# Online Proceedings

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## MOLECULAR AND CELLULAR BIOLOGY

*Session Moderator: Hannele Ruohola-Baker, Biochemistry*

**111 JHN**

*1:15 PM to 2:45 PM*

* Note: Titles in order of presentation.

**Patterns of Importin Evolution in *Drosophila***

*Emily Hsieh, Senior, Biochemistry, Biology (Molecular, Cellular & Developmental)*

*Levinson Emerging Scholar, Mary Gates Scholar, Undergraduate Research Conference Travel Awardee*

*Mentor: Harmit Malik, Basic Sciences (UW Genome Sciences), Fred Hutchinson Cancer Research Center*

*Mentor: Nitin Phadnis, Division of Basic Sciences, Fred Hutchinson Cancer Research Center*

The nuclear transport pathway performs the fundamental function of moving cargo between the cytoplasm and nucleus in all eukaryotes. Nuclear transport is carried out through a highly conserved mechanism across all eukaryotes. Yet, in *Drosophila*, several components of the nuclear transport apparatus, including importins, evolve rapidly under positive selection. Genetic conflict with selfish elements has been suggested as a possible cause for this pattern of rapid evolution. Here, we present a comprehensive phylogenomic analysis of importin gene evolution in *Drosophila*. Importins are adapter molecules that directly mediate the transport of cargo into the nucleus. Our analysis reveals a recurrent pattern of gain and loss of importin paralogs across independent lineages of *Drosophila*. Interestingly, we discovered that almost all new copies of importins have acquired a testes-specific expression pattern since their birth through gene duplication. This pattern of repeated gains of testes-specific copies of importins suggests a function in suppressing genetic conflicts such as segregation distortion in the male germline. Segregation distorters such as *SD* in *Drosophila melanogaster* act by impairing nuclear transport in the testes. We are currently performing functional tests for the hypothesis that an increased dosage of these testes-specific importins may serve a role in suppressing segregation distortion in the male germline by restoring nuclear transport during spermatogenesis.

**Exploring the Roles of C*oncertina* (*cta*) and its Partial Duplicate *CG40005* in *Drosophila* Tubulogenesis**

*Dorothy Glyze (Dorothy) Abuan, Senior, Biochemistry*

*Mentor: Celeste Berg, Genome Sciences*

*Mentor: Ariel Altaras, Genome Sciences*

Tubes are the foundation for many organs in all animals. Although tube formation is critical for development, relatively little is known about how cells coordinate their behaviors to form tubes. To investigate this process, we are studying the development of the dorsal appendages (DAs), two respiratory structures that are formed in *Drosophila* ovaries and protrude from the fly egg. We use a genetic approach to disrupt gene function, then study cell behaviors during tube formation. Previously, our lab showed that trimeric G proteins play a role in DA formation. Trimeric G proteins act as signal transducers and G$\alpha$, one subunit of the trimeric G-protein complex, is responsible for signal specificity. Although in situ hybridization showed that six of seven *Drosophila* G$\alpha$s are expressed in the ovary, functional studies demonstrated that G$\alpha$12 was most important. RNAi against G$\alpha$12 in a subset of DA-forming cells resulted in 90% of egg chambers exhibiting abnormal DAs. We hypothesize that *concertina* and its partial duplicate *CG40005*, the genes coding for G$\alpha$12, work together in DA-forming cells to coordinate cell behaviors. To test this hypothesis, we generated null mutations in *cta* and *CG40005*. We induced random deletions in the DNA via irradiation and screened for loss of a visible marker carried on a transposon near cta. Through complementation tests, we showed that 22 deletions extended into *cta*, or the neighboring essential gene, *light*. Although the genomic region contains many repeated sequences, I developed PCR tests and found 18 deletions disrupting *cta*, *CG40005*, and *light*, and one deleting only *CG40005*. Finally, rescue studies using large BAC clones generated by the P[acman] project will let us examine cell behaviors during DA formation in the absence of *cta* and *CG40005*. Since DA formation resembles neural-tube formation in vertebrates, our studies will give insight into tube-forming processes in other animals.

**The Role of Metabolites in Stem Cell Differentiation**
*Sandra Shannon, Senior, Biology (General)*
*Mentor: Hannele Ruohola-Baker, Biochemistry*
*Mentor: Henrik Sperber, Biochemistry*

Recent reports have shown that metabolic signatures are highly characteristic for a cell and may act as a leading cause for cell fate changes. In particular, we have shown that during development from naïve to primed embryonic stem cells, the cells undergo a dramatic transition from metabolically bivalent to highly glycolytic. However, this state of inert mitochondria rapidly changes to highly potent mitochondria during further differentiation. It is not yet understood how and why the pluripotent cells enter the highly glycolytic, metabolically cancer-like (Warburg effect) stage and how a differentiating cell leaves this stage. We have now performed metabolomics profiling of naïve and primed pluripotent cells using mass spectrometry. Interestingly, primed cells accumulate the tryptophan degradation product kynurenine. Kynurenine (KY) can act as a ligand for the transcription factor AHR. In cancer cells AHR activation by KY incudes growth while in surrounding T-cells KY based AHR activation will inhibit T-regulatory cells. My hypothesis is that kynurenine is a key metabolite in determining stem cell stage and in helping protect the primed stage embryo against the mother's immune cells. I will now test this hypothesis by analyzing the levels of the key KY metabolism enzymes by qPCR. My prediction is that the tryptophan degrading enzyme IDO1 will be unregulated in the primed stem cells. To test whether kynurenine can change the state of naïve stem cells, I will add different concentrations of kynurenine to the stem cell media and test potential cell fate changes. Since primed ESC-like metabolism is also observed in cancer cells, it is of great interest to understand how the cellular transitions in and out of this metabolic state are controlled during normal development. These studies will shed light on the function and regulation of metabolism during normal and disease developments.

**Sequencing DHODH in Persons with Miller Syndrome: Expanding the Spectrum of Mutations**
*Naomi Tweyo (Naomi) Nkinsi, Freshman, Pre-Sciences*
*Mentor: Heidi Gildersleeve, Department of Medicine Pediatrics Genetics*

Miller syndrome is a rare autosomal recessive disorder resulting from mutations in the DHODH gene. DHODH encodes the enzyme dihydroorotate dehydrogenase, which is involved in de novo pyrimidine biosynthesis. Clinical characteristics of Miller syndrome include craniofacial abnormalities, coloboma of the eyelids, cleft palate, and missing postaxial digits on the hands and feet. To broaden the spectrum of causal mutations, DNA samples obtained from two unrelated affected probands were Sanger sequenced to screen for mutations in DHOHD. In family 1, the affected individual was a compound heterozygote for a mutation in one of the canonical splice bases of intron 6-7 (c.820+1G>A), and a missense mutation in exon 8 (c.C1036T, p.R346W). DNA samples obtained from both parents of the affected proband were screened using Sanger sequencing and each parent was found to be heterozygous for one of the DHODH mutations. The c.C1036T mutation has been reported now in three families with Miller syndrome and functional studies have demonstrated a reduction of DHODH activity by 40%-70% in yeast with this mutation. No pathogenic mutations in DHODH were found in family 2. The next steps we will take include whole genome sequencing of the proband and parents in family 2 to determine if there are mutations in other genes that cause Miller syndrome. Our results mark the first report of a splice site mutation in a patient with Miller syndrome and further expand the spectrum of causative mutations in DHODH.

**Pax5 Movement in B-cell Differentiation**
*Eric Yuki (Eric) Helm, Non-Matriculated,*
*Mentor: Daniel Strongin, Basic Sciences*

Genes crucial for cell differentiation have been shown to change position within the nucleus at the appropriate stages of development. For example, it's been shown that the beta-globin locus moves from the periphery to the interior as transcription increases. The nuclear periphery is considered a repressive compartment, where silenced genes are located. Expression of Pax5, which encodes for the B-cell lineage-specific activator protein (BSAP), is vital for the commitment to the B-cell lineage and development. However, Pax5 is repressed during the differentiation of B-cells into plasma cells. In this study, we used fluorescence in-situ hybridization (FISH), to look at the position of the Pax5 locus at various stages of B-cell differentiation in order to determine if Pax5 position is correlated with its expression. We then performed RNA/DNA FISH to visualize the actively transcribing allele position.

**An Analysis of the Conservation of Phosphorylation Patterns across Yeast Species using Comparative Phosphoproteomics**
*Joanne Ino (Joanne) Hsu, Senior, Neurobiology*
*Howard Hughes Scholar, Levinson Emerging Scholar, Mary Gates Scholar*
*Mentor: Judit Villen, Genome Sciences*
*Mentor: Danielle Swaney, Genome Sciences*

The reversible phosphorylation of proteins mediates a wide range of biological processes that range from signal transduction cascades to regulation of protein abundance. However, little is known about the mechanisms and evolution of phosphorylation networks. Despite the extraordinary advances in genome sequencing of many yeast species, evolutionary studies on the phosphoproteome of yeast species have

been limited to the experimental analysis of phosphorylation in one species and theoretical analysis of the conservation of phospho-acceptor residues with other species. To properly study the evolutionary conservation phosphorylation in yeast, we are utilizing mass spectrometry to study and compare the phosphoproteome of 21 species of yeast, including representative species from each clade of the yeast phylogenetic tree. Most of these species have not been studied before by proteomics. This high-throughput phosphoproteomic study on the yeast species will contribute to the construction of phosphoproteome datasets, which can be exploited for comparative analysis of phosphorylation between the species. One of main aims is to identify phosphorylation sites that show a high degree of conservation across the 21 species of yeast. These highly conserved phosphorylation sites likely regulate the same functions across the different species, and these functions can be further studied and correlated to our understanding of the mechanistics of phosphorylation in key biological processes, as well as the diseases that occur when the signaling pathways that involve these sites are defective. The second aim of this project is to study the correlation between the conservation of the phosphoproteome with the conservation of the genome and transcriptome, which will expand our understanding of the evolution of phenotypic diversity. Finally, the large-scale dataset of the proteomes and phosphoproteomes holds a great potential for other types of computational analysis, and also serves as a foundational reference for future experiments.

**Heteroduplex Rejection and Infertility in Fission Yeast**

*Jiwon Yeo, Senior, Anthropology: Medical Anth & Global Hlth, Biology (General)*
*Mentor: Sarah Zanders, Basic Sciences*

Speciation is the process by which one species becomes two species that can no longer interbreed. A common hypothesis is that infertility between closely related species is due to the rapid evolution of DNA double-strand-break (DSB) hotspots that are active in meiosis. DNA DSBs play an important role in meiosis by initiating recombination after chromosome replication and can be repaired as crossovers. During DNA DSB repair, the broken strand of a homologue invades and repairs its break with the strand of the aligned homologue by matching complementary nucleotides. The repair can result in a heteroduplex which refers to a mismatch, such as adenine paired with cytosine. Heteroduplex rejection occurs when the cell's recombination machinery rejects recombination at sites where heteroduplex forms, and it is known to require the *msh2* gene. We hypothesize that heteroduplex rejection inhibits DSB repair in interspecies hybrids and can result in infertility and thereby the isolation of species. *Schizosaccharomyces kambucha* and *Schizosaccharomyces pombe* are two very closely related fission yeast species that are 99.5% identical at the DNA sequence level. Despite their similar genetic makeup, we found that their hybrids show decreased fertility and decreased recombination frequencies. We are testing whether inhibiting heteroduplex rejection will increase the fertility and recombination rates of the fission yeast hybrids. For this experiment, we have created *msh2*$\Delta$ *Schizosaccharomyces pombe* and *Schizosaccharomyces kambucha* mutants that cannot do heteroduplex rejection so the cells should go through DNA DSB repair even if there are mismatches. We expect to see an increase in fertility and recombination in the *msh2*$\Delta$ hybrids. This research may be extended further to explain speciation in other organisms or unexplained infertility between human couples.

**Molecular Fishing Rod: Tri-Functional Probes that Profile Kinase Conformations**

*Guillermo Sergio (Guillermo) Romano, Senior, Public Health-Global Health, Biochemistry*
*Amgen Scholar, Mary Gates Scholar*
*Mentor: Dustin Maly, Chemistry, UW, Seattle*

Cells process an enormous amount of information from their environment and translate this information into phenotypic behaviour that will dictate critical biologic activities. To do this, cells must collect, store, and respond to information both inside and outside the cell. This information is stored in the chemical composition of its cytoplasm, and these conditions will regulate complex biochemical pathways that relay the information from the cell's exterior to the effector proteins that modulate behaviour. These pathways, known as signal transduction pathways, are largely regulated by phosphorylation. Protein kinases are the class of enzymes responsible catalyzing the phosphorylation of intermediary signaling enzymes (resulting in their activation), and thus, stimulate the transduction of an external stimulus to intracellular machinery. As cellular conditions change, protein kinases sensitive to these conditions will modulate their activity through a change in shape (conformation). Research shows that disregulating signaling pathways through aberrant kinase activity can lead to grievous disease, making them good drug targets for cancer treatments and other conditions. Selective pressure has lead to the 500+ kinases in the proteome to adopt well-conserved active and inactive conformations, making it difficult to understand how nature can distinguish between them. Due to low abundance of kinases, quantitative analysis of kinase conformations is difficult with existing methodology. To this end, we have developed a catch-and-release system that allows us to enrich and quantitatively distinguish between kinases of specific conformations. This project will focus on the development trifunctional probe that serves as a chemical handle to enrich kinases in the novel system. The new catch-and-release system employs improvements over existing biotin-steptavidin systems by taking advantage of bioorthogonal chemistry and specific elution conditions that increase signal-to-noise ratios. Our probe marks significant

progress towards a panel of probes that will profile kinases for diagnostic means and drug target identification.