (aECM), which targets a larger number of cells in *Drosophila simulans* and hybrids compared with *Drosophila mauritiana*, and has been previously shown to underlie lobe development in *Drosophila melanogaster*. Finally, we measured similar differences in the gene expression pattern of *dumpy*, a component of the aECM, which suggests that this diverged developmental process is at least partially controlled at the level of gene regulation. This work suggests that extracellular forces can contribute to morphological diversification, and specifically indicates that changes in *dumpy* gene regulation may alter the cellular dimensions as well as the overall number of cells in the developing posterior lobe.

1285A Evolving a novel trait through co-option of the *shavenbaby* gene regulatory network *Gavin Rice*[1], Kenechukwu Charles-Obi[1], Mark Rebeiz[1] 1) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA.

In order to understand the mechanisms that led to the diversity of forms found across the tree of life, it is vital to determine how new traits are generated. Network co-option is a process thought to facilitate novelty through the deployment of a set of interacting genes into a new developmental context . Most models of co-option suggest the existence of an upstream factor whose ectopic expression is sufficient to initiate the activation of an entire network in a new tissue. However, these transcription factors have seldom been identified, and their sufficiency to induce a trait has rarely been established. **Here, we identify and validate an upstream network that was redeployed from a well characterized network to generate a novel morphological feature.**

Among *Drosophila* species, a wide variation of genital morphologies exist, including the phallus of *Drosophila eugracilis*, which is covered with over a hundred spike shaped structures that have been implicated in wounding females during copulation. The homologous tissue in *Drosophila melanogaster* lacks these spikes, providing a convenient outgroup for comparisons of development and genetic manipulations. We have found that the spikes in *Drosophila eugracilis* are produced by single cell extensions, similar to the small hairs (trichomes) that adorn the body in *Drosophila*. The transcription factor *shavenbaby* activates a well-studied network for trichome formation. We find that *shavenbaby* and its downstream targets are expressed in spike-forming tissues, suggesting the co-option of this network underlies this dramatic phenotype. We have also discovered that ectopic expression of *shavenbaby* in the phallus of *Drosophila melanogaster* induces phallic spikes. These results indicate that co-option of the *shavenbaby* network is sufficient for the gain of this dramatic novel trait.

1295B Determining the risk of hemiplasy in the presence of incomplete lineage sorting and introgression *Mark Hibbins*[1], Matthew Gibson[1], Matthew Hahn[1] 1) Indiana University, Bloomington, IN.

The incongruence of character states with phylogenetic relationships is often interpreted as evidence of convergent evolution. However, trait evolution along discordant gene trees can also generate these incongruences – a phenomenon known as hemiplasy. Classic phylogenetic comparative methods do not account for discordance, resulting in incorrect inferences about the number of times a trait has evolved, and therefore about convergence. Biological sources of discordance include incomplete lineage sorting (ILS) and introgression, but only ILS has received theoretical consideration in the context of hemiplasy. Here, we derive expectations for the probabilities of hemiplasy and homoplasy with ILS and introgression acting simultaneously. We find that introgression always makes hemiplasy more likely, suggesting that methods that account for discordance due to ILS alone will be conservative. We also present a method for making statistical inferences about the relative probabilities of hemiplasy and homoplasy in empirical datasets. Our method is packaged in software dubbed *HeIST* (Hemiplasy Inference Simulation Tool), and provides information on the most probable number of transitions among character states given a set of relationships with discordance. *HeIST* can accommodate ILS and introgression simultaneously, and can be applied to large phylogenies. We apply this tool to a phylogeny of New Guinea lizards that have evolved green blood from a red-blooded ancestor, and find that hemiplasy is likely to explain the observed trait incongruence.

Friday, May 1 12:00 PM - 12:30 PM

Evolutionary and Population Genetics 6 - Poster Q&A 1214B Intraindividual sequence variation in pre-rRNA cistons of the ectoparasitic plant *Cuscuta* *David A Johnson*[1] 1) Samford University.

Cuscuta, commonly called dodder (family Convolvulaceae), is an ectoparasite of numerous plants, sucking nutrients from its hosts using haustoria, which penetrate into the vascular tissue of the host. We have demonstrated that several species of *Cuscuta*, especially *C. pentagona*, show considerable variation in the sequence of its pre-rRNA cistrons. The phylogenetic relationship between these sequences is presented and the possible significance of this diversity is discussed.

1219A A study on genetic patterns of eye color and wing presence in *Drosophila melanogaster* *Yusheng Wu*[1] 1) Universtiy of the Southwest Hobbs, NM.

The traditional genetic patterns of eye color and wing presence in *Drosophila melanogaster* were studied using the commercial strains, apterous (wingless, on chromosome 2), brown eyes (on chromosome 2) and scarlet eyes (on chromosome 3) from Carolina Biological Supply Company in 2018. To observe the gene segregation in the first dihybrid, brown eye, winged strain was mated by red eye, wingless strain. To study gene interaction in the second dihybrid, brown eye strain was crossed by scarlet eye strain. The χ^2 statistical test was used to determine whether there was a good fit for two dihybrids. In monohybrids of these two traits, the segregation of male flies followed 3:1 ratio, but that of female flies didn't. All χ^2

test results from the first dihybrid indicated that the segregations of male, female and overall flies didn't fit 9:3:3:1 ratio. The number of brown and wingless flies was too far from expected number. The same situation occurred in the second dihybrid of the different eye colors. The number of two phenotypes, red and white eye, was far from expected number. The classical gene interaction was not observed in the cross. Meiotic drive elements and environmental factors may play the role in the variations.