24th Annual Biochemistry Research Day

Tuesday May 12, 2026, 8:00am- 5:30pm

JSMBS 2220A: Keynote Seminar Presentation; Oral Student Presentations

JSMBS 2nd Floor Atrium: Poster Presentations

Luncheon: 1220 JSMBS Active Learning Center

Catering Provided By Pita Gourmet

Hosted by the Biochemistry Graduate Student Association

Biochemistry Research Day

Tuesday, May 12th, 2026

8:00am-8:30am	Breakfast & Poster Set-Up	2nd Floor Atrium
8:30am	**Introduction:** Dr. Rebekah Charney	JSMBS 2220 A
8:35am-9:50am	**Session I Oral Presentations:** Katherine Casazza, Moderator	JSMBS 2220 A
8:35-8:50	Michelle Goldberg, Ph.D. Student	
8:50-9:05	Shane Fraher, Ph.D. Student	
9:05-9:20	Bridget Varner, Ph.D. Student	
9:20-9:35	Sandy Enriquez, Ph.D. Student	
9:35-9:50	Shuojie Teng, Ph.D. Student	
9:50-10:00am	***Break***	2nd Floor Atrium
10:00am-11:00am	**Session II Oral Presentations:** Janie McGlohon, Moderator	JSMBS 2220 A
10:00-10:15	Adeeb Saleh, Undergraduate Student	
10:15-10:30	Emma Murray, Ph.D. Student	
10:30-10:45	Sheana Ramcharan, Ph.D. Student	
10:45-11:00	Andra Dean, Ph.D. Student	
11:00am-12:00pm	**Poster Session I:** **Undergrads:** Arielle Arbel, Kyle Brown, Matthew Leyberman, Yichu Huang, Aisha Makama, Jisiah Wheeler **Graduates:** David Homer, Jennifer Sosa **Post-Docs/Research Staff**: Pat Wilson, Soumyadip Chakraborty	2nd Floor Atrium
12:00pm-1:15pm	**Lunch & Student Recognition:** Dr. Thomas Hohle, Dr. Fernando Estrada	JSMBS 1220
1:15-2:15pm	**Session III Oral Presentations:** Michelle Goldberg, Moderator	JSMBS 2220 A
1:15pm-1:30pm	Sean Johnson, MS Student	
1:30pm-1:45pm	Ben Bahun, Ph.D. Student	
1:45pm-2:00pm	Ashley McDougall, Ph.D. Student	
2:00pm-2:15pm	Amra Vranjkovina, MD/Ph.D. Student	
2:15pm-2:30pm	***Break***	2nd Floor Atrium
2:30pm-3:30pm	**Session IV Oral Presentations:** Bridget Varner, Moderator	JSMBS 2220 A
2:30pm-2:45pm	Gabby Budziszewski, Research Scientist	
2:45pm-3:00pm	Layne Jensen Ph.D. Student	
3:00pm-3:15pm	Xuejian Huang Ph.D. Student	
3:15pm-3:30pm	Bill Reid, Research Scientist	
3:30-4:30pm	**Poster Session II:** **Undergrads:** Adeeb Saleh, Samantha Arnone, Li Yi Hu, Nandini Kodi, Hailey	2nd Floor Atrium

	Leyberman, Leighton Lee, Lena Sparcino **Graduates**: Olivia Herman, Veronica Nickerson, Ben Bahun	
4:30pm-5:20pm	**Dr. Turchi Introduction: Katherine Casazza** ***Elizabeth Olmsted Ross Keynote*** **Speaker-** ***Dr. John Turchi*** Professor and Chair Biochemistry, Molecular Biology and Pharmacology Indiana University School of Medicine	JSMBS 2220 A
5:20pm-5:30pm	**Closing Remarks & Research Day Awards:** Dr. Jennifer Surtees	JSMBS 2220 A

Jacobs School of Medicine and Biomedical Sciences
University at Buffalo

Biochemistry Research Day
Tuesday, May 12, 2026 - JSMBS 2220A
Keynote Seminar Presentation

4:30pm – 5:20pm

John Turchi, PhD
Chair, Biochemistry, Molecular Biology & Pharmacology
Robert A. Harris Professor of Biochemistry & Molecular Biology
Tom & Julie Wood Family Foundation Professor of Lung Cancer Research
Professor of Biochemistry, Molecular Biology & Pharmacology
Department of Biochemistry
Indiana University, School of Medicine

"***Targeting Protein-DNA interactions in the DNA Damage Response for Cancer Therapy***"

Speaker Introduction by Katherine Casazza

Department of Biochemistry

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Session I: Oral Presentations

Transcription factor Ets1 regulates the expression of CD122 (IL-2/15Rβ) subunit) in developing thymic Vγ5+ dendritic epidermal T cell (DETC) progenitors

Michelle B. Goldberg, Michael Battaglia, and Lee Ann Garrett-Sinha

Dendritic epidermal T cells (DETC) are a subset of γδ T-cells found in the epidermis of the skin of mice, where they are thought to contribute to epidermal homeostasis and wound healing. Recently, DETC were also shown to play a contributing role in the clearance of skin *Staphylococcal* infections. DETC progenitors are the first lymphocyte cells to develop in the fetal thymus, arising as early as E14 during mouse gestation. These DETC progenitors mature in the thymus and then migrate to the epidermis where they are maintained by self-renewal. Recently, we showed that the loss of transcription factor Ets1 leads to an absence of DETC in the skin. In this report, we examine fetal thymic development of DETC progenitors in Ets1 knockout (Ets1KO) mice. While Ets1KO embryos have DETC progenitors in the fetal thymus at embryonic day 16.5, these cells are characterized by significant changes in the expression of multiple maturation markers. In particular, expression of CD122 (IL-2/IL-15 receptor β-chain) is decreased in DETC progenitors in Ets1KO mice. CD122 is required to maintain DETC cells in skin and indeed no DETC are found in the skin of Ets1KO mice at postnatal day 5, despite presence of progenitors of these cells in fetal thymus. DETC progenitors from Ets1KO fetal thymus show impaired survival when cultured in media containing IL-2 and IL-15, despite the preferential proliferation conditions. Further, Runx3, a master regulator in DETC development and which has been shown to be regulated by Ets1 while itself regulating CD122, is also significantly decreased in Ets1KO DETC progenitors. These data indicate that the main role of Ets1 in regulating DETC numbers may be in its ability to directly bind to and to upregulate the expression of the Runx3 and/or the CD122 genes, which are required for the maintenance of DETC in the skin.

Neuroinflammation links the neurogenic and neurodegenerative phenotypes of *Nrmt1*$^{-/-}$ mice

Shane Fraher, James P. Catlin, and Christine E. Schaner Tooley

It is widely thought that age-related damage is the single biggest contributing factor to neurodegenerative diseases. However, recent studies are beginning to indicate that many of these diseases may have developmental origins that become unmasked overtime. It has been difficult to prove these developmental origins, as there are still few known links between defective embryonic neurogenesis and progressive neurodegeneration. We have created a constitutive knockout mouse for the N-terminal methyltransferase NRMT1 (*Nrmt1*$^{-/-}$ mice). Previously, it has been shown that *Nrmt1*$^{-/-}$ mice display phenotypes associated with premature aging. Specifically in the brain, they exhibit age-related striatal and hippocampal degeneration, which is accompanied by both motor and memory impairments. These phenotypes are preceded by depletion of the postnatal neural stem cell (NSC) pools, which undergo premature differentiation and migration. However, this differentiation is often incomplete, as many resulting neurons cannot permanently exit the cell cycle and ultimately undergo delayed apoptosis. Here, we now show that these cell cycle defects in *Nrmt1*$^{-/-}$ brains coincide with an increase in pro-inflammatory cytokine signaling and are proceeded by the initiation of astrogliosis, microglia activation and complement deposition, all of which indicate an activated neuroimmune response. We also find *Nrmt1*$^{-/-}$ mice do not upregulate transcription of corresponding anti-inflammatory cytokines. Mechanistically, we show that the onset of neuronal apoptosis is preceded by the deposition of complement C3 along the postsynaptic density of striatal neurons. This is reminiscent of activity-dependent synaptic pruning, where damaged and dysfunctional synapses are marked by C3 and eliminated by microglia-mediated phagocytosis. These data indicate that abnormal neurogenesis can trigger neuroinflammation, which in the absence of compensatory anti-inflammatory signaling, could lead to neuronal apoptosis and progressive neurodegeneration.

Semi-conserved Active site Phenylalanine Modulates Ligand Binding and Positioning in *P. aeruginosa* Originating Cytochrome P450

Bridget M. Varner, D. Fernando Estrada

Cytochromes P450 (CYPs) are a family of heme enzymes, with human CYPs playing major roles in drug metabolism. Recently, CYP168A1 and CYP107S1, two of three confirmed CYPs in the opportunistic pathogen *Pseudomonas aeruginosa*, have been found to metabolize several clinically utilized drugs in a manner resembling human drug metabolism. While human drug metabolism is well studied, how pathogen-mediated metabolism modulates drug concentrations is still largely an open question. Both CYP168A1 and CYP107S1 are hypothesized to have large active sites and broad substrate specificities as they bind ligands of diverse sizes and drug classes. This includes, but is not limited to, fluoroquinolones (a class of antibiotics prescribed for *P. aeruginosa* infections), cystic fibrosis drug ivacaftor, SERM adjuvant raloxifene, and fatty acids. Molecular docking studies of CYP107S1 bound to several substrates revealed that active site F387 is predicted to form pi-stacking interactions with compounds, stabilizing and orienting them. This residue is conserved between *P. aeruginosa* CYPs. Phenylalanine gating is known to be involved in proper ligand positioning for catalysis in several human drug-metabolizing CYPs.

Here we utilized UV/Vis binding assays and ^{19}F NMR to characterize substrate and inhibitor interactions. By ^{19}F labeling of cysteines, including one in the dynamic B-C region and a cysteine introduced at the ligand sensitive F-G loop, a series of ^{19}F-NMR spectra were acquired to monitor resonance changes that indicate binding. To evaluate the necessity of the semi-conserved potential “gating” phenylalanine in CYP168A1 (F425), two mutations were made. A leucine substitution negates aromatic interactions but preserves hydrophobicity. We determined that while UV/Vis binding is relatively unchanged, ^{19}F NMR indicates that F425L alters ligand positioning in the CYP168A1 active site. Then, an alanine mutation was made to shorten the hydrophobic sidechain. This significantly affected ligand binding in addition to protein stability, illuminating the importance of F425 in CYP168A1 structure.

The SoxC transcription factors regulate the retinal ganglion cell (RGC) lineage and function in parallel to Atoh7

Sandy Enriquez, Yichen Ge, Tao Liu, Jonathan E. Bard, Veronique Lefebvre, and Xiuqian Mu.

The development of retinal ganglion cells (RGCs) is tightly regulated by several classes of transcription factors. RGCs, along with the other retinal cell types, arise from undifferentiated retinal progenitor cells (RPCs), yet the gene regulatory mechanisms that direct these distinct fates remain poorly understood. Previous studies have shown that *Sox4*, *Sox11*, and *Sox12*, members of the SoxC family, are enriched in transitional RPCs (tRPCs) and RGCs, and that their expression is unchanged in *Atoh7*-null retinas. Based on this, we hypothesize that Sox4 and Sox11 act in parallel with, rather than downstream of, Atoh7 in establishing RGC competence. Here, we use molecular labeling along with bulk and single-cell sequencing approaches to examine the transcriptome during early RGC development and define the molecular programs guiding RPCs toward the RGC lineage. Our results show that Sox4 and Sox11 function alongside Atoh7 to promote RGC fate specification. Immunohistochemistry and bulk RNA-seq analyses of *Sox4/Sox11* double conditional knockouts (dcKO) demonstrate that loss of these factors impacts not only RGCs, but also horizontal, amacrine, and photoreceptor populations. Comparative analysis of the *Sox4/Sox11/Atoh7* triple knockout further reveals both cooperative and independent roles in coordinating RGC lineage specification. In the triple knockout retinas, RGCs are essentially abolished, and the increase in photoreceptors is more pronounced. Both knockout models also show reduced proliferation and increased apoptosis. Our RNA-seq results support the IHC findings and further demonstrate how Sox4/Sox11 and Atoh7 work together and independently to regulate the RGC transcriptional network. Single-cell RNA-seq supports these findings and highlights shifts in retinal lineage trajectories. Ongoing epigenetic studies will examine SoxC-dependent chromatin changes.

Chromatin Regulators CTCF and CHD8 are critical during retinal development

Shuojie Teng, Dahong Chen

During developmental remodeling, neurons remove their juvenile features to make way for mature adult traits at both cellular and nuclear levels. Proper chromatin organization is critical during this process, and the architectural protein CCCTC-binding factor (CTCF) and the chromatin remodeler Chromodomain Helicase DNA-binding protein 8 (CHD8) regulates chromatin architecture and accessibility, respectively. We found that loss of CTCF and CHD8 in mouse photoreceptor cells results in morphological defects in the retina. The depletion of both factors leads to exacerbated phenotypes, suggesting a synergetic relationship. Molecularly, CTCF depletion leads to dysregulation of global gene expression and chromatin accessibility prior to any prominent cellular phenotype. CTCF has also been shown to antagonize compartmentalization during neuronal remodeling via immunohistochemistry. Oligopaint will be conducted to verify and quantify changes in chromatin architecture during rod remodeling at a sub chromosomal resolution, with cellular specificity. This finding on chromatin architecture, along with previous data on CTCF's regulation of global gene expression and chromatin accessibility will be the first to provide a comprehensive model on its molecular function across any *in vivo* animal systems. Global molecular changes discovered using developmentally synchronized post-mitotic rods emphasize the need to consider cellular heterogeneity and cell-type specificity when performing multiomics analyses.

Session II: Oral Presentations

Titration and Histological Validation of Lipopolysaccharide- and HCl-Induced Preclinical Models of Acute Lung Injury for the Evaluation of Treatment X

Adeeb Saleh, Anna Blumental-Perry, Ph.D.

Acute respiratory distress syndrome (ARDS) is characterized by dysregulated inflammation in the alveoli, leading to immune cell infiltration and disruption of the alveolar-capillary barrier. This form of non-cardiogenic pulmonary edema severely impairs the ability of oxygen exchange, often leading shortness of breath, wheezing, and cyanosis. A moderate form of ARDS, clinically known as Acute Lung Injury (ALI) is relatively common among intensive care unit patients receiving mechanical ventilation. While many patients often recover from ALI, elderly patients (> 65 years) have a mortality rate exceeding 40-50%. Lipopolysaccharide (LPS) derived from *E. coli* and hydrochloric acid administered to mice are widely used as models of ALI; however, injury severity and reproducibility are highly dependent on experimental parameters. In this study, mice were administered via oropharyngeal aspiration with varying doses and instillation volumes of LPS (*E. coli* O111:B4) as well as hydrochloric acid (HCl) and were subsequently analyzed at 24hr, 48hr, and 1week post-induction intervals. Disease severity was assed using longitudinal body weight changes as an indicator of systemic illness, alongside bronchoalveolar lavage (BAL) and histological analysis of lung tissue to assess immune cell infiltration and structural injury. Our ALI model demonstrated consistent weight loss and pronounced inflammatory infiltration within the lung parenchyma, confirming a reliable induction of ALI. Furthermore, treatment X was administered to our LPS-ALI model, showing potential in lowering the overall inflammatory response. By establishing clear injury parameters and the testing of a novel treatment, future studies include expanding the use of this preclinical ALI model for further mechanistic and therapeutic studies.

Myo9b's Motility in Epithelial Cells

Emma C. Murray, Leighton Lee, Lynn Ziegler, and Andrew Lombardo

Myosin-9b (Myo9b) is a motorized RhoA-specific GAP that localizes to sites of actin polymerization, including ruffles, junctions, and the apical cortex of polarized cells, where it suppresses RhoA-ROCK signaling and non-muscle myosin-2 contractility. Human variants of Myo9b contribute to celiac disease, Crohn's disease, and irritable bowel syndrome. Despite decades of work, Myo9b motility has not previously been observed in cells. Using live-cell super-resolution SORA confocal microscopy, we visualized Myo9b processive motility in cells for the first time, capturing fluorescently tagged Myo9b moving toward actin bundle plus-ends at microvilli tips. Endogenous Myo9b localization in intestinal tissue showed Myo9b at brush border microvilli tips, validating this finding. We quantified motility and found that while purified single-headed Myo9b moves at ~20 nm/s in vitro, in cells Myo9b particles travel at 295 ± 78 nm/s with run lengths exceeding 1 μm, limited by microvilli length. A point mutation in the motor ATPase salt bridge abolished motility, confirming its dependence on motor activity.

To probe function, we generated the first Myo9b CRISPR knockout in human epithelial cells and observed microvilli defects by electron and super-resolution microscopy. Finally, using a spatiotemporal RhoA biosensor and three-color live-cell super-resolution SORA confocal microscopy, we demonstrated that Myo9b locally regulates RhoA signaling within microvilli.

Together, these results redefine the model by which Myo9b acts as a processive motor in cells, linking its motility and RhoA regulatory activities.

Identifying Translesion Synthesis (TLS) DNA polymerases and their contribution to persister recovery in *Pseudomonas aeruginosa*

Sheana S. Ramcharan, Natalie Popielski, Kyle Brown, Farhang H. Kermany, and Mark D. Sutton

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen that infects humans, is able to persist after rigorous antibiotic treatments and has many mechanisms conferring drug resistance. It does this through bacterial pathoadaptations such as activating virulence factors, efflux pumps and increasing its mutagenesis. A major source of mutations occurs from replication errors by translesion synthesis (TLS) DNA polymerases after stalling of the replicative polymerase at the replication fork. TLS is an error-prone process by which specialized DNA polymerases (Pols) are able to replicate damaged or imperfect DNA templates. *P. aeruginosa* contains 5 known DNA pols (Pol I, Pol II, Pol III, Pol IV, ImuABC) in which Pol I, Pol IV and ImuABC are thought to be specialized DNA pols acting through TLS. However, it is still not known which DNA pols are TLS pols or which lesions they bypass. *P. aeruginosa* is able to adapt and persist long after rigorous antibiotic treatments conferring drug resistance. Persisters are a sub-population of bacteria that are able to decrease their metabolic pathways and lay dormant under stressful conditions and subsequently recover after treatment forming a chronic infection. Though the bacteria are not actively replicating and dividing, cells are still acquiring DNA damage while the host is being treated with antibiotics. It has been demonstrated in *E. coli* that TLS DNA Pol V is able to cope with DNA damage from antibiotic treatment allowing persisters to recover after antibiotic removal and generate mutations leading to antibiotic resistance. However, the exact function and mechanism of TLS pols and their contribution to persisters and recovery of persister populations is unknown in *P. aeruginosa.* This project will focus on first determining which DNA pols are specialized TLS polymerases and their function and determining if TLS pols are important for persistence and recovery of persister populations in *P. aeruginosa.*

Nα-methylation of ZHX2 regulates the mRNA transcript selection of FMRP

Andra Dean, Meghan Conner PhD, and Christine Schaner Tooley PhD

N-terminal RCC1 methyltransferase 1 (NRMT1) catalyzes the addition of trimethylation to the N-terminus of its target proteins. This post-translational modification promotes protein-DNA interactions for proper regulation of mitosis, chromatin organization, and transcriptional regulation. *In vivo*, loss of NRMT1 results in severe liver necrosis and misregulation of cytochrome P450 (CYP) and major urinary protein (MUP) liver metabolic genes, which are two gene families regulated by the transcription factor zinc fingers and homeoboxes 2 (ZHX2). We have identified ZHX2 as a target of NRMT1 and determined that N-terminal (Nα) methylation of ZHX2 regulates its nuclear role as a transcription factor. While ZHX2 is a well-characterized transcription factor in the liver, it mislocalizes to the cytoplasm in hepatocellular carcinoma (HCC), where its functions remain unknown. To identify cytoplasmic interactors of ZHX2, we performed a mass spectrometry screen with ZHX2 immunoprecipitated from the cytoplasm of HEK293 cells. We have identified a novel cytoplasmic interaction between ZHX2 and fragile X mental retardation protein (FMRP), which is stabilized by Nα-methylation of ZHX2. FMRP is an oncogenic RNA-binding protein that regulates *Stat3* mRNA localization and subsequent translation in HCC cell protrusions and promotes HCC metastasis. We went on to show that loss of NRMT1 results in reduced FMRP binding to its target mRNAs in HEK293 cells, and that Nα-methylation of ZHX2 regulates FMRP binding to cell protrusion-localized transcripts including *Stat3* mRNA. We will now determine how Nα-methylated ZHX2 regulates FMRP transcript selection in HCC cell lines and HCC migration. These studies will begin to characterize the novel cytoplasmic functions of ZHX2 and regulation of FMRP function in HCC progression.

Session III: Oral Presentations

Anatomical basis of endolysosome genesis and trafficking in the vertebrate photoreceptor

Sean Overocker, Ramachandra Rao Lab

Photoreceptor (PR) cells are highly polarized, terminally differentiated retinal neurons that are indispensable for visual transduction. These modified pseudo-unipolar neurons possess a distinct morphology and highly organized organelle distribution, rendering it as a useful *in vivo* neuronal system to investigate organelle trafficking mechanisms. The inner segment (IS) of the PR is divided into ellipsoid and myoid layers, the latter containing the actin cytoskeleton-rich cell cortex of the PR. The mitochondrial population of the PR primarily resides in the IS ellipsoid with a subpopulation found within the synapses. Conversely, PR endoplasmic reticulum (ER) and Golgi apparatus are exclusively localized to the myoid layer. Previous studies have identified phagosome and lysosome enrichment in the PR myoid and external limiting membrane (ELM). We decided to test if the myoid serves as the primary site of endocytosis, endolysosome biogenesis, and initiation of endolysosomal trafficking events within the cell. We refined a pharmacological methodology (vinblastine treatment) to investigate microtubule-dependent trafficking dynamics of endolysosomes, phagosomes, and mitochondria. Our study demonstrated significant differences in PR mitochondrial and lysosomal trafficking mechanisms. We applied this understanding to a mouse model of *CRB1*-associated retinopathy to test the role of the actin cytoskeleton in endolysosome biogenesis in the PR myoid. This demonstrated a critical role of myoid actin cytoskeleton in PR endocytosis and endolysosome genesis. Lack of lysosomal biogenesis is key signature of *CRB1*-retinopathy. To further delineate the mechanisms of mitochondria-lysosome contacts and mitophagy, we have engineered a novel tandem mitochondria and phagolysosome tracker mouse model. In summary, this investigation utilized transgenic, pharmacological, histological, and simple image-analysis based methods to provide critical insights into the mechanisms of microtubule-dependent organelle trafficking, organellar distribution in PRs.

ZEB2 and SMAD1 direct target genes reveal co-regulation of the human neural crest gene regulatory network

Benjamin J. Bahun, Maneeshi S. Prasad, and Rebekah M. Charney

The neural crest (NC) is a multipotent and migratory embryonic cell population that contributes to many cell types, including craniofacial bone and cartilage, odontoblasts, and peripheral neurons and glia. Many birth defects are associated with abnormal development of NC-derived structures, including cleft lip and palate, malformed teeth, and improperly formed facial features. Neurocristopathies can manifest as complex syndromes and aggressive pediatric cancer, thus highlighting the significance of investigating fundamental mechanisms of NC biology. Using a model of human NC formation, we previously revealed ZEB2 is an essential factor in the NC gene regulatory network (GRN). Notably, mutations in *ZEB2* underlie Mowat-Wilson syndrome; however, the mechanisms disrupted within the NC lineage of this neurocristopathy remain incompletely understood. ZEB2 is a transcriptional repressor and SMAD-interacting co-factor that is thought to negatively regulate TGF-β superfamily signaling. It is imperative that TGF-β superfamily signaling and SMAD-mediated transcriptional outputs are tightly regulated throughout NC formation. Given this, we first investigated SMAD signaling in human NC derived from Mowat-Wilson syndrome patient-derived induced pluripotent stem cells. We observed changes in levels of phospho-SMAD1/5/9 as well as dysregulation of BMP ligand and SMAD co-factor gene expression. Critically, we lack an understanding of the direct target genes of ZEB2 and the SMADs as key regulators of the human NC-GRN. To address this, we performed CUT&RUN using our human NC model. We identified ZEB2 and SMAD1 occupancy at both unique and overlapping direct genomic targets, which is being coupled with motif analysis to identify additional factors involved in regulating these genes. Together, these results reveal novel ZEB2 and SMAD1 direct targets while suggesting a co-regulatory mechanism within the human NC-GRN. We are currently advancing these findings by addressing the precise role of ZEB2/SMAD and SMAD co-factors in wild-type and pathological NC.

Elevated and Skewed dNTP Pools Alter Replication Progression and Okazaki Fragment Processing

Ashley S. McDougall, Natalie Lamb, Sushma Sharma, Andrei Chabes, Jennifer A. Surtees

During DNA replication, cells must maintain proper levels of deoxynucleotide triphosphates (dNTPs), the building blocks of DNA. Decreased dNTP pools lead to replication fork stalling, whereas elevated dNTP pools increase fork progression rate, leading to rapid, error-prone DNA synthesis. dNTP levels are controlled by ribonucleotide reductase (RNR), which is highly conserved across eukaryotes. In humans, mutations to RNR's catalytic subunit (RRM1) have been identified in tumor samples, and elevated dNTP levels have been observed in cancer cell lines. In *S. cerevisiae,* point mutations in the activity and specificity sites of RRM1's homolog (Rnr1) result in defined dNTP pools alterations, allowing us to use *rnr1* mutants as a genetic tool to study altered dNTP levels. We previously performed SGA screens in *S. cerevisiae* backgrounds with defined dNTP pools changes (*rnr1D57N, rnr1Y285A*, *rnr1Y285F*) to identify pathways beyond polymerase fidelity that may become implicated when dNTP pools are elevated/skewed. This screen identified significant negative genetic interactions with nearly all genes involved in mismatch repair, as well as genes whose products are involved in Okazaki fragment processing and replisome progression. This suggests that there are changes to these processes in the presence of elevated/skewed dNTP pools. We have performed deep sequencing of Okazaki fragments in all three of these *rnr1* mutants and have identified changes to Okazaki fragment processing and replication fork movement, including potential changes to origin usage. Currently, we are working toward better understanding these changes at a mechanistic level. In doing so, we will gain insight into key pathways and proteins that are essential under these replication stress conditions, providing the opportunity to exploit these findings to identify chemotherapeutic targets.

Cell-Type Specific APA Changes are Associated with Gene Dysregulation and Predict Survival Outcome in Head and Neck Cancer

Amra Vranjkovina, Dr. Jonathan Bard, Dr. Michael Buck

Alternative polyadenylation (APA) occurs in over 70% of human genes and results in the formation of multiple mRNA isoforms with varying 3' untranslated region (UTR) length. While single-cell transcriptomic techniques have improved over the years, cell-type specific alternative polyadenylation in head and neck squamous cell carcinoma (HNSCC) remains poorly characterized. We utilize a computational workflow to investigate alterations in 3'UTR length in single-cell RNA sequencing (scRNA-seq) HNSCC data, aiming to determine APA-mediated gene dysregulation and potential oncogenic advantages due to inclusion or exclusion of specific miRNA binding sites. Our analysis has identified distinct populations of genes undergoing 3'UTR shortening and lengthening in cancer epithelial cells relative to normal epithelial cells. To assess the clinical relevance of these findings, we extended this to TCGA HNSC bulk RNA-seq data and computed per-gene 3'UTR length changes relative to GTEx minor salivary gland as a normal reference. Lasso-penalized Cox modeling identified APA events associated with worse overall survival, with significant interaction by molecular subtype. We hypothesize that cell-type specific 3'UTR APA alterations lead to an oncogenic advantage by modulating miRNA binding sites and result in gene dysregulation in HNSCC. These findings establish a clinically relevant APA regulatory landscape in HNSCC linking tumor specific 3'UTR changes to dysregulated gene expression, molecular subtypes, and poor patient survival.

Session IV: Oral Presentations

Burkholderia pseudomallei **rubrerythrin binds metals promiscuously in a pre-formed four-helix bundle**

Gabrielle R. Budziszewski, Miranda L. Lynch , M. Elizabeth Snell, Diana C. F. Monteiro, Sarah E. J. Bowman

In many bacteria, rubrerythrins are Ferritin-like superfamily proteins that participate in the oxidative stress response. *Burkholderia pseudomallei* are a bacterial species that cause the human disease meliodosis, a serious bacterial infection that requires an arduous course of antibiotics to clear. Formerly confined to the tropics, meliodosis cases have been reported in recent years in the southeastern United States as a result of altered climate circumstances. Rubrerythrin (Rbr) in *B. pseudomallei* is expressed under oxidative stress conditions, likely contributing to the ability of the organism to survive in soil environments and withstand the onslaught of the human immune system. Here, we characterize *Bp*Rbr as a promiscuous metal-binding protein which stably interacts with redox-capable first row transition metals manganese, cobalt, and iron. We confirm the presence of single metal species with the use of *in-crystallo* energy dispersive spectroscopy (EDX). Analysis of a metal-nonbinding variant of *Bp*Rbr reveals that the four-helix bundle structure of *Bp*Rbr is pre-formed, reconciling our observation that all metal binding sites are only partially occupied. Further work will explore the enzymatic activity of *Bp*Rbr, as other rubrerythrins have been observed to perform catalase, peroxidase, pyrophosphatase and superoxide dismutase functions. We anticipate that defining the mechanism of *Bp*Rbr and its required di-metal cofactor will enable therapeutic approaches to bacterial infections that target oxidative response pathways.

Modeling human Cytochrome P450 recognition of vitamin D through the bacterial enzyme CYP107-sb3a from *Sebekia benihana*

Layne Jensen, D. Fernando Estrada

Cytochrome P450 enzymes (CYPs) are notorious for their role in xenobiotic metabolism and steroidogenesis, which are essential reactions across all kingdoms of life. Despite low sequence similarity, they maintain a similar tertiary fold and catalyze a wide array of reactions. We are particularly interested in human vitamin D metabolism, which begins with carbon 25 hydroxylation of vitamin D_3 on the aliphatic tail by hepatic CYP2R1 or CYP27A1. The 25-OH D_3 is then activated by 1α hydroxylation in the kidney by CYP27B1, forming the active metabolite 1α,25-$(OH)_2$ D_3. However, how each CYP recognizes vitamin D in the proper orientation remains unknown. Here, we utilize the carbon-25 hydroxylase CYP107-sb3a from soil bacterium *Sebekia benihana* as a model for understanding CYP-mediated recognition of vitamin D_3. Molecular docking using the Alphafold2 model of CYP107-sb3a identified two polar side chains, Gln-79 and Ser-383 that appear to be implicated in vitamin D_3 recognition. We hypothesized that alanine mutagenesis of either Gln-79 or Ser-383 would disrupt recognition, binding, and therefore catalysis of vitamin D_3 compounds. However, S383A unexpectedly displays increased catalysis of both vitamin D_3 and 1α-OH D_3, while Q79A displays increased activity (several-fold) only on 1α-OH D_3. We posit that Q79A reduces the productive binding of vitamin D_3 due to removal of hydrogen bonding potential with the 3ß hydroxyl of vitamin D_3, and that both mutants potentiate nonpolar recognition of the aliphatic vitamin D tail. Taken together, this outcome provides a context for understanding the basis of recognition of vitamin D by mammalian CYP enzymes.

Cell type-specific functions of ATF6 coordinate mitochondrial homeostasis in lung parenchyma progenitor cells and extracellular matrix remodeling in the lung pericytes.

Xuejian Huang, Jonathan Bard, Bat-Ider Tumenbayar, Roy Miller[2], Yongho Bae, and Anna Blumental-Perry

Activating transcription factor 6α (ATF6), retained in the endoplasmic reticulum (ER) under basal conditions, is a principal regulator of the ER stress response. ATF6 activity is required for the maintenance of alveolar epithelial type II cells (AEC2s), local lung parenchyma progenitors, and its nuclear localization has been observed in lungs during smoke inhalation. We therefore investigated the effect of ATF6 deficiency on physiological and smoke inhalation lung homeostasis. Alveoli of ATF6-deficient mice exhibited elevated numbers of TUNEL-positive cells and accelerated alveolar simplification compared to wild-type (WT) counterparts. Alveolar simplification was further exacerbated by smoking. Notably, during repeated smoke exposures, ATF6-deficient mice were protected from the small airway vascular fibrotic disease that developed in WT animals. We analyzed single-cell RNA sequencing (scRNA-seq) datasets from human and mouse lungs with and without chronic smoke exposure or COPD to evaluate cell type-specific effects. Statistically significant differences in ATF6 expression were detected in lung pericytes and human AEC2s, with similar trends in mouse AEC2s. Ridge plots revealed heterogeneity in ATF6 expression, identifying subpopulations of high expressors in both cell types that may correspond to cells in more affected areas. Transcriptomic analyses demonstrated pathway reprogramming due to smoke exposure in both AEC2s and pericytes. In smokers' AEC2s, high ATF6 expression correlated with altered mitochondrial unfolded protein response, reduced oxidative phosphorylation (OXPHOS), and increased expression of senescence and inflammation genes. In smokers' pericytes, elevated ATF6 levels were associated with PI3K-AKT signaling, wound healing, and collagen deposition pathways. Ingenuity Pathway Analysis (IPA) predicted ATF6 as an upstream regulator of extracellular matrix genes in pericytes. Given the presence of AEC2 and pericyte subpopulations differing in ATF6 expression levels, and to assess potential discrepancies between mRNA and protein levels due to ATF6 absence, we performed spatial proteomic with panels design based on scRNAseq data, comparing WT and ATF6-deficient AEC2s and lung pericytes. In ATF6-deficient mice, the population of AEC2s with high bioenergetic capacity (progenitor pools) was reduced, the mitophagy receptor BNIP3 was elevated, and high Sox9 levels indicated abnormal repair leading to cellular senescence. These findings suggest distinct cell type-specific roles of ATF6: maintaining ER-mitochondrial homeostasis and regenerative capacity in AEC2s, while modulating extracellular matrix remodeling through pericyte activation during smoke-induced lung injury.

Establishing non-model insect transgenesis at UB

William Reid and Marc S. Halfon

The activity of the *cis*-regulatory elements that drive gene expression (enhancers) in a gene locus is governed by complex, largely unknown, rules referred to as “enhancer grammar”. Within the model organism *Drosophila melanogaster*, more than 58,000 enhancers have been identified, with >17,000 validated *in vivo* using reporter gene assays. The wealth of foundational knowledge in *Drosophila* provides a platform upon which comparative biology approaches to better understand enhancers can be taken. Studies to fully characterize conserved gene loci between species allows for the identification of enhancer commonalities shared across evolutionary time, providing powerful data towards better understanding the rules of enhancer grammar. Central to this approach is the capacity to generate and test enhancers in other transgenic animals, that is: non-model insects. We therefore established non-model insect transgenesis at UB using two Dipteran species: the yellow fever mosquito *Aedes aegypti* and the scuttle fly *Megaselia abdita*. Along with *D. melanogaster*, these three species span roughly 250 MY of evolution, illuminating the natural experiment of how enhancers evolved among conserved genes in the true flies.

Graduate Students, Post Docs and Research Staff Poster Abstracts

ZEB2 and SMAD1 direct target genes reveal co-regulation of the human neural crest gene regulatory network

Benjamin J. Bahun, Maneeshi S. Prasad, and Rebekah M. Charney

The neural crest (NC) is a multipotent and migratory embryonic cell population that contributes to many cell types, including craniofacial bone and cartilage, odontoblasts, and peripheral neurons and glia. Many birth defects are associated with abnormal development of NC-derived structures, including cleft lip and palate, malformed teeth, and improperly formed facial features. Neurocristopathies can manifest as complex syndromes and aggressive pediatric cancer, thus highlighting the significance of investigating fundamental mechanisms of NC biology. Using a model of human NC formation, we previously revealed ZEB2 is an essential factor in the NC gene regulatory network (GRN). Notably, mutations in *ZEB2* underlie Mowat-Wilson syndrome; however, the mechanisms disrupted within the NC lineage of this neurocristopathy remain incompletely understood. ZEB2 is a transcriptional repressor and SMAD-interacting co-factor that is thought to negatively regulate TGF-β superfamily signaling. It is imperative that TGF-β superfamily signaling and SMAD-mediated transcriptional outputs are tightly regulated throughout NC formation. Given this, we first investigated SMAD signaling in human NC derived from Mowat-Wilson syndrome patient-derived induced pluripotent stem cells. We observed changes in levels of phospho-SMAD1/5/9 as well as dysregulation of BMP ligand and SMAD co-factor gene expression. Critically, we lack an understanding of the direct target genes of ZEB2 and the SMADs as key regulators of the human NC-GRN. To address this, we performed CUT&RUN using our human NC model. We identified ZEB2 and SMAD1 occupancy at both unique and overlapping direct genomic targets, which is being coupled with motif analysis to identify additional factors involved in regulating these genes. Together, these results reveal novel ZEB2 and SMAD1 direct targets while suggesting a co-regulatory mechanism within the human NC-GRN. We are currently advancing these findings by addressing the precise role of ZEB2/SMAD and SMAD co-factors in wild-type and pathological NC.

TBL1XR1 functions as a chromatin-associated cofactor to regulate transcriptional programs in head and neck squamous cell carcinoma

Soumyadip Chakraborty, Jennifer Sosa, Tithi Patel, Kirsten Smalley, and Satrajit Sinha

Oncogenic transcription factors (TF) are master regulators of cell fate that drive cancer by modulating gene expression programs that favor tumor growth, survival, and metastasis. One such TF, p63, is crucial for maintaining squamous epithelial identity and is often amplified in head and neck squamous cell carcinoma (HNSCC), yet the underlying molecular mechanisms by which p63 drives oncogenesis remain largely elusive. To probe the p63-chromatin complexes and identify potential cofactors directing the p63 cistrome in HNSCC, we performed rapid immunoprecipitation followed by mass spectrometry of endogenous proteins (RIME) in a HNSCC patient-derived cell line. Our analysis identified a cohort of known and novel p63-interactors, including TFs and co-factors involved in chromatin remodeling, including Transducin Beta-Like 1 X-Linked Receptor 1 (TBL1XR1). Follow-up examination of patient datasets revealed that TBL1XR1 is broadly amplified across squamous malignancies, including HNSCC, with tumor cells being a key source of its dysregulation in HNSCC patients. To further delineate the functional role of TBL1XR1, we depleted TBL1XR1 in representative HNSCC cell lines using lentiviral-mediated shRNA transduction. Loss of TBL1XR1 resulted in widespread transcriptomic reprogramming and affected crucial sets of genes, including those linked to epithelial–mesenchymal transition, extracellular matrix remodeling, and inflammatory signaling. Genome-wide profiling of TBL1XR1 binding sites further linked these transcriptional changes to a broad gene regulatory landscape that is co-dependent on several oncogenic TFs such as AP-1 and p63. Together, our data suggest that TBL1XR1 acts as a rheostat to fine-tune the expression of a crucial subset of p63 target genes representing key hallmarks of oncogenesis and offers a potential therapeutic target that can be exploited to harness cancer vulnerability to transcriptional addiction.

Characterization of rad1-rad10 protein-protein interactions and protein-dna binding

Olivia Herman, Javier Rodriguez-Gonzales, Alba Guarn, Jennifer A Surtees

Rad1-Rad10 is a structure specific heterodimeric endonuclease that plays a critical role in DNA repair pathways. It displays specificity for cleavage of double strand/single strand (ds/ss) DNA junctions with 3' single-stranded DNA, structures that occur in multiple DNA repair pathways, including nucleotide excision repair (NER). Rad1-Rad10 is recruited to the upstream side of DNA bubbles that form during NER through interactions with Rad14. These ds/ss DNA structures with 3' ssDNA also form in some double strand DNA break repair (DSBR) pathways, including single-strand annealing (SSA) and a subset of gene conversion events with non-homology at the 3' end of the invading strand [1]. When recombination intermediates with 3' nonhomologous tails are formed, 3' nonhomologous tail removal (3' NHTR) is required before repair can proceed via DNA synthesis. Rad1-Rad10 is also required for 3' NHTR. Notably, previous work has demonstrated that Rad1-Rad10 requires Saw1, as well as the mismatch repair complex Msh2-Msh3, for its recruitment these recombination intermediates. Saw1 also binds ds/ssDNA junctions, forms a complex with Rad1-Rad10 and directly stimulates Rad1-Rad10 endonuclease activity,[1] promoting cleavage of the 3' tails. Thus Rad14 and Saw1 recruitment proteins confer repair pathway specificity on Rad1-Rad10. Saw1 (and Msh2-Msh3) is not required for NER; Rad14 is not required for 3' NHTR. Here we present a high resolution cryo-EM structure of Rad1-Rad10-Saw1 and a predictive model of Rad1-Rad10-Rad14. This structural data indicates that Saw1 and Rad14 interact with distinct regions of Rad1.To test our models, we identified and mutated Rad1-Rad14 and Rad1-Saw1 interface residues and tested physiological NER or SSA function *in vivo*. We hypothesized that mutations in *RAD1* that are predicted to mediate the Rad1-Rad14 interface will impair NER activity, leaving SSA intact, while Rad1-Saw1 interface mutations will impair SSA activity, leaving NER intact. We also assess Rad1-Rad10/Saw1 DNA substrate specificity, using Biolayer Interferometry

(BLI) to measure complex binding affinity to non-specific homoduplex, 3' NHTR Y-Splayed and MMR +8 Loop DNA substrates. Our data indicate that Rad1-Rad10/Saw1 displays the highest specificity for the 3' NHTR substrate.

Investigating the role of the Msh3 N-Terminal region in DNA repair

David Homer, Mia Piede, Gloria Opuni, Linh Le, Charanya Kumar, Fernando Estrada, Jennifer A. Surtees

The mismatch repair (MMR) pathway maintains genome stability by recognizing and directing removal of nucleotide misincorporation events (DNA mismatches). In *Saccharomyces cerevisiae* and humans, MMR is initiated by one of two heterodimeric complexes, Msh2-Msh6 or Msh2-Msh3, that recognize separate but overlapping classes of mismatches. Msh2-Msh3 preferentially binds insertion/deletion loops (IDLs). Once bound, Msh2-Msh3 recruits an MLH complex and activates its latent endonuclease activity to continue MMR. Msh2-Msh3 is also important in a subset of double-strand break repair pathways that involve recombination intermediates with 3' nonhomologous tails. Msh2-Msh3 binds to the double-strand/single-strand DNA junction that includes the 3' ssDNA tail, recruiting the Rad1-Rad10/Saw1 endonuclease to remove these tails in 3' nonhomologous tail removal (3' NHTR). While the crystal structure of human Msh2-Msh3 was solved, providing important structure-function information, the long N-terminal region (NTR) of Msh3 was deleted for crystallization. Msh3 NTR is predicted to be highly flexible and encodes several important features, including overlapping PCNA and MLH interaction motifs. Intriguingly, there is an abundance of charged residues in Msh3 NTR, including many positively charged lysines and an acidic patch of negatively charged residues. *In vivo* experiments highlighted the important, complex role of the acidic patch. When the acidic patch was deleted, we found that it was essential for MMR, but dispensable for 3' NHTR. We hypothesize that the Msh3 NTR functions as a DNA mimic regulating Msh2-Msh3 DNA-binding activity. We generated mutations that alter the acidic patch and tested them in MMR and 3' NHTR *in vivo*. To date, the Msh3 NTR mutations in *Saccharomyces cerevisiae* exhibited only modest changes to MMR and DSBR. In parallel, we successfully purified Msh3-NTR via Ni-NTA and ion exchange chromatography. Biolayer interferometry experiments indicate that Msh3-NTR does not bind DNA. Preliminary NMR results are consistent with an expected disordered structure. Longer term plans include *in vitro* characterization of Msh3 NTR.

Buildup of polyprenol in rod photoreceptors does not affect protein glycosylation

Veronica Nickerson, Sean M. Johnson, Seul Kee Byeon, Steven J. Fliesler, Sriganesh Ramachandra Rao

Dolichol (Dol) phosphate is the obligate oligosaccharide carrier required for protein glycosylation. The first step of the *de novo* Dol synthesis pathway is the condensation of farnesyl-pyrophosphate (PP) with isopentenyl-PP to generate a long chain polyprenol (Pol)-PP. The terminal α isoprene unit of Pol then undergoes reduction by steroid reductase 5 alpha-3 (SRD5A3) to generate DolPP. Mutations in the *SRD5A3* gene cause a congenital disorder of glycosylation (CDG) due to decreased Dol content and is accompanied by early onset retinal dystrophy. Here we aimed to model the effects of inhibition of Pol synthesis specifically in the retina to study the lipid-based mechanism of protein glycosylation in photoreceptors (PRs), and mechanism of SRD5A3 CDG-related retinal dystrophy. A conditional allele model targeting *Srd5a3* Exon 4 was developed. *Srd5a3* was deleted in rod PRs using a Rho-iCre mouse line. Immunohistochemical analysis of opsin and GFAP was done to assess rod photoreceptor integrity and retinal gliotic response. *In Situ Hybridization* (ISH) was performed to test PR-specific *Srd5a3* deletion. Electroretinogram (ERG) was performed at PN 9 months. TUNEL analysis was performed to assess PR cell death. Retinal Pol and Dol content was measured using LC-MS/MS analysis. ISH suggested prompt deletion of *Srd5a3* in PRs. TUNEL analysis and ONL thickness measurement suggested gradual loss of PRs beyond PN 6 mo., with >70% reduction in PR layer thickness by PN 12 months. Retinal Pol/Dol ratio increased significantly in KO vs. controls at all tested time points. Severe gliosis was observed beyond PN 8 months. Scotopic a-wave and b-wave were significantly reduced by PN 8 months. Protein glycosylation in PRs was not unaffected despite verified Pol buildup. Specific inhibition of Dol synthesis only led to slow PR loss, without concomitant protein glycosylation defects, highlight the role of Pol and DolP recycling in PR protein glycosylation.

FOSL1 acts as a tumor-intrinsic transcriptional sensor that integrates CAF-derived signals to enhance tumor plasticity at the leading edge in HNSCC

Jennifer Sosa; Sinha, Satrajit

Head and Neck Squamous Cell Carcinoma (HNSCC) is a highly heterogeneous malignancy arising from squamous epithelial cells lining mucosal tissues of the head and neck. Cancer stem cells (CSCs) are key drivers of this heterogeneity due to their capacity to adapt to both tumor-intrinsic and extrinsic signals. However, how the tumor microenvironment regulates CSC states to promote invasion, therapy resistance, and metastasis remains largely unknown. Here, we integrated published single-cell RNA sequencing (scRNA-seq), single-cell ATAC sequencing (scATAC-seq), and spatial transcriptomics datasets to define the transcriptional, epigenomic, and spatial landscape of CSCs in HNSCC. Our multiomic approach identified a 144-gene signature that enabled robust CSC identification across independent datasets and predicted poorer patient survival. We found elevated expression and activity of 28 Transcription factors (TFs) in CSCs, including the AP-1 family member FOSL1, an emerging central regulator of cellular plasticity. Spatial analyses further showed preferential CSC enrichment at the tumor leading edge, an invasive niche characterized by partial epithelial-to-mesenchymal transition (pEMT) and heightened stromal interactions. Notably, cell–cell communication analyses identified preferential signaling from cancer-associated fibroblasts (CAFs) to CSCs, with TGFB2-high CAF populations predicted to activate TGFBR1-expressing CSCs. Single-cell analysis of HNSCC–CAF co-cultures further confirmed that CAFs reinforce CSC programs through regulation of CSC-TFs including FOSL1, p63, and ETS1. Finally, integrative analysis of the FOSL1 cistrome and transcriptome revealed that CSCs preferentially leverage FOSL1-bound genomic regions marked by accessible chromatin. Collectively our data support a model in which FOSL1 acts as a crucial player in maintaining the accessible regulatory chromatin landscape that underlies CSC identity and serves as a central node integrating intrinsic and extrinsic cues to drive CSC plasticity.

Cooperative FoxA1–Hnf4α binding emerges from motif spacing and nucleosome architecture

Patrick D. Wilson and Michael J. Buck

The pioneer factor hypothesis holds that specialized transcription factors clear nucleosomal barriers so non-pioneer factors can subsequently bind. Recent K562 experiments expressing FOXA1 and HNF4α individually and together challenged this categorical view, identifying a cooperative subset of co-bound sites that requires both factors; how sequence and nucleosome context specify this cooperativity has been unresolved. Here we re-classify K562 dual-induction CUT&Tag at peak resolution, dissect sequence determinants with a dual-head convolutional neural network, and measure TF binding to defined nucleosomes in vitro with Pioneer-seq. Cooperative sites carry FOXA1 and HNF4α motifs at 15–60 bp inter-motif spacing, a cis-grammar absent from sites where either factor binds alone and consistent with soft-syntax cooperativity on positioned nucleosomes. The pattern reproduces at endogenously co-bound FOXA1–HNF4α sites in HepG2 and survives removal of primate-specific retrotransposon families that contribute a parallel, TE-derived pattern of motif co-occurrence. FoxA1 binds reconstituted nucleosomes more strongly than Hnf4α at most positions, and cobinding strength on natural nucleosomes tracks the distance of the FOXA1 motif from the nucleosome dyad. Cooperative FOXA1–HNF4α engagement reflects a structural arrangement of motifs on nucleosomes acting as a scaffold, rather than a barrier one factor clears for the other.

Undergraduate Student Poster Abstracts

Exploring the functional consequences of NRMT1 deletion on muscle differentiation and cell fate decisions

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Skeletal muscle differentiation is a tightly regulated, complex process requiring regulation of gene and protein expression and suppression of alternative cell fates to ensure proper lineage commitment. Despite extensive study, the specific mechanisms which lead to myogenic differentiation while suppressing alternative lineage fates remain incompletely defined. One known regulator for muscle differentiation is N-terminal RCC1 methyltransferase 1 (NRMT1), an important downstream target of CREB-mediated transcription that is required for proper myogenic differentiation. When there is a loss of NRMT1 function, myogenic differentiation of C2C12 mouse myoblasts is disrupted, leading to reduced expression of early myogenic regulators, impaired myotube formation, and activation of alternative lineage pathways. To better understand how these phenotypes occur with loss of NRMT1, both RNA-sequencing (RNA-seq) and ribosome-sequencing (ribo-seq) were performed. The RNA-seq experiments indicated altered transcription of very early myogenic regulators and markers of other lineages. The ribo-seq experiments indicated altered translation of membrane and secreted proteins. This project aims to validate the RNA-seq and ribo-seq findings by using quantitative PCR (qPCR) and Western blotting. qPCR analysis confirmed the RNA-sequencing results that showed a reduced expression of early myogenic regulators such as Pax7 and Myf5 and an increased expression of alternative lineage markers such as Ccn5 in NRMT1 knockout cells. Western blot analysis did not detect altered levels of membrane proteins in the NRMT1 knockout cells but did detect decreased levels of overall secreted proteins. These findings indicate NRMT1 is regulating muscle cell differentiation through both transcriptional and translational mechanisms.

Analyzing the structure and function of rubrerythrin from Streptococcus equinus

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Introduction:

A growing concern in medicine today is the dramatic rise in antibiotic resistance. Investigating proteins that are involved in the ability of bacteria to respond to oxidative stress and to withstand the onslaught of the human immune system is important for responding to this growing public health crisis. Rubrerythrins (Rbr) are members of the ferritin-like superfamily and are non-heme di-metal binding proteins found in bacteria and archaea. Many Rbr proteins have a di-iron center capable of reducing H2O2, which offers protection against radical oxidative damage. We hypothesize the Rbr protein enzymatic reaction contributes to bacterial ability to resist antibiotic treatments. Our project specifically examines the function and structure of rubrerythrin from the bacterium Streptococcus equinus (SeRbr) a gram-positive facultative anaerobic bacteria found in the digestive tracts of horses. We aim to answer several outstanding questions: What is the structure of SeRbr? What is the function of rubrerythrin from S. equinus? Does SeRbr function as a peroxidase? We have designed enzymatic assays to examine the function of SeRbr and will present evidence that it functions as a peroxidase enzyme. We will also present our recent structural work on SeRbr in different oxidation states. These experiments lay the foundation for future work understanding the function of rubrerythrin proteins in pathogenic bacteria.

Experimental Methods and Results:

We utilized X-Ray crystallography along with enzymatic assays that probe NADH consumption to evaluate SeRbr's metal-binding capacity, structure, and functional activity across various oxidative states. Our results indicate that SeRbr binds iron. Additionally, SeRbr's structure undergoes conformational changes in reduced (Fe(II)) and oxidized (Fe(III)) states. We have determined that SeRbr functions as a peroxidase by reducing H2O2. Furthermore, we performed these same experiments within the anaerobic chamber and observed slight differences in anoxic environments.

Conclusions and Future Directions:

Prior to our research, rubrerythrin from Streptococcus equinus had no known structure or validated function. We have determined SeRbr's structure in both the reduced and oxidized states, noting differences in the di-iron active site depending on the oxidation state. Further, we have confirmed that SeRbr functions as a peroxidase, contributing to

the oxidative stress pathway in bacteria. To follow these findings, our future research will involve designing new variants with different iron binding affinities.

A Protease Deficient Strain of Pseudomonas aeruginosa to Improve Purification Yields of an Error Prone Polymerase

Kyle Brown, Mark D. Sutton, Natalie H. Popielski

ImuABC is a translesion synthesis (TLS) polymerase highly conserved across bacterial species. A critical element of the SOS response, the low-fidelity ImuABC polymerase facilitates rapid mutagenesis in response to severe DNA damage within a bacterial cell, making ImuABC expression a critical mechanism via which pathogens such as Mycobacterium tuberculosis increase virulence and develop drug resistance. This makes ImuABC a promising therapeutic target to improve efficacy of antibiotic treatment and curb the emergence of multidrug-resistant pathogens. However, up to this point efforts to study and characterize ImuABC in vitro have been difficult, owing in large part to low cytosolic concentrations of the protein, making efficient purification extremely challenging. One potential cause of these low cytosolic concentrations is the active repression of ImuABC levels within the cell by proteases to mitigate its mutagenic activity outside of stressful conditions. Here, we work in the model organism Pseudomonas aeruginosa, ideal for its well-characterized genetics and native expression of ImuABC. We aim to develop a strain in which the protease genes lon, clpX, and clpP have been knocked out, which we hypothesize will result in greater concentration of cytosolic ImuABC and therefore improve protein purification yields. In pursuit of this end, we have thus far developed the genetic materials necessary to construct and characterize a mutant strain and continue to work to optimize reaction conditions.

Shifting Keratinocyte States: The Role of Follistatin in Proliferation and Differentiation

Li Yi Hu

Atopic dermatitis (AD; eczema) is a chronic inflammatory skin disease characterized by impaired barrier integrity, with an incompletely understood etiology and no definitive cure. Keratinocytes maintain epidermal integrity through tightly regulated proliferation and differentiation, processes essential for epidermal homeostasis and barrier function. The transcription factor p63 is a key regulator of epithelial stem cell maintenance and keratinocyte differentiation. Prior analysis revealed follistatin (FST), a secreted glycoprotein that regulates TGF-β family signaling, as a transcriptional target of p63 in head and neck squamous cell carcinoma. While the loss of p63 disrupts epithelial homeostasis, the role of its downstream target FST in keratinocyte differentiation remains unclear. This study investigates how FST regulates keratinocyte differentiation and its relationship to key epidermal markers. Differentiation was assessed in vitro using HaCaT cells with FST manipulation. Transcriptomic analysis of FST knockdown revealed downregulation of cell cycle and mitotic pathways, alongside enrichment of epidermal differentiation and barrier-associated processes. These findings indicate a shift from a proliferative basal state toward a more differentiated keratinocyte phenotype. Consistent across independent knockdown conditions, differentiation-associated genes were upregulated, including barrier and structural markers such as filaggrin (FLG) and desmoplakin (DSP). Additionally, regulators of keratinocyte maturation and signaling, including LAMTOR1, as well as inflammatory genes such as NLRP1, were upregulated, supporting a coordinated program of differentiation, structural maturation, and barrier formation. Collectively, these results suggest that FST functions to maintain keratinocytes in a proliferative, basal-like state, and that its loss promotes differentiation and epidermal maturation. These findings provide insight into mechanisms of skin homeostasis and identify FST as a potential regulator of keratinocyte proliferation and differentiation, with relevance to barrier dysfunction in inflammatory skin diseases such as AD, where disrupted keratinocyte differentiation contributes to disease pathology.

Biochemical and Biophysical Analysis of the Antiviral SLFN14 Endoribonuclease

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The SLFN14 endoribonuclease is a post-transcriptional regulator that imposes translational repression. In response to a viral infection (e.g. influenza), the slfn14 interferon stimulated gene targets the translation machinery and its associated RNAs for cleavage to suppress viral protein synthesis. SLFN14 must dimerize to form an extended RNA binding cleft capable of cleaving tRNA of the acceptor stem. Previous structural and proteomic studies have revealed a conserved phosphorylation site within other SLFN family members, suggesting the equivalent uncharacterized threonine residue in SLFN14 may be a site for post-translational regulation. We developed a procedure to isolate recombinant human SLFN14 protein free of contaminating nucleic acids, making it suitable for quantitative biochemical studies. We reconstituted full-length wild-type and phosphomimetic SLFN14 variants and performed SDS-PAGE analysis and Western blot analysis to confirm sample purity and protein identity, respectively. Thermal stability of phosphomimetic SLFN14 variants was compared to wild-type protein. Mass photometry analysis reveals a prominent SLFN14 dimeric population for both wild-type and phosphomimetic variants as well as a shift in molecular mass corresponding to tRNA binding with a 1:1 stoichiometry. Electrophoretic mobility shift assays reveal a difference in binding affinity of each variant. We performed in vitro tRNA cleavage assays to measure the impact of phosphomimetic variants on tRNA cleavage efficiency and site-specificity. SLFN14 phosphomimetic variants also exert altered effects in the repression of protein synthesis in an in vitro translation assay system. Together, these data establish an important regulatory site on SLFN14 that may modulate its catalytic function through protein phosphorylation.

Linking SOX2 to Tumor Progression: The Role of TP63 and ETV4 in Head and Neck Squamous Cell Carcinoma

Nandini Kodey, Satrajit Sinha

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. Characterized by its high rate of recurrence in patients, lack of early-detection biomarkers, strong resistance to therapy, and tumor heterogeneity, HNSCC shows a trend of poor prognosis in those diagnosed. Despite advances in treatment strategies, overall survival rates for HNSCC patients have remained relatively unchanged, highlighting a need for better molecular targets and therapeutic approaches. The transcription factor SOX2 has been identified as a diagnostic marker for HNSCC patients, with high SOX2 expression linked to improved survival in HNSCC. It acts as a biomarker for a favorable treatment response, particularly in radiotherapy. SOX2 is frequently amplified in HNSCC and plays a vital role in maintaining cancer stemness and promoting tumor progression. However, its direct downstream targets remain undefined, limiting the understanding of SOX2's role in tumor progression. This study investigates SOX2-regulated targets contributing to oncogenic transcription signaling in HNSCC using an integrative analysis of RNA sequencing and chromatin immunoprecipitation sequencing datasets. It identifies genes directly bound by SOX2 and those differentially expressed following SOX2-modified expressions, such as knockdown conditions. This analysis identifies transcription factor ETV4 as a key downstream component within the SOX2–TP63 regulatory network, which plays a central role in transcription regulation in HNSCC. ETV4 is downregulated following SOX2 and TP63 knockdowns, linked to cell proliferation and tumor progression pathways, and showing its role as a downstream transcriptional target. This SOX2–TP63–ETV4 regulatory system contributes to HNSCC progression and provides specific targets for further functional validation studies. These findings identify ETV4 as a downstream effector of the SOX2–TP63 regulatory network and a candidate target for further functional validation in HNSCC.

Class 9 Myosin Regulation of Epithelial Cell Polarity

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Introduction.

Cell polarity is defined by the asymmetric distribution of cellular components and the alignment of cytoskeletal filaments along the axis of polarity. A major contributor to cell polarity is the actin cytoskeleton, which is essential for maintaining proper cellular structure and dynamics. The actin cytoskeleton is regulated through two mechanisms: force production by myosin motor proteins and signaling molecules. Myosin 9b (Myo9b) is a unique cytoskeletal regulator as it combines both an N-terminal motor domain and a C-terminal Rho GAP signaling domain. However, how Myo9b uses each domain to regulate the actin cytoskeleton is unknown. Understanding these domain functions is critical because mutations in the Myo9b gene have been shown to increase the risk of celiac disease and intestinal barrier disorders, and the detailed mechanism by which these mutations alter epithelial cell polarity remains unknown.

Experimental Methods and Results.

Our lab confirmed Myo9b expression in epithelial cells and found that it localizes to microvilli tips using SORA confocal microscopy. CRISPR-Cas9 knockout of Myo9b caused a substantial increase in microvilli and had basal actin and morphological defects. Supporting this, SEM and confocal images of microvilli in Jeg3 WT and Myo9b KO cells show that microvilli are more abundant in the KO compared to WT cells. Furthermore, expression of a motor-dead Myo9b construct eliminated microvilli formation, whereas a Myo9b GAP dead construct resulted in a partial restoration of the wild-type microvilli phenotype.

Conclusions and Future Directions.

Myo9b KO cells exhibit more abundant microvilli than WT cells, indicating that Myo9b regulates microvilli formation. Additionally, domain specific rescue experiments show that the GAP and motor domains have distinct functions, suggesting that Myo9b may have two potential mechanisms to regulate the actin cytoskeleton. Future studies will focus on identifying molecular interactions between Myo9b and its regulatory partners and defining the mechanisms how Myo9b regulates actin organization and epithelial cell polarity.

Potential Effects of a Semi-Conserved Active Site Phenylalanine on CYP107S1 Mediated Drug Metabolism in Pseudomonas aeruginosa.

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Introduction. Cytochrome P450 (CYP) enzymes are heme-containing proteins that catalyze oxidative metabolism and clearance of potentially toxic compounds. There are three known CYPs in Pseudomonas aeruginosa, a common hospital acquired pathogen, which are CYP107S1, CYP168AS1 and CYP169A1. CYP107S1 can metabolize the cystic fibrosis drug ivacaftor to hydroxymethyl ivacaftor, as done by the human hepatic CYP3A4.1 Alphafold3 models show evidence of a semi-conserved phenylalanine residue in the active site. The purpose of this study is to test the importance of F387's π-stacking interactions and bulky hydrophobicity for substrate turnover in guiding and properly positioning compounds for catalysis. The mutant F387L removes π-stacking interactions while preserving bulky hydrophobicity, and the mutant F387W preserves π-stacking interactions, but reduces hydrophobicity.

Experimental Methods and Results. Both mutants were purified via Ni-NTA and size exclusion chromatography, then analyzed via SDS-PAGE. To determine importance of π-stacking interactions and bulky hydrophobicity in CYP107S binding, UV-VIS absorbance binding assays of wild-type, F387L, and F387W were compared. Preliminary findings indicate binding of the ligands ivacaftor and raloxifene with F387L is similar to wild type. Binding of ivacaftor with F387W is significantly different than wild type.

Conclusion and Future directions. CYP107S1 F387L and F387W were successfully purified via Ni-NTA chromatography and had a high yield, resembling wild type. SDS-PAGE gel analysis showed prominent bands around 50 kDa and is relatively pure, similar to wild type. Next, CO assays show high absorbance around 450 nm and low absorbance at 420nm which indicates that the protein is stable and still functional after being stored in -80◦C. CYP107S1 F387L tightly binds ivacaftor and raloxifene, with the differences of Kd between mutant and wild-type being negligible. CYP107S1 F387W shows significantly less binding to ivacaftor than wild-type, and a Kd was unattainable. π-stacking interactions between active site F387 and the ligands ivacaftor and raloxifene aren't necessary factors for substrate binding. Future experiments will be done to further test the hypothesis. A functional assay via HPLC will be performed to test if binding is productive. Additionally, binding of Raloxifene with the Trp mutant will be done to see if the results are specific to ivacaftor or if they can be generalized to other ligands. Last, NMR will be used to examine structural changes within the two mutant proteins compared with wild type.

Investigating the Role of Diploidy in Buffering Genome Instability from dNTP Pool Imbalance and Mismatch Repair Deficiency in Saccharomyces cerevisiae

Matthew Leyberman, Dr. Jennifer Surtees

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Introduction

Genome stability depends on both balanced deoxynucleotide (dNTP) pools and an intact mismatch repair (MMR) system to maintain high-fidelity DNA replication. Disruption of either pathway increases mutation rates, but combined defects can result in catastrophic genome instability. The rnr1Y285A mutation skews dNTP pools, causing a 20-fold increase in dCTP and dTTP levels, which promotes replication errors, while deletion of MMR genes such as MSH2 or MSH6 prevents correction of these errors. Previous work in our lab has shown that haploid rnr1Y285A msh2Δ and rnr1Y285A msh6Δ strains exhibit synthetic lethality, likely due to accumulation of mutations in essential genes. The objective of this study is to determine whether diploidy can buffer this lethal mutational burden by providing a second copy of essential genes, thereby allowing survival despite increased genome instability.

Experimental Methods and Results

To test this, we compare haploid and diploid Saccharomyces cerevisiae strains carrying combinations of rnr1Y285A and MMR gene deletions. Cell viability is assessed using colony-forming unit (CFU) assays and DNA damage and replication checkpoints are analyzed through Western Blots. Preliminary observations indicate that haploid double mutants accumulate in G2/M phase with abnormal DNA content and morphology, consistent with replication stress and checkpoint arrest. In contrast, diploid double mutants are expected to exhibit improved viability but with an increased mutational load, causing increased stress and compromised fitness.

Conclusions and Future Directions

These studies could provide insight into how diploidy lessens the lethal consequences of combined dNTP imbalance and MMR deficiency. We expect that diploid cells will tolerate higher mutational loads, allowing survival under conditions that are lethal in haploids, as the presence of a second allele of essential genes can buffer against otherwise lethal mutations. At the same time, we anticipate that mutation rates will be further elevated, which could activate a DNA damage checkpoint. This work provides a model for understanding how cells manage replication stress and DNA repair defects, with direct relevance to cancer biology, where similar conditions are common. Future work will focus on further characterizing mutation spectra and identifying pathways that enable survival under extreme genomic stress, potentially revealing targets for synthetic lethal therapeutic strategies.

Prognostic Significance of Pre-Treatment Neutrophils, Lymphocytes, and Monocytes in Stage IV Head and Neck Cancer Patients

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Introduction: Head and neck cancer comprises a group of biologically related malignancies arising in the upper aerodigestive tract and is associated with poor prognosis in advanced stages. Identifying reliable and accessible prognostic biomarkers remains critical for improving patient stratification and clinical decision-making. Peripheral blood cell populations, including neutrophils, lymphocytes, and monocytes, have been increasingly studied for their potential to reflect systemic inflammatory and immune responses in cancer. This study aims to evaluate the relationship between pre-treatment neutrophil, lymphocyte, and monocyte levels and overall survival in patients with Stage IV head and neck cancer, and to assess their utility as prognostic biomarkers.

Experimental Methods: A retrospective chart review was conducted using clinical data from patients with Stage IV head and neck cancer. Patient information was extracted from Electronic Health Records (EHR), including physician notes, treatment records, laboratory values, and survival outcomes. Pre-treatment neutrophil, lymphocyte, and monocyte values were collected and entered into REDCap to create a secure research database. The dataset was then exported into Excel for data cleaning and organization. Patients were stratified into high and low groups based on median pre-treatment blood cell percentages. Kaplan-Meier survival analysis was used to compare overall survival between groups, and Fisher's Exact Test was performed to assess statistical significance.

Results and Conclusion: Results: A total of 498 patients with Stage IV head and neck cancer were analyzed. Patients with lower pre-treatment neutrophil percentages (8.0–65.2%) had significantly improved overall survival compared to those with higher neutrophil percentages (65.3–91.2%) ($p < 0.05$). Similarly, patients with higher lymphocyte percentages (22.2–83.4%) had better survival than those with lower lymphocyte percentages (4.2–22.1%) ($p < 0.05$). In contrast, monocyte percentages showed no statistically significant association with overall survival when comparing low (1.0–6.4%) and high (6.5–17.5%) groups. Pre-treatment neutrophil and lymphocyte levels are associated with higher overall survival in patients with Stage IV head and neck cancer. This suggests that routine blood counts offer a cost-effective method for prognostic assessment. Future studies should explore the mechanistic role of peripheral blood in cancer progression.

Titration and Histological Validation of Lipopolysaccharide- and HCl-Induced Preclinical Models of Acute Lung Injury for the Evaluation of Treatment X

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Acute respiratory distress syndrome (ARDS) is characterized by dysregulated inflammation in the alveoli, leading to immune cell infiltration and disruption of the alveolar-capillary barrier. This form of non-cardiogenic pulmonary edema severely impairs the ability of oxygen exchange, often leading shortness of breath, wheezing, and cyanosis. A moderate form of ARDS, clinically known as Acute Lung Injury (ALI) is relatively common among intensive care unit patients receiving mechanical ventilation. While many patients often recover from ALI, elderly patients (> 65 years) have a mortality rate exceeding 40-50%. Lipopolysaccharide (LPS) derived from *E. coli* and hydrochloric acid administered to mice are widely used as models of ALI; however, injury severity and reproducibility are highly dependent on experimental parameters. In this study, mice were administered via oropharyngeal aspiration with varying doses and instillation volumes of LPS (*E. coli* O111:B4) as well as hydrochloric acid (HCl) and were subsequently analyzed at 24hr, 48hr, and 1week post-induction intervals. Disease severity was assed using longitudinal body weight changes as an indicator of systemic illness, alongside bronchoalveolar lavage (BAL) and histological analysis of lung tissue to assess immune cell infiltration and structural injury. Our ALI model demonstrated consistent weight loss and pronounced inflammatory infiltration within the lung parenchyma, confirming a reliable induction of ALI. Furthermore, treatment X was administered to our LPS-ALI model, showing potential in lowering the overall inflammatory response. By establishing clear injury parameters and the testing of a novel treatment, future studies include expanding the use of this preclinical ALI model for further mechanistic and therapeutic studies.

Investigating mutagenesis over time in Saccharomyces cerevisiae homologs of msh3 mutations identified in African American colorectal cancer patients

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The mismatch repair (MMR) system contributes to the maintenance of replication fidelity by recognizing and directing repair of errors during DNA replication. In eukaryotes, including humans and Saccharomyces cerevisiae, MMR is initiated by one of two MutS homolog (MSH) heterodimeric complexes, MutSα (Msh2-Msh6) and MutSβ (Msh2-Msh3). These complexes have separate but overlapping specificities for distinct DNA errors. Deficiencies in MMR are implicated in multiple human cancers, notably a hereditary colorectal cancer (CRC) and endometrial cancer syndrome called Lynch Syndrome and drive classic microsatellite instability (MSI) in tumors. Hereditary CRCs have been primarily associated with Msh2-Msh6, but alterations in Msh3 specifically have been linked to a specific form of MSI overrepresented in African American patients and associated with poorer prognosis. Six pathogenic variants in the MSH3 gene of African American CRC patients were previously identified; the human residues at these positions are conserved in yeast and equivalent mutations were constructed and tested for mutation rate as a measure of MMR functionality. Five of these mutations demonstrated no significant phenotype, and three demonstrated an intermediate phenotype, perhaps suggesting that these mutations are not related to the patients' cancers. However, cancer development is a multistep process; mutations accumulate gradually over time until genes important for cell cycle control are compromised, promoting tumor initiation. We hypothesize that, despite the low mutation rate in our reporter assay, time-dependent mutation accumulation will be increased in the msh3 mutants compared to wild-type MSH3. To test this hypothesis, we designed a mutation accumulation (MA) experiment where we created MA lines over bottleneck passages to minimize natural selection and enable us to attribute mutation accumulation to differences in the starting gene. We performed microsatellite instability assays on each cell line at specific cell passages to characterize mutation rate over time and identify potential trends. Our reporter assay data indicated that the MSH3 (WT) mutation rate remained consistent over time, while the msh3Δ and all three msh3 alleles exhibited a decrease in mutation rate. Future directions include continuing the MA cell lines and performing additional phenotypic assays that test cellular fitness. We also plan to perform whole genome sequencing on each MA line to identify specific genomic mutations that may be present.

FURIN Recovers Aged Myoblast's Ability to Differentiate

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Introduction. Sarcopenia, the loss of muscle mass and function with age is caused by both environmental and genetic factors. Currently there are no FDA drugs approved for treatment of sarcopenia. A major cause of sarcopenia is that as muscle cells start to age they no longer develop the same as younger cells. Young muscle cells start as myoblasts that can fuse into myotubes in a process known as differentiation. These myotubes form in long strands that are responsible for contraction and large scale movement. As muscle cells age, they lose the ability to differentiate into myotubes leading to loss of muscle function. Initial silica discovery identified FURIN as a possible factor involved in sarcopenia. The objective of our research is to explore the role of FURIN in muscle differentiation and how it changes with age.

Experimental Methods and Results. Our initial in silica discovery came from the Adult Genotype-Tissue Expression (GTEx) Portal dataset. Following this, qPCR and Western blots were used to measure mRNA and protein expression changes of FURIN and NOTCH with increasing passage numbers in our C2C12 mouse skeletal muscle cell line. Finally we restored FURIN expression in aged C2C12 cells using plasmid transfection.

Conclusions and Future Directions. Our work showed a negative correlation between age and expression of FURIN and NOTCH. Along with this we found that when restoring FURIN expression in aged mouse skeletal muscle cells they regained the ability to differentiate suggesting FURIN plays a critical role in this process. Ongoing and future experiments are aimed at identifying the underlying mechanism FURIN uses to regain differentiation ability through analysis of related genes and proteins. Furthermore, we have obtained an IBR approval to analyze human tissue and plan to translate our experiments from the C2C12 model into a human one.