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Online Proceedings

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From Yeast to Mice: Insights into Human Disease and Stress Response

Session Moderator: Sarah Pierce, Medical Genetics

JHN 022

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Utilizing Budding Yeast as a Model Organism for Dwarfism

Haley Minami (Haley) Amemiya, Senior, Biochemistry, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar, UW Honors Program
Mentor: Bonita Brewer, Genome Sciences

Meier-Gorlin Syndrome (MGS) is a rare form of primordial dwarfism characterized by a short stature, small external ears, and absent kneecaps. Recently, mutations in the pre-replication complex (pre-RC) were linked to MGS. The pre-RC is composed of multiple proteins that must be present in order to initiate DNA replication. Although a genetic link has been made for MGS, it is unclear how these mutations cause the phenotypes observed in humans. To better understand the mutations' phenotypes on a cellular and molecular level, our lab is utilizing the budding yeast, *Saccharomyces cerevisiae*. The MGS mutation in the Origin Recognition Complex (ORC) subunit six (*ORC6*) of the pre-RC occurs in a highly conserved region of the protein where there is a single amino acid substitution (Tyrosine to a Serine) in the C-terminal helix. Since the mutation is conserved from human to yeast, it is predicted that the function of the mutations is also conserved. I introduced the equivalent mutations into budding yeast ($orc6^{MGS}$), to investigate the consequences of harboring the MGS mutation on a molecular and cellular level and to elucidate complex genetic interactions these proteins may be involved in. The residues in ORC that recognize origins are unknown. Investigation into this mutation has the potential to illuminate the mechanism behind ORC in eukaryotic cells. The assays performed in this project have exciting implications for the model by which replication initiates and the consequences of a single mutated protein. This genetic approach will hopefully provide insight to how the conserved $orc6^{MGS}$ mutation is functioning, which has the potential to be translated to the human condition.

A Genome-Wide Screen for Suppressors of the *S. cerevisiae**FMO1* Deletion

Nicholas Dobroslaw (Nicholas) Rintala, Senior, Microbiology
Mary Gates Scholar, UW Honors Program
Mentor: Matt Kaeberlein, Pathology
Mentor: Ryan Rossner, Pathology

The transcription factor hypoxia inducible factor-1 (HIF-1) plays a central role in responding to changes in environmental oxygen levels in both mammals and nematodes. HIF-1 stabilization in *Caenorhabditis elegans* via knockout of the E3 ubiquitin ligase *von Hippel-Lindau 1* gene (*vhl-1*) improves proteostasis and lifespan. In humans, however, stabilization of HIF-1 via loss of VHL results in von Hippel-Lindau syndrome, a disease characterized by angiomas and renal carcinomas. Because it is a transcription factor, HIF-1 activates multiple genes that are likely responsible for its effects – whether they are improved proteostasis and lifespan in worms or neoplastic disease in humans. Previously, my lab performed a screen to determine which genes downstream of hypoxic signaling are responsible for the beneficial effects of HIF-1 stabilization in worms. This screen yielded a list of genes which were subsequently tested for whether they were necessary and/or sufficient to promote health and longevity, leading to the isolation of flavin-containing monooxygenase-2 (FMO-2), as a protein that increases lifespan and improves multiple measures of healthspan in nematodes. My project involves first characterizing the sensitivity of the yeast *FMO1* deletion strain (*fmo1*Δ) to specific forms of toxic stress. Having established these phenotypes, I am performing a screen to find genes that, when transgenically expressed, rescue the effects of the *FMO1* deletion. My strategy is to overexpress a library of other yeast genes as well as orthologous *FMO* genes to isolate those that rescue the *fmo1*Δ phenotypes. Additionally, I will overexpress *FMO1*, and any genes of interest from the screen, for analysis in replicative lifespan assays, testing whether they also play a role in increasing yeast longevity.

This screen and the associated replicative lifespan assays will help us characterize the role of yeast *FMO1* and the conserved role of FMOs in general, furthering our understanding of the process of aging.

The Role of TSPO in Regulating Heme Excess in Early Red Blood Cell Development

Ishan Vimalkumar Bhatt, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Janis Abkowitz, Medicine
Mentor: Raymond Doty, Medicine

To support the increased production of hemoglobin, the oxygen-binding protein in red blood cells, erythroid progenitor cells need large amounts of its functional constituent biochemical, heme. Despite this increased demand for heme, excessive amounts of free (unutilized) heme form reactive oxygen species which causes damage to cellular components and even apoptosis. To understand the decisions individual cells make to compensate for induced excessive heme, we are analyzing expression data from a pilot single cell RNA-sequencing study. Alongside various exploratory revelations, we identified Tspo (Translocator Protein), a poorly characterized mitochondrial transmembrane protein as one with significant expression changes in cells with heme excess. I have been conducting bioinformatics analyses on the transcriptomic data to classify it's expression pattern and find possible correlations with the expression of other genes. As per my initial findings, it's expression resembles that of a ROS-response gene, which will guide my further molecular/ biochemical experiments into determining causation vs. correlation between Tspo expression and heme excess. I intend to measure TSPO expression alongwith several other genes implicated in pathways related to it under conditions of upregulated heme synthesis. I will also quantify expression patterns through erythroid development in vivo with induced heme overload. I hope to better characterize the role of Tspo, if any, in heme-response pathways with these experimental investigations, together with bioinformatics analysis on large-scale transcriptomic data.

Development of a Polymerase Chain Reaction-Based Genotyping Assay to Identify Cyp4v3 Gene Knockout Mice

Audrey Tay, Junior, Environmental Science & Resource Management
Mentor: Edward Kelly, Pharmaceutics

Bietti crystalline dystrophy (BCD) is an autosomal recessive eye disease caused by mutations in the human *CYP4V2* gene. To understand the biochemistry and physiology associated with BCD, our lab has developed a "knockout" of the orthologous mouse gene, *Cyp4v3*. The gene targeting vector that was used to disrupt the *Cyp4v3* gene incorporated a *LacZ* gene reporter and a neomycin antibiotic resistance gene to replace the majority (exons 2-10) of the *Cyp4v3* gene. To differentiate endogenous mouse sequence from the targeting vector we designed primers to amplify by polymerase chain reaction (PCR) portions of the *Cyp4v3* and *LacZ* genes, respectively. The experiments conducted for the *LacZ* primers failed to yield any PCR product when tested on samples of genomic DNA from *Cyp4v3* heterozygous mice. However, we did get PCR products of the expected size from the same DNA samples for both *Cyp4v3* a loading control (fatty acid binding protein-FABP). Because of the technical difficulties encountered with the *LacZ* primers, we designed new primers targeting the neomycin resistance gene sequence. When these new primers were tested, we were able to amplify the expected targets for all 3 genes when testing genomic DNA from a *Cyp4v3* heterozygous mouse. We have developed a robust and reproducible method to genotype mice that are either *Cyp4v3* +/+ (wild type), +/- (heterozygotes) or -/- (homozygous knockouts). Ongoing studies in the lab with these knockout mice are focused on characterizing the age-related progression of BCD ocular pathologies, including crystalline deposit chemical composition and defects in lipid metabolism.

Adaptive Regulation of Glutathionylation in *Gclm* Null Mice

Claire Chisholm, Senior, Environmental Health, Microbiology
UW Honors Program
Mentor: Terrance Kavanagh, Environmental and Occupational Health Sciences
Mentor: Gary Merrill

Protein glutathionylation is a post-translational modification that can take place under mild oxidative stress. It occurs when the antioxidant glutathione (GSH) forms mixed disulfide bridges with low pKa cysteine residues on proteins. This may play a role in reversibly regulating the activity of proteins that have key cysteine residues or protecting those cysteines from further irreversible oxidation if oxidative stress worsens. GSH itself is synthesized in a two-step process, and the rate limiting step is catalyzed by glutamate-cysteine ligase (GCL), a heterodimer composed of the catalytic subunit (GCLC) and modifier subunit (GCLM). GCLM acts to increase the activity of GCLC. Thus, in the absence of GCLM, GCLC is less efficient in making GSH. We have generated *Gclm* null mice and these mice have liver GSH levels that are about 10% of normal, resulting in an oxidized thiol redox environment. An important question that has not been answered is whether *Gclm* null mice have different levels protein glutathionylation than *Gclm* wild-type mice. The oxidized thiol redox status of *Gclm* null mice is predicted to increase the level of protein glutathionylation. On the other hand, there may in fact be less protein glutathionylation in *Gclm* null mice because they

have less GSH to begin with and there is a compensatory increase in sulfiredoxin and thioredoxin reductase expression, both of which are known to reverse glutathionylation. Using assays of protein glutathionylation, this project seeks to provide confirmation of the hypothesis that *Gclm* deficiency results in adaptive regulation of glutathionylation under different degrees of oxidative stress.

Characterizing Drug-Metabolizing Enzymes and Transporters that are *bona fide* CAR-Target Genes in Mouse Intestine

Claire Shinhee Park, Senior, Biochemistry
Undergraduate Research Conference Travel Awardee
Mentor: Julia Cui, Environmental and Occupational Health Sciences
Mentor: Sunny Lihua Cheng, Environmental and Occupational Health Sciences

Intestine is responsible for the biotransformation of many orally-exposed chemicals. The nuclear receptor constitutive androstane receptor (CAR/Nr1i3) can be activated by various drugs and environmental chemicals, and in turn it is known to up-regulate the expression of many drug-processing genes (DPGs) in liver to facilitate xenobiotic biotransformation. However, relatively less is known regarding the effect of CAR on the basal and inducible expression of DPGs in various sections of intestine. To fill this critical knowledge gap, sixty-day-old wild-type and CAR-null mice were administered the CAR-ligand TCPOBOP (3mg/kg, i.p.) or vehicle once daily for 4-days. CAR-null mice had altered basal expression of many DPGs in a section-specific manner in intestine. Consistent with the liver data (Aleksunes and Klaassen, 2012), TCPOBOP up-regulated many DPGs in specific sections of small intestine in a CAR-dependent manner. In contrast, the mRNAs of certain DPGs were previously known to be up-regulated by TCPOBOP in liver but were not altered in intestine. Interestingly, many known CAR-target genes were highest expressed in colon where CAR is minimally expressed, suggesting that additional regulators are involved in regulating their expression. In conclusion, the present study has shown that CAR is critical in both maintaining the basal expression as well as the pharmacological regulation of certain DPGs in a section-specific manner of the intestine, and the CAR activation in liver and intestine produces overlapping but not identical results. Therefore, Identification of the xenobiotic responses to CAR activation in intestine is critical for further understanding the adverse drug-drug interactions for many orally-exposed chemicals.