RUTGERS
Molecular Biosciences
Graduate Student Organization

17th Annual MBGSO Research Symposium

Friday March 22, 2024

9:30 AM-4:30 PM

Life Sciences Building Atrium

Busch Campus

Rutgers University

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A Letter from the Organizers

Welcome to the 17th Annual Graduate Student Research Symposium hosted by the Rutgers University Molecular Biosciences Graduate Student Organization (MBGSO). We are delighted to have you join us today to support the outstanding research produced by graduate students in the Molecular Biosciences Graduate Program and related graduate programs. We are fortunate to be able to foster a scientific community here at Rutgers.

As a student-led organization, the goal of MBGSO is to facilitate the professional development of graduate students and provide opportunities for continued growth, learning, and networking. By hosting the MBGSO Annual Symposium we hope to not only showcase graduate student research but also to encourage collaboration and interdisciplinary discourse. With support from the university community our presenters receive valuable feedback and input on their work as well as an opportunity to disseminate their research to a broader audience. With these goals in mind, we organize the annual symposium and look to the university community to make it a success.

We would like to express our gratitude to our faculty advisor, **Dr. Janet Alder**, for her tireless work on behalf of the graduate students and for providing valued guidance and support. We would also like to thank **the administrative personnel supporting the Rutgers School of Graduate Studies** for their role in helping the MBGSO coordinate this and several other events throughout the year. We gratefully acknowledge the **Molecular Biosciences faculty** for their ongoing support and commitment to our graduate program and especially to those of them that volunteered their time to participate in the symposium to judge student presentations and offer valuable feedback and insight. We are also grateful to our keynote speaker, **Dr. Christina Montagna**. And finally, this event would not be possible without the participation of the graduate student presenters, and so **we offer a hearty thanks to our peers** for helping create and nurture the vibrant intellectual and social community within the Molecular Biosciences Graduate Program. We are proud and honored to be at the service of the Molecular Biosciences community.

Sincerely,
MBGSO Executive Board 2023-2024

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Vice President: **Ryan Fink**

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Secretary: **Noah Mac**

Program Coordinator: **Melinda Liu**

Content Creator: **Eton Victor**

Symposium Schedule

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10:00AM - 11:00AM……**Oral Presentations I**

11:00AM - 12:00PM……**Poster Presentations I**

12:00PM - 1:00PM……**Keynote Address**

1:00PM - 2:00PM……**Lunch & Networking**

2:00PM - 3:00PM……**Poster Presentations II**

3:00PM - 4:00PM……**Oral Presentations II**

4:00PM - 4:30PM……**Award Ceremony**

4:30PM - 5:30PM……**Networking Event**

Keynote Address

""Oncogene Discovery and Mechanisms of Genomic Instability on Tumor Initiation and Progression"

Dr. Christina Montagna, PhD

Dr. Cristina Montagna is a Professor in the Department of Radiation Oncology at the Cancer Institute of New Jersey at Rutgers University, New Brunswick. Dr. Montagna is a leader in the field of genomic instability and particularly aneuploidy. She received her PhD from the University of Milano, training in the lab of Dr. Renato Dulbecco lab, who received the 1975 Nobel Prize for his discoveries related to DNA virus induced transformation of healthy cells. From there, Dr. Montagna went on to do a postdoc at the National Institute of Health with Dr. Thomas Reid, who specializes in fluorescent in situ hybridization assays to "paint chromosomes", termed spectral karyotyping. Her seminal work in the Reid lab characterized chromosomal aberrations using spectral karyotyping in breast cancer and she discovered new genes involved in genomic instability such as Septin9.

Dr. Montagna went on to found her lab at Albert Einstein College of Medicine where she continued investigating oncogene discovery including mechanisms of genomic instability on tumor initiation and progression with a special emphasis on women's health. Dr. Montagna's work pioneered the discovery that somatic aneuploidy is observed frequently in disease-free tissues. Her laboratory was the first to report accumulation of somatic aneuploidy with aging and to demonstrate that developing aneuploidy in wild type cells causes senescence.

Today, Dr. Montagna is a leader in her role here at Rutgers. Her lab continues to use cytogenetic approaches and has incorporated single-cell and next-gen sequencing techniques as primary tools of study. Dr. Montagna has amassed over 6,000 citations in her career and is relentless in her pursuit of high-quality science, demonstrated by recent publications in Cell, Nature and Science-affiliated journals.

Most importantly, and in accordance with the theme of this symposium, Dr. Montagna has a profound record with mentorship. Dr. Montagna writes, "I am strongly dedicated to mentorship, and I am committed to providing mentees with a challenging intellectual environment and solid training in molecular genetics with a translational focus". She is actively involved in mentorship including undergraduates, PhD candidates, clinical fellows, and postdoctoral scholars.

*Adapted from Rutgers CINJ Faculty Profile & Molecular Bioscience Graduate Program Profile

ORAL PRESENTATIONS

Morning Presentations

10 AM-10:20 AM: Enhancing the Anti-Tumor Efficacy of Nucleotide Synthesis Inhibitors By Targeting the DNA Replication Stress Response

Presenter: Masuda Akther

Loss of function mutations in the tumor suppressor genes TSC1 and TSC2 cause the genetic tumor syndrome Tuberous Sclerosis Complex (TSC), and are also associated with some cancers. TSC1/2-deficient tumors exhibit increased cellular anabolism driven by the mechanistic Target of Rapamycin Complex 1 (mTORC1). We previously discovered that TSC2-deficient cells and tumors are highly dependent on rate limiting enzyme in de novo guanine nucleotide synthesis, inosine-5′-monophosphate dehydrogenase (IMPDH). This metabolic vulnerability can be exploited using clinically approved IMPDH inhibitors which selectively induce DNA replication stress and DNA damage in TSC2-deficient cells, culminating in cell death. However, these cells can also adapt to IMPDH inhibitor therapy by activating the replication stress response (RSR) and DNA damage response (DDR) pathways, which can promote their survival and reduce IMPDH inhibitor efficacy. We hypothesized that inhibiting RSR and DDR pathway effectors can enhance the efficacy of IMPDH inhibitors. We combined the clinically approved IMPDH inhibitor Mizoribine with inhibitors of the ATM, ATR, CHK1, CHK2, DNA-PK, and WEE1 kinases, to determine which combinations are most effective at selectively killing TSC2-deficient cells relative to wild-type. Our preliminary findings indicate that combining Mizoribine with the ATR inhibitor AZD6738, which is currently in clinical trials for oncology, significantly increases apoptotic cell death in TSC2-deficient cells compared to Mizoribine treatment alone, while maintaining selectivity. Additionally, inhibitors targeting WEE1 (AZD1775) and a dual CHK1/ CHK2 inhibitor (AZD7762) also enhanced mizoribine-induced cell death. Thus, we propose a novel combinatorial treatment strategy targeting the RSR/DDR pathways in conjunction with IMPDH inhibition to enhance the anti-tumor efficacy of these therapies.

10:20 AM-10:40 AM: Ubiquitin E3 Ligase OSTM1 Regulates The cAMP/PKA/CREB Pathway And Suppresses B-cell Malignancies

Presenter: Muhammad Usama Tariq

B cell malignancies (BCM) is a group of B lymphocytes specific cancers that arise because of faults introduced during different stages of B-cell development in primary and secondary lymphoid organs. Here, we performed a whole genome targeting CRISPR/Cas9 knockout screen in the immortalized yet non-transformed IL-3-dependent Ba/F3 murine pro-B cell line to identify novel tumor suppressors in BCM. One of the candidate genes is osteoporosis-associated transmembrane protein 1 (OSTM1), a putative E3 ubiquitin ligase that is known to regulate bone homeostasis. Interestingly, genetic deletion of OSTM1 is highly frequent and correlates with poor prognosis. Genetic silencing of OSTM1 in Ba/F3 cells conferred IL-3-independent survival and proliferation. Tail vein injection of OSTM1 KO Ba/F3 cells resulted in reduced animal survival that was accompanied by splenomegaly, lymphadenopathy, and abnormal peripheral blood cell count. Using mass spectrometry screens, we identified phosphodiesterase 3B (PDE3B) as an interacting partner of OSTM1. OSTM1 ubiquitylates PDE3B and promotes its proteasomal degradation. Interestingly, non-glycosylated OSTM1 preferentially interacts with PDE3B for its degradation. As PDE3B is a negative regulator of the tumor-suppressive cAMP/PKA pathway, overexpression of PDE3B in DLBCL lines accelerated cell proliferation while knockout of PDE3B in OSTM1 KO cells led to phenotypic reversal both in cell lines and in-vivo. Modulation

of cAMP/PKA signaling was seen by altering both OSTM1 and PDE3B in malignant cells, and the expression of cAMP/PKA pathway target genes was strongly correlated with OSTM1 expression in patients across BCM. Finally, B-cell specific deletion of OSTM1 cooperated with CDKN2A deletion and allowed spontaneous lymphoma formation in transgenic mice. Collectively, these evidences suggest that OSTM1 targets PDE3B for proteasomal degradation and relieves the suppression on cAMP/PKA pathway that allows growth inhibition in B-cells; hence acting as a tumor suppressor in BCM.

10:40 AM-11:00 AM: Dynamic RNA polymerase II recruitment drives intestinal differentiation under the direction of HNF4
Presenter: Kiranmayi Vemuri
The mammalian intestinal epithelium is one of the most aggressively self-renewing tissues in the body. It comprises of villi which primarily contain absorptive enterocytes, and crypts which harbor adult stem and progenitor cells, crucial for differentiation. Understanding transcriptional regulation in intestinal differentiation is imperative for overall intestinal health but remains incomplete. Terminal differentiation requires a massive restructuring of the transcriptome. During intestinal differentiation, the expression patterns of nearly 4000 genes are altered as cells transition from progenitor cells in crypts to differentiated cells in villi. We identified dynamic recruitment of RNA Polymerase II (Pol II) to gene promoters as the primary driver of transcriptomic shifts during intestinal differentiation in vivo. Changes in enhancer-promoter looping interactions accompany dynamic Pol II recruitment and are dependent upon HNF4, a pro-differentiation transcription factor. Using genetic loss-of-function, ChIP-seq and IP mass spectrometry, we demonstrated that HNF4 collaborates with chromatin remodelers and loop-stabilizing proteins and facilitates Pol II recruitment at hundreds of genes pivotal to differentiation. We also explored alternate mechanisms which drive differentiation gene expression and find pause-release of Pol II and post-transcriptional mRNA stability regulate smaller subsets of differentially expressed genes. These studies provide insights into the mechanisms of differentiation in a renewing adult tissue.

Afternoon Presentations

3 PM-3:20 PM: Genetic Blueprint of Congenital Muscular Dystrophies with Brain Malformations
Presenter: Kyle Flannery
Congenital muscular dystrophies (CMDs) are autosomal recessive disorders characterized by infantile hypotonia, motor delay, and progressive muscle atrophy. Severe brain involvement is also observed in CMD subtypes such Muscle Eye Brain disease (MEB) and Walker Warburg syndrome (WWS), which have 18 known disease genes, and merosin-deficient CMD (MDC1A), which is caused by mutations in LAMA2. Whole exome sequencing (WES) is a remarkable tool for identifying genetic variants that cause CMDs, yet many individuals remain genetically undiagnosed. In addition, rare variants that do not directly impact protein-coding sequence are often overlooked in WES analyses, and novel disease-gene associations continue to emerge as next generation sequencing (NGS) becomes more common in research and clinical practice. Here, we present a WES cohort of 105 patients presenting with CMD and brain malformations: 57 individuals from 53 families with WWS, 22 individuals from 20 families with MEB, and 26 individuals from 25 families with MDC1A. In addition to standard analyses of exonic, protein-disrupting variants, we leveraged bioinformatic tools including SpliceAI, RegVar, and SynVep to analyze synonymous and noncoding variants, as well as candidate gene sharing platforms such as GeneMatcher. Overall, our comprehensive strategy achieved a genetic diagnosis in 83% (44/53) of WWS families, 80% of MEB families (16/20), and 100% of MDC1A families (25/25), which is improved

compared to other CMD cohorts. However, it is likely that additional NGS strategies such as whole genome sequencing and transcriptome sequencing, especially long-read strategies, are needed to capture the remainder of disease-causing variants.

3:20-3:40 PM: Genomic Instability in HPV Tumorigenesis: Functional Impact of TP63 Variant Expression Changes in Non-Transformed Keratinocytes
Presenter: Eleanor Agosta
Human papillomavirus (HPV) is associated with over 90% of cervical cancers and 70% of head and neck cancers with viral DNA often integrated into human DNA in one of five recurrent locations. One of these locations is near the TP63 gene, a transcription factor in the p53 family, which is an important regulator of differentiation in keratinocytes. However, the molecular mechanisms underlying HPV-induced tumorigenesis and behind the preferential integration of HPV near specific loci such as TP63 remain unknown. My working hypothesis is that the integration of HPV into these loci provides a selective advantage to host cells leading to cellular transformation and tumor progression. To test my hypothesis, I analyzed publicly available databases of cervical tumors and healthy cervical tissues for differential TP63 expression. Through my analysis of the Genotype Tissue Expression Project (GTEX) and The Cancer Genome Atlas (TCGA), I found that prevalent splice variants of TP63 in healthy cervical tissues include a long variant, V1, and one truncated variant, V4. However, in cervical tumors, expression of V1 is virtually absent, and patients instead predominantly express one of three truncated variants: V4, V5, or V9. I then designed functional studies to introduce these variants into non-transformed keratinocytes and monitor them for tumorigenic characteristics such as changes in proliferation, colony formation, and differentiation. Due to the lack of commercially available constructs harboring TP63 variants, I meticulously cloned each variant into a lentivirus construct resulting in new tools for use in functional studies examining HPV-induced tumorigenesis.

3:40 PM-4:00 PM: Single Nucleotide Variants of RIG-I Reported in Severe COVID-19 Patients Reveal Dysregulated Autoinhibitory Mechanisms Leading to Aberrant Immune Responses
Presenter: Mihai Solotchi
The symptoms of COVID-19 infection are unpredictable and vary widely, however severe disease manifestations are often associated with extreme innate immune responses in the form of hyperinflammation and cytokine storms. Retinoic acid-inducible gene I (RIG-I) is a cytosolic innate immune receptor that plays a frontline role in defending against RNA viruses, such as SARS-CoV-2. RIG-I acts as a pattern recognition receptor (PRR), identifying and binding to viral RNA with high affinity, thereby triggering a robust downstream antiviral interferon signaling response. Previous studies have identified and characterized a molecular gating mechanism within RIG-I that critically regulates RNA binding and interferon activation. Recently, three single nucleotide variants of RIG-I were discovered in severe COVID-19 patients, gain-of-function (GOF) mutations (N573S, E583G) and a loss-of-function (LOF) mutation (G731R). Through a series of cellular and biochemical assays complemented by structural hydrogen-deuterium exchange mass spectrometry (HDX-MS), we demonstrate that the distinct phenotypes of these mutants result from dysregulation in the gating mechanism. GOF mutations weaken the gating mechanism, reducing RNA specificity and promoting hyperactive immune responses. Conversely, the LOF mutation strengthens the gating mechanism, maintaining highly-specific RNA binding, albeit in a signaling-inactive conformation, thereby interfering with the normal immune responses of wild-type RIG-I. These studies were highly informative in uncovering the vulnerability of RIG-I's inherent regulatory mechanisms to single-point mutations, and our findings contribute to the structure-based development of small molecule RIG-I modulators.

POSTER PRESENTATIONS

Morning Session

Presenter: Maria Ibrahim

Poster #1: Loss of CCL1/MCP1 Rescues Systemic Metabolic Reprogramming during Autophagy Deficiency

Authors: Maria Ibrahim, Laura Perez, Zhixian Hu, Eduardo Cararo Lopes, Maria Gomez, Eileen White

Lung cancer is a deadly disease and one of the leading causes of death worldwide, and thus needs new therapeutic approaches [1]. Autophagy is the mechanism by which cells recycle proteins and organelles to maintain cellular homeostasis during stress and starvation [4]. Under normal conditions, autophagy functions at a low basal level to remove damaged cellular components, thus preventing the gradual accumulation of toxic, intracellular waste material [4]. Cancer cells rely on autophagy -- in many cases, they are more autophagy dependent than normal cells and tissues. This is due to the inherent deficiencies in the surrounding microenvironment caused by increased metabolic and biosynthetic demands imposed by deregulated cell proliferation. A major limitation is that most cancer models have addressed the role of autophagy only in tumors without drawing a direct comparison to autophagy deficiency in normal tissues. We propose to use a GEMM of systemic ablation of essential autophagy gene 7 (Atg7) to explore the underlying metabolic phenotype associated with autophagy deficiency and the tumor microenvironment. We hypothesize that loss of autophagy causes systemic metabolic defects that block tumor growth by limiting tumor nutrients and promoting an anti-tumor immune response. For the first aim, we hypothesize that the overall energy balance, expenditure, and respiratory exchange rate might regulate the metabolic phenotype underlying autophagy deficiency. We propose to characterize overall energy consumption, with OxyMax/CLAMs, in Atg7, Atg5, and Fip200 mice. Hence, we propose that the overall alterations in energy balance, consumption, and macro-fuel combustion contribute to the metabolic phenotype underlying autophagy deficiency. For the second aim, we will investigate the role of interferon induced cytokine signaling in the tumor microenvironment and lung cancer upon loss of autophagy. From previously established serum cytokine and chemokine profiling, it determined an increase in pro-inflammatory cytokines CCL2, CXCL1, and CXCL10 in conditional whole-body Atg7-deficient mice (Atg7Δ/Δ) in comparison to wild-type (Atg7+/+) mice. We propose to generate two knockout models, CCL2 -/-, and CXCL10 -/-, each in the background of an autophagy deficient mouse to investigate if we can rescue tumor growth. To do this we will implant YUMM 1.3 melanoma and carcinogen induced MB49 urothelial carcinoma cancer cell lines as flank tumors. Additionally, to investigate the role of these cytokines in lung cancer we will generate two whole-body knockout model of CCL2 -/- and CXCL10 -/- in lung cancer. With these specific aims, we will investigate the mechanism for the phenotype of autophagy deficient mice contributing to neuro-degenerative defects in behavior and motors skill, shorten life span, and liver damage

Presenter: Noah Mac

Poster #2: Development of an in vitro system to measure thrombin-mediated protease-activated receptor-1 signaling in hepatic stellate cells

Authors: Mac N., McClenaghan C., Poole L.

Clinical and experimental evidence suggests that activation of the blood clotting cascade plays a pathologic role in hepatic fibrosis, i.e., scarring of the liver. One hypothesis connecting blood coagulation proteases to liver fibrosis focuses on thrombin-mediated activation of protease-activated receptor-1 (PAR-1) on hepatic stellate

cells (HSCs), the primary collagen-producing cells in the fibrotic liver. In fact, studies suggest that thrombin treatment of cultured HSCs increases expression of pro-fibrotic markers in these cells. However, the precise mechanisms linking PAR-1 activation to induction of pro-fibrotic mediators in HSCs remain unclear. The aim of this study is to develop an in vitro system to measure PAR-1-dependent downstream signaling in human HSCs. To determine the impact of thrombin treatment on expression of pro-fibrotic mediators, LX-2 cells were cultured for 48h then treated with thrombin (1 U/mL) or control (serum-free media). Thrombin treatment resulted in a concentration-dependent increase in fluorescence compared to control, achieving saturation at 1 U/mL. Pre-treatment with the PAR-1 antagonist vorapaxar (0.01-10 10 μM) caused complete suppression of thrombin-mediated calcium mobilization. Surprisingly, thrombin treatment for 24h had no significant effect on expression of pro-fibrotic or pro-inflammatory markers in HSCs. This study indicates that although LX-2 cells mobilize calcium in response to thrombin in a PAR-1 dependent manner, thrombin has no impact on induction of pro-fibrotic or pro-inflammatory mediators after 24h. In conclusion, this in vitro system affords the opportunity to elucidate the precise mechanisms linking thrombin-mediated PAR-1 activation to the induction of pro-fibrotic markers and demonstrates the ability to unveil novel thrombin-mediated pro-fibrotic pathways.

Presenter: Jay Joshi

Poster #3: mTORC1 activity oscillates throughout the cell cycle promoting mitotic entry and differentially influencing autophagy

Authors: Joshi J., Lerner A., Scallo F., Grumet A., Valvezan A.

Mechanistic Target of Rapamycin Complex 1 (mTORC1) is a master metabolic regulator that integrates nutrient and growth factor signals to promote anabolic cell growth and proliferation. mTORC1 dysregulation contributes to the development and progression of many diseases, including most human cancers. mTORC1 is activated in most, if not all, proliferating eukaryotic cells, but whether mTORC1 is regulated in a cell cycle-phase specific manner is unknown. We hypothesized that mTORC1 activity changes throughout the cell cycle to meet the unique metabolic and biosynthetic demands of each cell cycle phase. Using multiple independent methods to track mTORC1 activity throughout a complete cell cycle, we find that mTORC1 activity oscillates from lowest in mitosis/G1 to highest in S and G2. These changes correlate with the induction of Cyclin E and Cyclin A in S and G2 respectively, suggesting Cyclin E/Cyclin A could promote mTORC1 activation during this time. The interphase oscillation in mTORC1 activity is mediated through changes in the subcellular localization of an essential negative regulator of mTORC1, the TSC Complex. By contrast, mTORC1 activity is suppressed during mitosis in both control and TSC Complex-deficient cells, in a CDK1-dependent manner. We demonstrate that mTORC1 also promotes progression through S and G2 and into mitosis by satisfying the Wee1-/Chk1- dependent G2/M checkpoint. We also find that low mTORC1 activity in G1 sensitizes cells to autophagy induction. Taken together, our data uncover cell cycle-dependent oscillations in mTORC1 activity and suggest that mTORC1 has previously unappreciated phase-specific functions in promoting cell growth and proliferation.

Presenter: Prateeksha Rout

Poster #4: Gene regulation mediating alternative stem cell fate switching during intestinal regeneration

Authors: Rout, P., Verzi, M.

Under normal conditions, the small intestine undergoes cell turnover every 3-5 days. When injury or damage is inflicted upon the intestinal epithelium, a subset of rare, highly enriched regenerative genes are activated, and facilitate repair and regeneration. Distinct from genes involved in homeostatic turnover, we see that this injury induced change in gene expression leads to unique intestinal organoid and tissue morphology. Furthermore, the promoter-enhancer looping and 3D chromatin contacts that take place during regeneration are poorly

understood. By stimulating a wound healing state in intestinal tissue through irradiation (IR) exposure, we observe the emergence of key regenerative gene signatures, and visualize these changes using qRT-PCR, RNA-seq and ATAC-seq. An increase in regenerative genes such as Clusterin, Annexin 1, 5 and 8, Ctgf and Ly6a was observed across in vivo and ex vivo models of the intestinal epithelium. We aim to streamline mouse and organoid models to better capture regenerative phases of growth post injury, and expand on how these regenerative models can be harnessed for underlying disease conditions in the intestine as a whole. In future studies, we plan to include Hi-C experiments to further elucidate chromatin looping with higher resolution. New knowledge of gene regulatory mechanisms driving the regenerative stem cell fate switch will point us to pathways with which to manipulate and improve regeneration of the intestine following injury from chemotherapy, radiation damage, and inflammatory bowel diseases.

Presenter: Maddy Terry

Poster #5: Investigating the roles of kinases and phosphatases in meiotic biorientation and spindle assembly

Authors: Terry, M. McKim, K

During female meiotic cell division, the interactions between the kinetochores and the spindle are responsible for the correct segregation of chromosomes, independent of centrosomes. Correct genome partition relies on the chromosomes and their kinetochores establishing proper end-on attachments connected to microtubule fibers stemming from opposite spindle pole ends. The capture of spindle microtubules to kinetochores is error prone, which can lead to incorrect segregation of chromosomes. Unequal distribution of chromosomes can ultimately lead to spontaneous abortions, birth defects, and infertility. We are interested in determining the mechanisms for how kinases Aurora B, Aurora A, and Mps1 regulate error correction and end-on attachments to ensure the accurate biorientation of chromosomes during meiosis. Kinetochore subcomplex NDC80, recruited by SPC105R, is associated with the conversion of lateral to end-on attachments and is a target of Aurora B kinase during error correction. Aurora B, Mps1 and Aurora A localize to the central spindle, kinetochores and spindle poles respectively. Protein phosphatase 2A (PP2A) antagonizes the kinases and is necessary for NDC80 to establish stable end-on interactions with microtubules. How the kinases and PP2A interact to regulate biorientation, is not known. To better understand the relationship between the kinases and the shifting of attachments from lateral to end-on, we intend to confirm NDC80's ability to regulate end-on attachments through phosphomimetic and phospho-null mutants. To determine the mechanisms behind the kinases and their roles in spindle assembly, we intend to manipulate their locations by targeting the various kinases to either the spindle poles, central spindles, or the chromosomes, as well as generate knockdowns of spindle assembly regulators. We will examine these phenotypes through confocal microscopy. We predict that NDC80 regulates end-on attachments and the kinases in three different locations are responsible for mediating correct microtube-kinetochore attachments.

Presenter: Andrew J Boreland

Poster #6: Dysregulated neuroimmune interactions and sustained type I interferon signaling after human immunodeficiency virus type 1 infection of human iPSC derived microglia and cerebral organoids

Authors: Boreland, AJ; Stillitano, AC; Hsin-ching, L; Abbo, Y; Hart, RP; Jiang, P; Pang, ZP; Rabson, AB

Human immunodeficiency virus type-1 (HIV-1) associated neurocognitive disorder (HAND) affects up to half of people living with HIV-1 and causes long term neurological consequences, including dementia. There are no effective therapeutics for HAND because the pathophysiology of HIV-1 induced glial and neuronal functional deficits in humans remains enigmatic. To bridge this knowledge gap, we established a model simulating HIV-1 infection in the central nervous system using human induced pluripotent stem cell (iPSC) derived microglia

combined with sliced neocortical organoids. Upon incubation with two replication-competent macrophage-tropic HIV-1 strains (JRFL and YU2), we observed that microglia not only became productively infected but also exhibited inflammatory activation. RNA sequencing revealed a significant and sustained activation of type I interferon signaling pathways. Incorporating microglia into sliced neocortical organoids extended the effects of aberrant type I interferon signaling in a human neural context. Collectively, our results illuminate the role of persistent type I interferon signaling in HIV-1 infected microglial in a human neural model, suggesting its potential significance in the pathogenesis of HAND..

Presenter: Sree Varshini Murali

Poster #7: CX3CR1 Fate Mapping Reveals Heterogeneity in Cochlear Macrophages and Blood Circulating CCR2-Expressing Infiltrated Macrophages Promote Neuron Survival After Acoustic Trauma

Authors: Murali S., Stothert A., Pereyra E., Manickam V., Batalkina L., Cardona A., Kaur T.

Cochlear trauma activates resident macrophages (RM) and allows infiltration of blood circulating monocytes and monocyte-derived macrophages (Mo-M). The chemokine fractalkine receptor (CX3CR1), expressed on both RM and Mo-M, influences macrophage density and promotes neuron survival in the injured cochlea. However, it is unknown if CX3CR1-RM and CX3CR1-Mo-M differentially promote neuron survival after cochlear injury. We used tamoxifen inducible CX3CR1YFP−CreER/YFP−CreER mouse crossed with Rosa-lsl-tdTomato reporter line wherein CX3CR1-RM and CX3CR1-Mo-M are differentially fluorescent labeled to define their origin, spatiotemporal distribution, morphology, fate, states, and function. Also, to determine the precise role of CX3CR1-Mo-M in neuron survival, CCR2 knockout mice were used. Mice were exposed for 2 hours at 112 dB SPL noise level at 8-16 kHz and subjected to hearing function assessment followed by euthanasia and tissue collection. After acoustic trauma, the distinctly labeled but morphologically similar RM (YFP+RFP+) and Mo-M (YFP+RFP-) were observed in the spiral ganglion neurons, lateral wall, and organ of Corti, whereas sham exposed mice showed only RM. Both RM and Mo-M were Ki67 positive, suggesting both subtypes contributed to the increased macrophage numbers in the noise-damaged cochlea. CX3CR1-Mo-M expressed CCR2 receptor, absence of which was associated with increased loss of sensory hair cells and neurons after acoustic trauma. Fibrinogen probing showed its presence inside the cochlea after acoustic trauma, suggesting a leaky vasculature. The data suggest that macrophages are heterogeneous, and CX3CR1-Mo-M may contribute to the survival of neurons in the noise-injured cochlea.

Presenter: Jingyun Qiu

Poster #8: Identifying CHD4 Enrichment at Enhancers During Inner Ear Stem Cell Differentiation

Authors: Qiu, J; Kim, J; Martinez, E; Ni, J; Kwan, K

Spiral ganglion neurons (SGNs) are essential for hearing. Since these neurons do not regenerate, SGN death contributes to hearing loss. Stem cell replacement is a potential strategy to replace lost neurons. Stem cells or fate-restricted progenitors can be guided to differentiate into specific cell types to replace lost SGNs. Understanding the molecular mechanisms that govern SGN differentiation will advance regenerative therapies. Using the immortalized multipotent otic progenitor (iMOP) cell line as a model system, we identified the chromodomain helicase DNA-binding protein 4 (CHD4) as a candidate that drives SGN differentiation. Knockdown of Chd4 affects neuronal differentiation of iMOPs. I hypothesize that CHD4 regulates cell fate decisions by functioning at regulatory elements such as enhancers to control gene expression. Epigenetic alterations at enhancers modulate the different gene expression patterns and help specify cell identity. However, CHD4 function at enhancers during the neuronal differentiation is unknown. To identify CHD4-enhancer regulatory networks, Cleavage Under Targets & Tagmentation (CUT&Tag) was used to identify

the genome-wide binding of different proteins on the chromatin. NEUROD1 CUT&Tag from iMOP-derived neurons was used to define neuronal promoters and enhancers. Comparing the deposition of primed (H3K4me1) or active (H3K27ac) histone marks in undifferentiated iMOPs to iMOP-derived neurons revealed that neuronal enhancers reside in different states during neuronal differentiation. CHD4 is enriched at both primed and active neuronal enhancers and may facilitate epigenetic changes at the enhancer networks during neuronal differentiation. These results suggest that CHD4 may function at enhancers to drive the neuronal differentiation of iMOPs.

Presenter: Gustavo Rios-Delgado

Poster #9: Small RNA IsrR represses TCA cycle during iron limitation in Staphylococcus aureus

Authors: Rios-Delgado, Gustavo; Norambuena Javiera; Boyd, Jeff

Host tissues sequester iron to protect against infections in a process known as nutritional immunity. In response, pathogenic bacteria alter gene expression to adapt to iron starvation. This change in gene expression is mediated, in part, by the ferric uptake regulator (Fur), which acts as an iron-dependent transcriptional repressor. Under iron starvation, Fur repression is alleviated, allowing for increased expression of iron uptake genes. In addition, in its apo-form, Fur also decreases the expression of iron-requiring processes such as the tricarboxylic acid cycle (TCA) resulting in a growth defect. Similar regulation has been described in Escherichia coli, which uses the small RNA RyhB to mediate mRNA degradation of non-essential genes encoding iron-utilizing enzymes. S. aureus does not have a RyhB. We hypothesized that Fur conducts positive regulation of TCA cycle in S. aureus using a small non-coding RNA. Using a suppressor screen, we selected strains with a second site mutation that suppressed the growth defect of a Δfur mutant and had increased TCA cycle activity. Whole-genome sequencing of the suppressors revealed that all selected strains contained a mutation in the sRNA Tsr25 (renamed IsrR). We created a ΔisrR mutant and determined that it could suppress the varied phenotypes of the Δfur mutant. We also genetically complemented the ΔisrR mutants, showing that the altered function of isrR mutant alleles functioned to conduct regulation in the Δfur mutant. Results support that IsrR is the staphylococcal iron sparing regulator and represses TCA cycle to affect intracellular iron homeostasis.

Presenter: Lingjun Lu

Poster #10: Dietary Timing- and Protein Quality-Responsive Liver Circadian Clock and its Function on Ribosome Biogenesis

Authors: Lu, L., Li, Y., Levy, J.L., Anthony, T.G., Androulakis, I.P.

Circadian rhythms are critical in regulating various physiological processes and maintaining homeostasis. Disruption of circadian rhythms has been linked to diverse pathological conditions including metabolic disorders and neurological diseases. Independent of the central clock, peripheral clocks, especially in the liver, can be strongly entrained by metabolic inputs. The entrained circadian clocks integrate signal sensing and stress response pathways to maintain downstream metabolic activities. Both the timing (feeding/fasting cycle) and composition or quality (nutrient variation) of metabolic inputs contribute to clock function and dysfunction. However, the nutrient entrainment and its regulation on the circadian metabolism remain to be fully elucidated.

Essential amino acids (EAAs), as vital nutrients not endogenously synthesized in mammals, are fundamental to the regulation of metabolic events such as protein synthesis. Its dietary imbalances or deprivation can trigger the integrated stress response (ISR), specifically, GCN2-eIF2α-ATF4 pathway, regulating the

translation-regulatory processes, such as indirectly suppressing ribosome biogenesis (RiBi). In contrast, EAA abundance is sensed through the mTORC1 pathway, which is intertwined with the GCN2 pathway and positively regulates RiBi. Either of these EAA signaling pathways interacts with the core clock network and affects its intrinsic properties, along with which the clock genes and proteins also directly control RiBi. In this study, we propose a semi-mechanistic mathematical model to describe the dietary timing- and EAA availability-responsive liver circadian clock and downstream ribosomal protein (RPs) synthesis. Our in silico experimental results will shed light on the impact of dietary EAA manipulations or EAA sensory imbalances on hepatic clock synchronization, and the regulation principles of dietary cues and EAA-responsive clock on RPs production, under normal and disrupted circadian conditions. This can enhance the understanding of the dynamics of peripheral circadian rhythms and their links to metabolism, laying the foundation for studying the mechanisms driving multi-entrainer entrainment and designing effective personalized nutrition recommendations to alleviate chronic diseases associated with circadian disruption."

Presenter: Seanna Kelly

Poster #11: The Effects of Traumatic Brain Injury (TBI) on Sleep Regulation in Drosophila

Authors: Kelly, S and Barber, A

"Traumatic brain injury (TBI) is a multifaceted condition characterized by disruption of brain function due to brain injury caused by external forces. Among the common complaints reported by TBI patients, sleep disorders are the most prevalent, persisting for months to years following injury and can exasperate other pathologies following TBI. Sleep is critical for human health, and sleep and circadian rhythm disorders (SCRDs) are identified as a contributing factor in mood disorders, memory issues, headaches, and metabolic dysregulation, all of which are also common symptoms after TBI. Advances in medicine have increased survival after primary injury, but currently there are no pharmacological options to treat secondary injury pathologies present in the TBI survivor population. Using our Drosophila TBI model (dTBI2), we show that injured flies have a reduction in sleep and increased mortality. Using the Drosophila activity monitor (DAM) assay, we found that both mild (1.9V) and severe (2.4V) injuries produced significant increase in mortality compared to wildtype controls. Additionally, post-TBI flies had increased sleep fragmentation at night, causing a reduction in total nighttime sleep. Our results demonstrate that varying TBI severities using the dTBI2 model elicits conserved sleep dysregulation flies, making it a favorable model for behavioral genetics approaches. We anticipate to screen immune and stress response genes to further uncover the molecular mechanisms driving secondary injury sleep pathologies associated with TBI."

Presenter: Alexandra Logerfo

Poster #12: Intestinal knockout of BMP pathway transcription factors in mice disrupts nutrient absorption and alters transcriptional zonation of villus enterocytes

Authors: Logerfo, A; Iqbal, J; Kandalgaonkar, S; Verzi, M

Diseases of the gastrointestinal system are complex and poorly understood. Uncovering the molecular mechanisms by which transcription factors establish and maintain the homeostatic balance of proliferative stem cells and functional villus cells in the intestine is necessary to understand and treat these diseases. Recently, Bone Morphogenetic Protein (BMP) pathway signaling has been implicated in driving a gradient of functional cell types for intestinal villus enterocytes, the absorptive cells in the intestine. Improper differentiation of enterocytes can lead to metabolic dysfunction, malabsorption, and compromised barrier integrity. Despite the demonstrated importance of BMP signaling in appropriate differentiation, the specific

molecular mechanisms are not understood. Mothers against decapentaplegic homolog (SMAD) family proteins are transcription factors involved in the canonical transduction of signaling cascades for the BMP pathway to regulate and promote development of most tissues in the body. The roles of SMAD1 and SMAD5, the BMP pathway specific R-SMADs, have not yet been studied in the mammalian intestine. Our results from observational and metabolic studies, RNA-sequencing, and histology demonstrate that simultaneous loss of Smad1 and Smad5 expression in the murine intestinal epithelium leads to weight loss, intestinal lengthening, and nutrient malabsorption. Genomic profiling via CUT&RUN of SMAD1 binding in villus cells suggests a transcriptional hierarchy in which SMAD1 regulates the expression of secondary transcription factors to induce expression of nutrient absorption and processing genes. Additional replicates and analyses will provide further evidence toward this mechanism. In parallel, the redundancy of SMAD proteins in the small intestine will be investigated with additional mouse models.

Presenter: Trishna Das

Poster #13: Fasting and Caloric Restriction Suppresses Inflammation to Mediate Cancer-Associated Cachexia

Authors: Das,T, Gomez, M, White, E.

Cancer-associated cachexia is a multifactorial disabling syndrome that is characterized by the progressive depletion of lean mass, adipose tissue, and skeletal muscle mass. It is highly prevalent in lung cancer, with an estimated 60% of lung patients having cachexia. It significantly contributes to the morbidity and mortality of patients, causing weight loss, metabolic imbalances, and chronic inflammation, thereby compromising patients' quality of life, treatment tolerance, and overall survival rates. Pro-inflammatory cytokines such as IL-6 exhibit significant upregulation, identified as a contributing factor to the process of muscle wasting, as elucidated in previous research endeavours. Hence, nutritional interventions such as fasting and caloric restriction (CR) have shown promise in limiting the activities of pro-inflammatory cytokines, improving cancer-associated complications, potentially mitigating malignancy risks, and enhancing lifespan and health span.

In this study, we are comparing the metabolic profile of fasted Trp53flox/flox (P) mice to cachectic KrasG12D/+;trp53−/− (KP) NSCLC GEMM, crucial for understanding the differences in their key metabolic parameters. Additionally, we hypothesize that implementing a 30% CR regimen in KP mice will impact tumorigenesis and extend their lifespan. By employing Metabolomic Cages, Echo-MRI, and grip-strength assessments, we intend to monitor their energy expenditure, alterations in lean and fat mass, and grip strength, facilitating real-time evaluation of cachexia progression.

Prior investigations have highlighted the compromised autophagic activity in skeletal muscles as a contributor to wasting. Additionally, our study seeks to explore whether caloric restriction can modulate autophagy within the skeletal muscles of KP mice, potentially mitigating muscle wasting.

Presenter: Nicholas Pontillo

Poster #14: Dietary Choices Shorten Lifespan in a 5-HT2-Dependent Manner in Drosophila

Authors: Pontillo N, Lyu Y

Decades of research have interrogated the relationship between diet and longevity, but in both mammalian and invertebrate model organisms, diets are typically provided as holistic mixtures of nutrients without the “real-world” requirement for and ability of animals to choose what they eat. Dietary choice - the separation of nutrients into separate but equally accessible wells rather than the provision of a single, nutritionally complete food mixture - shortens lifespan in the male fruit fly Drosophila melanogaster while inducing widespread metabolomic changes. Dietary choice joins a growing list of other perceptive or psychological factors - including

hunger, sex pheromones, and the sight of dead conspecifics - that modulate aging in Drosophila. Remarkably, shortened lifespan from dietary choice appears to be independent of the chosen quantity or ratio of nutrients consumed, and is dependent on neuronal expression of 5-HT2 receptors. This suggests that the dynamic of choice itself, beyond merely choosing to consume a poor diet, modulates the activity of 5-HT2 neurons in a manner harmful to Drosophila health. However, the mechanism by which dietary choice accelerates aging and the neuronal circuit responsible remain unknown. Preliminary evidence suggests that the activity of a small number of 5-HT2A-expressing neurons in the Drosophila central nervous system may be required to mediate shortening of lifespan by dietary choice. Rigorously identifying these neurons will be the first step to understanding the neural circuit and physiological mechanisms that translate choice into accelerated aging.

Presenter#15: Juliana Choza

Poster: Alterations of the DNA modification, 5-hydroxymethylcytosine, in Parkinson's disease

Authors: Joseph Kochmanski, Juliana I. Choza, Mahek S. Virani, Nathan C. Kuhn, Marie Adams, and Alison I. Bernstein

Evidence for epigenetic regulation, particularly for DNA modifications, in Parkinson's disease (PD) is growing. The large majority of PD cases are due to a complex interaction between aging, genetics, and environmental factors and epigenetic mechanisms are thought to act as important mediators of the interactions between these risk factors. There are a small number of published epigenome-wide association studies in PD, but none explore the role of 5-hydroxymethylcytosine (5-hmC). All previous studies have utilized bisulfite (BS)-based methods, which do not differentiate between 5-m methylation (5-methylcytosine) and 5-hmC. Because, the highest levels of 5-hmC are in the brain and 5-hmC is thought to be particularly sensitive to environmental exposures, it is important to explore a potential role of 5-hmC in PD. A modification of BS conversion called oxidative BS (oxBS) conversion specifically measures 5-mC. When paired with BS data, levels of 5-hmC can be estimated. Here, we performed oxBS conversion and paired this data with our previously published BS-based EWAS in an enriched neuronal population from PD post-mortem parietal cortex. By pairing these two datasets, we have identified PD-associated changes in both 5-mC and 5-hmC.

Presenter #16: Maria Søgaard

Poster: T cell receptor profiling of blood improves lung cancer diagnosis

Authors: Søgaard, M. T., Tseng, D., Fitzgibbon, M., Gibbs, S., Nolan, L. G., Yang, P. Y., Lai, M., Sather, C., Steadele, M., Wu, W., Pipavath, S., Kinahan, P., Houghton, M., Payne, K. K., Chiou, S.H. & Nair, V. S.

Lung cancer is the leading cause of cancer-related deaths in North America and worldwide. Despite advances in treatment over the past decade, the 5-year relative survival remains low due, in part, to late diagnosis. In this work, we investigated the use of T cell receptor (TCR) sequencing for early diagnosis of lung cancer. To do so, we sequenced the TCR chain of the leukocyte fraction of peripheral blood from 633 subjects and derived The Immune Lymphocyte Score (TILS), a novel measure of the number of T cell clones associated with lung cancer in each subject. Integrated with currently known clinical variables of lung cancer risk, TILS significantly improves current methods of lung cancer detection as well as risk prediction of patients with solitary pulmonary nodule findings in CT scans. In addition, human leukocyte antigen (HLA) restriction of TILS further improves prediction in HLA-matched patients. Thus, profiling of the TCR repertoire in peripheral blood integrated with currently known clinical variables of lung cancer risk could be instrumental for the early detection of lung cancer.

Presenter: Gregory Marshall

Poster #17: HBV integrations into KMT2B Drive Hepatocellular Carcinoma

Authors: Marshall, Gregory; Cao, Jian

Liver Cancer is the second-leading cause of cancer related deaths worldwide. Of all liver cancers, hepatocellular carcinoma (HCC) comprises 80% of all cases. Of these HCC cases hepatitis B virus (HBV) infection is known to be the cause of about half of all the cases, making HBV-induced HCC a necessary field of research. Our lab has previously performed genomic analysis on HBV positive HCC samples, and recurrent HBV integrations were found to localize in two major loci, TERT and KMT2B (MLL4), where in the case of KMT2B all integrations are localized between exon 3 and 6. Though TERT is a well-established oncogene, the oncogenic function of HBV integration into KMT2B remains largely unknown. Our preliminary data suggests that these HBV integrations into KMT2B result in a C-terminal truncated version of KMT2B (KMT2B-T) and that KMT2B-T is oncogenic in vivo. Furthermore, our data also shows that KMT2B-T binds to tumor suppressor MENIN (Men1) and its binding partner LEDGF. The overexpression of KMT2B-T decreases the binding of MENIN to the endogenous KMT2A/B histone methyltransferase complex. Our data also shows that the differential length of truncated KMT2B influences tumorigenesis. Therefore, we hypothesize that C-terminal truncated KMT2B produced by HBV integrations between exons 3 and 6 does induces HCC by sequestering MENIN from the endogenous KMT2A/B complex. These studies provide the basis for targeting dysregulated KMT2B as a potential therapeutic approach in HBV caused liver cancer.

Presenter: Xia Qiu

Poster #18: Epithelial cellular responses to Serrated colon tumor initiation

Authors: X Qiu, M Verzi

Serrated colon tumors account for up to 20% of colon tumors, have a poor prognosis relative to conventional adenomas. A “sawtooth” pattern of epithelium infoldings in histopathology characterizes these tumors, and molecularly, Serrated colon tumors are characterized by the presence of BrafV600E mutation. However, the BrafV600E mutation inefficiently drives tumor formation on its own in mouse models, no tumor observed in 16 months after tamoxifen injection, herein we called Serrated tumor resistant condition. To effectively induce the development of serrated colon carcinomas, susceptibilities must be in place. Our lab found that the loss of the transcription factors Smad4 in epithelium, can create susceptible condition for Serrated oncogenesis, large tumors in intestine can be observed as soon as 2 months. In the susceptible condition, it seems that the number of stem cells remains relatively unchanged. Mitogen-activated protein kinase (MAPK) cascades are important pathway that orchestrate intestine development, homeostasis and cellular fate. Serrated colorectal cancers are hypothesized to be initiated by Braf V600E mutation and subsequent activation of MAPK/ERK pathway. There is a gap in understanding of the cellular origins responsible for initiating serrated carcinomas, and their regulatory mechanisms downstream of ERK activation (phosphate ERK1/2). I seek to elucidate the cellular dynamics following activation of BRAF-V600E in conditions of susceptibility to serrated tumors. Mechanistically, I also aim to understand the transcriptional regulatory mechanisms of downstream events by BRAF-V600E mutation during Serrated oncogenesis.

Presenter: Nusrath Yusuf

Poster #19: The role of Tex15 in stochastic olfactory receptor choice

Authors: Yusuf, N, Kahiapo, J, Brann, D, Irvine, A, Veera, P, Ruzina, I, Ravi, M, Datta, B, Monahan, K

The main olfactory epithelium (MOE) can detect a vast diversity of smells with incredible specificity through the

function of olfactory sensory neurons (OSN). Each OSN expresses olfactory receptor (OR) genes, which encode the proteins that bind chemical odorants. A mature OSN stochastically transcribes only one allele of an OR gene. It can choose to express any one of the approximately 1400 OR genes or 2800 alleles. This diversity of ORs expressed is important to the MOE's ability to detect a vast range of smells with precise specificity. The choice of an OR gene occurs as OSN progenitors mature into an OSN. Recent work has revealed remarkable remodeling of the local chromatin and co-expression of multiple OR genes during this period, but the molecular mechanisms governing these processes and their connection to a singular OR allele expression in a mature OSN remain unknown. Tex15 is transiently expressed during the critical gene regulation window in OSN progenitors. Tex15 protein has only been studied in the testes where it regulates methylation and silencing of transposons. We show that Tex15 is crucial for stochastic OR gene choice, where when knocked out there is a dramatic reduction in the diversity of expressed OR genes with a few class II OR genes dominating stochastic choice. These class II OR genes are the first to be transcriptionally activated in wild-type OSN progenitors. We hypothesize that Tex15 is regulating monoallelic olfactory receptor gene choice through either methylation patterns or through its effect on chromatin state of developing olfactory sensory neurons. We elucidate a novel aspect of how OSNs come to stochastically choose a single OR and how Tex15 gene and protein guides this specific yet diverse choice.

Afternoon Session

Presenter: Rachel Ofer

Poster #1: Urea cycle functional significance in regenerating intestinal epithelial cells

Authors: Ofer, Rachel

Under homeostatic conditions the urea cycle (UC) maintains cellular nitrogen balance by removing toxic ammonia. Evidence suggests UC contributes to intestinal stem cell proliferation and growth by regulating signaling pathways that are precursors for protein and polyamine biosynthesis and has protective effects on intestinal permeability. Compared to homeostatic proliferation, regenerating IEC occurs in response to injury, and is characterized by hyperproliferation, with significant increase in need for biomass production and energy, suggesting regenerating IEC undergo massive metabolic shifts to support these changes. IEC regeneration is a process that is poorly understood. Furthermore, the role of UC in IEC regeneration has not been well explored. Analysis of a single-cell RNA sequencing dataset of regenerating cells compared to cells under homeostatic conditions revealed significant alterations to the UC transcriptional expression. Mice were treated with irradiation (IR) to induce a regenerative response, and the actively proliferating cells were sequenced. The alterations observed suggest arginine accumulation, increase in nitric oxide production and upregulation in polyamine biosynthesis, implying that regenerating IECs undergo a massive metabolic shift in the UC to facilitate rapid growth associated with tissue repair, including significantly reducing urea synthesis, and instead utilizing nitrogen for cellular activities that promote growth. To further these findings, I will use mouse genetic models and metabolomics approaches in organoids cultures to test the role of UC during intestinal regeneration. By understanding the roles of UC enzymes and their modifications in response to acute injury we can enhance development of regenerative and radiation therapies.

Presenter: Byron Avihai

Poster #2: Longitudinal Single-Cell Genomic and Transcriptomic Analysis of Relapsed Pediatric AML

Authors: Avihai, B; Singh, A; Khiabanian, H; Herranz, D.

Background and hypotheses: Little is known about the mutations driving therapeutic resistance in pediatric AML, and diagnostic/therapeutic options for relapsing patients (with dismal survival) are lacking. We hypothesize that relapsed pediatric AML is associated with distinct genomic signatures detectable at the single-cell level at diagnosis and which correlate with changes in transcriptional profiles.

Methods: Using novel bioinformatics pipelines, we integrated single-cell DNA (MissionBio) and RNA (10x Genomics) data from 78 samples from 40 patients collected at diagnosis, remission, and/or relapse (including 9 trios), and identified genomic/transcriptomic features associated with relapse.

Preliminary results: Genomic data from one case revealed strong associations between a pathogenic KRAS mutation (KRASG13D) and leukemic subclones. The main diagnosis leukemic clone was KRAS heterozygous and was eclipsed at relapse by a homozygous KRAS clone. This suggests that either the homozygous KRAS mutation conferred resistance to chemotherapy or that resistance-conferring mutations arose in a homozygous KRAS subclone. In another case, transcriptomic data revealed an expression profile of a minor diagnosis subclone (0.9% cells) that mapped to an expanded relapse population (20.3%). Associated marker genes were enriched for erythrocytic cells and bone marrow pathways, indicating that relapsed cells correspond to a prominent leukemic clone originating from a minor diagnosis leukemic subclone.

Conclusions: We will next integrate such clonal (DNA) and biological pathway (RNA) findings for each patient and across patients to highlight the underlying mechanisms of therapy resistance in relapsing pediatric AML.

Acknowledgments: New Jersey Commission of Cancer Research (NJCCR) grant (COCR23PRG006).

Presenter: Mani Subramanian

Poster #3: Identifying Chronic Tourette Disorder subtypes using diagnostic data

Authors: Subramanian, Krishnamurthy; King, Robert; TIC Genetics; Tischfield, Jay A.; Heiman, Gary A.; and Xing, Jinchuan

Tourette's disorder (TD) is a heterogeneous, childhood-onset neurodevelopmental disorder. TD is characterized by the presence of motor and/or vocal tics and it affects 1-3% of the population. About 88% of the patients have other neurodevelopmental disorder comorbidities, suggesting shared genetic risk factors for these disorders. Because of the elevated level of heterogeneity and comorbidities, we hypothesize that distinct subtypes exist among TD patients. Here we identified TD subtypes and evaluated their discriminatory factors among patients in the Tourette International Collaborative Genetics (TIC Genetics) study. Using K-Means and Bayesian Hierarchical Clustering (BHC), we analyzed the TIC Genetics diagnostic data (16 variables) for 1,127 TD patients. K-Means identified six distinct clusters. BHC identified nine clusters, four of which corresponded to K-Means clusters. Of the remaining five clusters, three mapped on to one K-Means cluster and two mapped on to the final K-Means cluster. Four large clusters were common between the 2 methods. 1. Males with ADHD but not OCD (N=112); 2. Females with OCD but not ADHD (N=369); 3. patients with self-described White race, OCD, ADHD, and higher prevalence of Autism (N=233); 4. Patients with lower prevalence of other NDD comorbidities, such as OCD, ADHD and Autism (N=285); 5. Patients with Trichotillomania (N=46), which were split into two groups with higher and lower prevalence of OCD in BHC; and 6. Patients with self-described Asian race (N=82), which were split into 3 groups in BHC – with OCD, with ADHD but not OCD, and males with lower prevalence of ADHD, OCD, and Autism. In summary, our results show that distinct clusters can be identified among TD patients based on diagnosis data. In the future, we will conduct stratified analysis of genetic data (e.g., microarray and whole exome sequencing) based on these subtypes to determine their genetic etiology.

Presenter: Deimante Mikalauskaite

Poster #4: Transcriptional co-repressor Atrophin regulates Hippo pathway target genes in Drosophila

Authors: Deimante Mikalauskaite, Cordelia Rauskolb, PhD, Tom Lehan, Srividya Venkatramanan, PhD, Kenneth Irvine, PhD

The Hippo signaling pathway controls expression of target genes through its downstream effector, the transcriptional co-activator Yorkie (Yki). With our expanding knowledge of Hippo pathway activity, other components regulating expression of Yki target genes still need to be identified. Previous studies suggest that the transcriptional co-repressor Atrophin could regulate expression of the Yki target gene four- jointed.

Using gene knock down and overexpression approaches we investigated whether Atrophin contributes to Hippo signaling in the wing imaginal disc, and what molecular mechanisms underlie this regulation. We found that Atrophin regulates multiple Hippo pathway target genes. Interestingly, our results show that Atrophin represses the expression of Yki target genes in the distal wing and activates them in the proximal wing. When we investigated Atrophin's effect on Yki, we found that Atrophin knock down resulted in decreased Yki nuclear levels throughout the wing, while overexpression had an opposite effect, suggesting that Atrophin controls Yki nuclear localization. This result implies that Atrophin regulates Hippo pathway activity. We further identified that depletion of Atrophin leads to changes in component levels of Fat- Dachsous (Ft-Ds) signaling, which controls Hippo pathway activity.

We are now investigating how Atrophin regulates the levels of the Ft-Ds factors. In addition, we plan to identify direct transcriptional targets of Atrophin. These studies will help us understand how Atrophin exerts its regulatory function on the components of the Ft-Ds network and explain its downstream effects on Yki target gene expression.

Presenter: Nathalie Groot

Poster #5: Characterization of Calcium Signals and Oscillations in Dendritic Cells in vivo in Response to Bacteria

Authors: Groot, N., Barbet, G.

Dendritic cells (DCs) are disseminated throughout organisms and serve as sentinels, organizing immune responses to infection, tissue damage, and cancer. DCs play a crucial role bridging innate and adaptive immune systems and elicit tailored adaptive immune responses to threats they encounter. Manipulation of DCs is critical for vaccination, and DC-based immunotherapies have been tested in clinical trials as anti-cancer therapeutics and for treatment of infectious diseases. Calcium, a ubiquitous secondary messenger, regulates and modulates critical effector functions of DCs like phagocytosis, maturation, migration, and cytokine production. However, calcium regulation and mobilization in DCs remains poorly understood. To decipher calcium signals induced by bacterial infection in DCs intravitally, we generated CD11c-Salsa6f reporter mice that express the fluorescent protein (FP) construct tdTomato fused to GCaMP6f in CD11c expressing cells. Expression of TdTomato, a red FP, highlights the cell shape, and GCaMP6f, which fluoresces green upon calcium binding, visualizes intracellular calcium levels. We are using CD11c-Salsa6f mice to observe calcium signals in DCs in the periphery and in secondary lymphoid organs (SLOs), for example the gut and the spleen, respectively. Using two-photon microscopy to image the spleen, our preliminary experiments unveiled oscillatory calcium signals in DCs in response to whole bacteria with distinct calcium dynamics specific to the species of bacteria. This discovery led us to use our experimental system to investigate and compare calcium dynamics in DCs elicited by bacterial exposure at mucosal sites where DCs are continuously exposed commensals to DCs in the sterile environment of a SLO.

Presenter: Siwen Wu

Poster #6: HP1 Interacts with the Chromosomal Passenger Complex to Promote Spindle Assembly and Chromosome Segregation in Meiosis

Authors: Siwen Wu, Ryan Doherty, Manisha Persaud, Keara Greer, Janet Jang, Kim McKim

Chromosome segregation fidelity during female meiosis is critical for maintaining genome integrity, with aberrations causing infertility, miscarriages, and severe congenital anomalies. At the heart of this process, the chromosomal passenger complex (CPC), comprising the inner centromere protein (INCENP), Borealin, Survivin, and Aurora B kinase, functions as a central regulator of spindle assembly and ensures accurate bi-oriental chromosome segregation during meiotic cell division. In Drosophila oocytes spindle assembly, the CPC is required for recruitment of microtubules to the chromosomes, and regulating kinetochore-microtubule attachments during meiotic metaphase I. However, the mechanism of CPC recruitment to the chromosomes and movement to the spindle microtubules remain elusive. INCENP, the scaffolding protein, provides the platform for CPC assembly. We previously proposed that HP1 recruits the CPC to the chromosomes to initiate acentrosomal spindle assembly. To test this hypothesis, we developed HP1 RNAi reagents and generated Incenp mutants with a deletion of HP1 binding sites. Our results revealed that HP1 is required for spindle assembly and recruitment of the CPC to the chromosomes. Furthermore, an HP1-INCENP interaction may not be essential to initiate spindle assembly but is critical for chromosome biorientation. We further observed no spindle assembly, and a failure to recruit the CPC to the chromosomes, in oocytes with a deletion of the microtubule- and HP1-binding domains, suggesting these two domains' activities are additive for recruitment to the chromosomes. Overall, our study demonstrates that INCENP-HP1 interaction is essential for CPC chromosomal recruitment and spindle formation in female meiosis. We are currently performing experiments to determine which domains and CPC subunits mediate movement of the CPC from the chromosomes to the microtubules.

Presenter #7: Robert P. Madramootoo

Poster: Focused Mutagenesis of DNA Replication Factors Involved in Heterochromatin Inheritance

Authors: Madramootoo,R.P.

The goal of my work is to create a focused mutagenic screen to identify separation of function alleles for essential factors involved in DNA replication, to determine their role in heterochromatin silencing at the centromere. Centromeres contain satellite repeat DNA and play a key role in kinetochore assembly. If heterochromatin is not maintained at this locus post DNA replication, then homologous recombination between non-homologous chromosomes can occur, leading to chromosomal breaks and aneuploidy. These chromosomal abnormalities often lead to disease and many forms of cancer, so understanding the mechanisms and pathways which govern them can lead to the discovery of potential drug targets. We have identified a set of DNA replication factors whose role in heterochromatin silencing is largely unknown. But many of them are essential factors making them difficult to study. The allele swap technique that I am developing we hypothesize will allow us to identify which residues and/or domains of these essential factors are involved in heterochromatin silencing without affecting viability (separation of function). The Pop-in,Pop-out (PIPO) allele swap technique allows for the study of many base pair changes in a targeted high throughput manner. Which has advantages over current targeted mutagenic approaches such as CRISPR, because it is not limited to PAM sites or does not have off target affects.

Presenter #8: Leelabati Biswas

Poster: Maternal gene mutations in kinesin motor domains influence early embryonic aneuploidy risk

Authors: Biswas, L.; Tyc, K.; Aboelenain, M.; Sun, S.; Sosich, I.; Vukušić, K.; Liu, J.; Guo, V.; Xu, M.; Scott, Jr., R.; Tao, X.; Tolić, I.; Xing, J.; Schindler, K.

The female reproductive lifespan is highly dependent on egg aneuploidy, the presence of an abnormal number of chromosomes in an egg. However, knowledge of the precise genetic landscape that causes egg aneuploidy in women is limited, in part because phenotypic data on the frequency of human egg aneuploidy is difficult to obtain and, as a result, unrepresented in large, public genetic datasets. Here, we identify novel genetic determinants of reproductive aging via egg aneuploidy in women using a new biobank of individual maternal exomes linked with maternal age and embryonic aneuploidy data. We first prioritized 404 genes bearing variants enriched in individuals with pathological rates of egg aneuploidy. Within this group, we identify a functional network and specifically implicate variants in genes encoding the kinesin protein family as causal in egg aneuploidy via deep experimental perturbation. Finally, we demonstrate that a specific genetic variant in the kinesin KIF18A accelerates reproductive aging and diminishes fertility using a novel knock-in mouse model. These findings reveal new functional mechanisms of reproductive aging, how genetic variation underlies individual heterogeneity in the female reproductive lifespan, and how genetic variation might be leveraged to predict reproductive longevity.

Presenter #9: Hanna Caiola

Poster: Loss of Par1b/MARK2 results in increased neuronal excitability and dysregulation of transcriptional programs associated with ion homeostasis and synaptic transmission

Authors: Caiola H, Wu Q, Monahan K, Margolis D, Zhang H.

Neurodegenerative disorders are a major cause of death and disability in the United States and are increasing in prevalence at an alarming rate. Par1/MARK serine threonine kinases have been genetically linked to

neurodegenerative diseases such as Alzheimer's disease (AD). In addition, we and others have found that human AD patients have decreased levels of Par1b in the medial temporal lobe. Furthermore, we found that loss of Par1b in mice results in AD-related phenotypes such as memory impairments, age-dependent cortical and hippocampal degeneration, and synaptic dysfunction. Par1b is known to function upstream of several transcriptional regulators implicated in AD. Moreover, our RNAseq analysis in Par1b knockouts suggests that programs associated with synaptic transmission and ion homeostasis are highly dysregulated. This is interesting given that dysregulation of ion channels is highly associated with seizures, which has been proposed as an early biomarker of AD. Indeed we found that heterozygous knockout of Par1b in mice results in increased seizure susceptibility, suggesting underlying neuronal hyperexcitability. Indeed, preliminary evidence from in vitro calcium imaging in primary neurons supports the hypothesis that loss of Par1b results in increased neuronal excitability. Current efforts aim to use in vivo two-photon calcium imaging to determine the effect of Par1b knockout on neuronal excitability in vivo. Together, these data will help us understand how Par1b contributes to the regulation of neuronal excitability, which could give insight into potential mechanisms for Par1b in neurodegeneration and AD.

Presenter: Nuo Jia

Poster #10: The role of mitochondrial defects in tauopathy-linked autophagy dysfunction

Authors: Nuo Jia, Yu Young Jeong, Hongyuan Guan, Gavesh Rajapaksha, and Qian Cai*

Hyperphosphorylation and aggregation of microtubule-associated tau is a pathogenic hallmark of tauopathies and a defining feature of Alzheimer's disease (AD). Pathological tau is targeted by autophagy for clearance, but autophagy dysfunction is indicated in tauopathy. We have recently documented that Mitochondrial Rho GTPase (Miro) deficiency leads to mitochondrial deficits in early tauopathy. In the current study, we sought to address whether such early defects contribute to tauopathy-linked autophagy failure and thus tau buildup. Phosphatidylethanolamine (PE) is a major component of the autophagosome membrane, and the supply of PE constitutes a limiting factor for autophagy activity. Our pilot studies have shown that increasing Miro levels in tauopathy neurons enhances autophagosome biogenesis by restoring impaired PE supply from mitochondria, leading to attenuation of tau pathology in tauopathy mouse brains. We will next define this novel role of Miro in mitochondrial PE biosynthesis. Our study highlights the involvement of mitochondrial defects in tauopathy-associated autophagy dysfunction and may suggest a new therapeutic strategy for combating AD and other tauopathies.

Presenter: Katelyn VanderSleen

Poster #11: Lipidation modulates the inclusions formed through disruption of the α-synuclein tetramer

Authors: VanderSleen, K.; Kimelman, R.; Eubanks, E.; Patel, N.; Archakam, S.; Kara, E.

The neuropathological feature of Parkinson's disease is Lewy bodies, which are round inclusions of α-synuclein within neurons in the brain. While the exact pathogenetic mechanisms of Parkinson's disease are unknown, recent evidence implicates lipid dysregulation. The aim of this project was to assess the role of lipidation in the formation of α-synuclein inclusions. As a model system, we utilized a neuroblastoma M17D cell line expressing a triple mutant α-synuclein tagged with Venus YFP under a TET-ON inducible system which is unable to form tetramers. This mutant α-synuclein contains E to K amino acid substitutions in three of the six KTKEGV repeat motifs. Oleic acid was used to increase neutral lipid content in those cells. Lipid droplets were stained using LipidTox deep red neutral lipid stain (Thermofisher) and nuclei were stained with Hoechst. Images were acquired on a Zeiss LSM780 confocal microscope and analyzed in Python. We found that oleic acid treatment induces entrapment of neutral lipid droplets within α-synuclein inclusions, making them adopt a "swiss

cheese-like" appearance, in a dose-dependent manner. Knocking down several genes that we previously showed through a high-throughput screen to regulate α-synuclein propagation, modified the association between α-synuclein and neutral lipids. Our findings support a role of lipid dysregulation in the pathogenesis of Parkinson's disease.

Presenter: Reem Alatrash

Poster #12: Adaptive Immune Responses against La Crosse Virus Infection

Authors: Alatrash, R, Herrera, B

La Crosse virus (LACV) poses a significant threat of viral encephalitis, particularly affecting children in the United States. Infections are predominantly observed in individuals under 16 years of age, suggesting a heightened vulnerability among younger demographics. Studies using weanling mice (3 weeks), which exhibit heightened susceptibility to LACV-related illnesses, parallel this age-related susceptibility, contrasting with the resistance observed in adult mice (6 weeks). Presently, no authorized vaccines or treatments exist for LACV infections. The protective mechanisms that shield adult mice from LACV infection remain incompletely elucidated. While several hypotheses could help explain this phenomenon, a robust adaptive immune response is a possible explanation. Our experiments show that adult mice mount stronger and more polyfunctional CD4+ and CD8+ T cell responses against LACV structural and non-structural proteins as compared to weanling mice as early as 6 days post-infection. Furthermore, the protective potential of antibodies was explored through the transfer of serum from previously infected adult mice to weanlings. Notably, when weanling mice received serum antibodies verified to neutralize LACV in vitro 1 day prior or 1-3 days post-infection, they exhibited enhanced survival following exposure to a highly lethal dose. Our findings suggest that virus-specific T cells and antibodies play a pivotal role in the protection against LACV infection. Our results have potential to aid in the development of effective vaccines and/or therapeutics against LACV infection.

Presenter: Rebecca Shear

Poster #13: Trafficking of AMPA receptor subunits in Par1c/MARK1 knockout mice

Authors: Shear, R., Kelly-Castro, E.C., Sun, M., Runnels, L. and Zhang, H.

Dendritic spines are sites of postsynaptic excitatory input that are critical for learning and memory. Abnormalities in dendritic spine dynamics and morphology have been linked to neurodevelopmental disorders such as autism spectrum disorder (ASD). Our laboratory has recently generated a forebrain-specific conditional knockout (cKO) of partitioning defective 1 c (Par1c), also known as microtubule affinity regulating kinase 1 (MARK1), which is a serine/threonine kinase linked to ASD and bipolar disorder. Importantly, genetic evidence supports that Par1c functions in higher level cognition. Furthermore, MARK1 is highly expressed in forebrain pyramidal neurons and exhibits human-specific accelerated evolution, suggesting its importance in the development of cognition. We found that Par1c cKO mice show dendritic spine and behavioral abnormalities in vivo. To examine how loss of Par1c affects the postsynaptic receptors and scaffolding proteins, we performed Western blot analysis of synaptosomes. Interestingly, we found a significant increase in synaptic levels of the AMPA receptor subunit GluR2. To probe for the underlying mechanisms, we performed phosphoproteomic analysis of WT and Par1c cKO hippocampi. We discovered a significant decrease in the phosphorylation of S843 of RalGAPα1 in Par1c cKO hippocampi, which is a site that matches the Par1 phosphorylation consensus sequence. Indeed, in vitro kinase assay data show that Par1c phosphorylates RalGAPα1 on S843. Interestingly, we found that expression of a nonphosphorylatable (S843A) RalGAPα1 significantly increases surface GluR2 in primary hippocampal neurons and N2a cells. Thus, our current experiments are aimed at determining whether

RalGAPα1 regulates GluR2 trafficking downstream of Par1.

Presenter #14: Jaeyong Jung

Poster: Commensal bacteria are required for chronic inflammation and B lymphoma development in myeloid cell-specific TRAF3-deficient mice

Authors: Jaeyong Jung, Sining Zhu, Almin I. Lalani, Judith Shakarchi, Guojun Wu, Wei-Xing Zong, Liping Zhao, and Ping Xie

Myeloid cells are the major players of innate immunity and inflammation. The functionality of myeloid cells is controlled by innate immune receptor signaling, which is critically regulated by a cytoplasmic adaptor protein and tumor suppressor termed TRAF3. We previously reported that specific ablation of TRAF3 from myeloid cells leads to spontaneous chronic inflammation and B lymphoma in aging mice. In the present study, we sought to identify the internal trigger of chronic inflammation and aberrant B cell activation in myeloid cell-specific TRAF3-deficient (M-Traf3-/-) mice. We first noticed that M-Traf3-/- mice exhibited altered diversity and composition of gut bacteria and transmigration to the liver. To determine if gut dysbiosis and bacterial transmigration induce chronic inflammation and aberrant B cell activation, we treated mice with broad-spectrum antibiotics. We found such depletion prevented both chronic inflammation and B lymphoma development in aging M-Traf3-/- mice. Interestingly, sequencing of Igh gene revealed that many malignant B cell clones of M-Traf3-/- mice had CSR and contained SHM, indicating the germinal-center origins. Furthermore, the CDR3 sequences of malignant B cell clones from M-Traf3-/- mice showed high homology to prevalent bacteria-reactive Ig clonotypes, suggesting they are reactive against commensal bacteria. Indeed, aging M-Traf3-/- mice with spontaneous B lymphomas contained high titers of antibodies against commensal bacteria. Our findings suggest that commensal bacteria dysbiosis and transmigration can promote chronic inflammation and B lymphoma development in aberrant immune landscapes. Accordingly, inhibition of commensal bacteria may serve an effective therapeutic strategy for prevention and treatment of chronic inflammation/B lymphoma in patients.

Presenter: Kuan-Ying Lee

Poster #15: LGP2 prevents RIG-I promiscuous binding and signaling in an RNA-dependent and RNA-independent way

Authors: Lee, Kuan-Ying Craig, Candice Patel, S Smita

LGP2, a cytoplasmic RNA sensor within the RIG-I helicase family, is crucial in initiating antiviral immune responses by selectively detecting foreign RNAs. Despite lacking the immune activation signaling domain, LGP2 is a critical regulator in the RIG-I/MDA5-triggered antiviral signaling pathway. In this study, we unveil a novel physiological role of LGP2 in RIG-I-mediated IFN-β signaling. Our research demonstrates that LGP2, in the presence of ATP, competitively sequesters most self-RNA substrates from RIG-I, except for blunt-ended RNAs possessing a 5' triphosphate, which are recognized as non-self-mimicking viral RNA. Further analysis reveals that, unlike LGP2, ATP enhances RIG-I's affinity to RNA substrates, promoting helicase engagement, RNA binding, and multimer formation on non-self-mimicking RNA substrates. Thus, LGP2 and ATP jointly prevent RIG-I from associating promiscuously with RNA in an RNA-dependent manner. The reporter cell assay study of the impaired RNA binding capability of LGP2 demonstrates that LGP2 also precisely promotes RIG-I signaling, regardless of its RNA association ability. Our findings highlight the novel function of LGP2 in preventing RIG-I from engaging in promiscuous binding and signaling, involving both RNA-dependent and RNA-independent pathways. This discovery holds potential significance for the design of antiviral drugs and the development of immune therapies.

Presenter: Jiayu Shen

Poster #16: human mitochondria transcription initiation

Authors: Shen JY, Ajjugal Y, Goovaerts Q, Patel S, Das, K"Mitochondria are semi-autonomous organelles of the eukaryotic cells, possessing their own genome and the ability for independent replication and transcription. The human mitochondrial genome is double-stranded and circular, with both strands coding for x mRNAs, y tRNAs, and z rRNAs. These RNAs are transcribed by a distinct nuclear-encoded RNA polymerase, POLRMT, which shares similarities with the single-subunit bacteriophage T7 RNA polymerase. While T7 RNAP functions independently in transcription, POLRMT relies on mitochondrial transcription factor B2 (TFB2M) and mitochondrial transcription factor A (TFAM) for transcription initiation. Due to the lack of active and resolved transcription bubble structures, the mechanism by which POLRMT, TFAM, and TFB2M melt and promoter and initiate transcription remains unclear.

We have combined biochemistry and mutagenesis with single-particle cryo-EM to determine the mechanism of transcription initiation by the human mt RNAP. By mutating each base pair in the promoter sequence near the transcription start site (TSS), we identify promoter-specific interactions critical for promoter melting, de novo RNA synthesis, and slippage synthesis. The cryo-EM structures of IC3 and IC4 reveal a functional and fully resolved transcription bubble and RNA: DNA hybrids. The IC3 reveals the structure of a transcription slipped complex, and IC4 defines the +1 TSS and the upstream edge of the bubble at -4, five nucleotides preceding the TSS. Notably, our structure-function studies identify a unique loop (156 to 164 AA) in TFB2M (B2-loop) conserved in animals that engage the -1 A base of the non-template strand. The interactions are critical for catalyzing transcription bubble opening, stabilization, and TSS selection and are unique from the yeast homolog of TFB2M.

Presenter: Elmira Kirichenko

Poster #17: Investigating Jub-dependent phosphorylation and phase separation of Warts

Authors: Elmira Kirichenko, Dr. Kenneth Irvine

The Hippo signaling pathway is one of the major growth-regulating pathways, dysregulation of which results in cancer. The central events of this signaling pathway converge to regulate the activity of the transcription co-activator Yorkie with the help of tumor suppressor kinases Hippo and Warts. The status of Warts activity is one of the key steps regulating cell proliferation. While the activation of Warts has been linked to phosphorylation events induced by its association with proteins like Expanded, Hippo, and Mats, the regulatory mechanism involving its inhibitor Jub remains poorly understood. In this study, we have identified that Jub-dependent phosphorylation of Warts controls the growth of the Drosophila wing, and hence cell proliferation. We have found that it is the N-terminus of Warts that undergoes a robust Jub-dependent phosphorylation, and intriguingly also phase separation in association with Jub. Since Jub is only an adaptor protein and cannot phosphorylate Warts directly, we propose that Jub recruits a kinase. Investigation of the identified phosphorylation sites lead us to hypothesize that the kinase responsible for Jub-dependent Warts phosphorylation is from the Proline-directed family of kinases. We are conducting experiments to identify this kinase. The findings acquired from this study expand our understanding of the Hippo signaling pathway and the mechanism of Warts regulation by Jub.

Presenter: Mayra Romero

Poster #18: Unraveling the protective role of SIRT7 in the aging oocyte

Authors: Romero, M ; Schindler, K

A hallmark of reproductive aging is the decline of oocyte quality and quantity. Many age-associated molecular changes, like regulation of epigenetic marks, negatively affect the quality of the oocyte and its developmental competency. Sirtuins, a family of anti-aging proteins, play key roles in maintaining genome integrity. SIRT7, a member of the sirtuin family, holds particular significance in the context of female reproductive aging. Previous studies on Sirt7 knockout mice demonstrated premature age-related infertility, linked to reduced oocyte quantity and increased poor-quality oocyte production. We hypothesized that SIRT7's deacetylase activity protects oocyte quality during aging. To test this, we analyzed 6-month-old WT and Sirt7-/- mice for markers of oocyte quality decline. Sirt7-/- mice exhibited higher percentages of aneuploid Metaphase II eggs, abnormal spindle morphology, and increased DNA damage compared to WT, indicating compromised genomic integrity. We focused on SIRT7's role in histone H3 lysine 36 (H3K36) deacetylation during oocyte maturation. Sirt7-/- oocytes retained H3K36 acetylation at Metaphase I, whereas WT oocytes deacetylated this residue. In WT mice, H3K36 acetylation persists with age, suggesting a link between H3K36 deacetylation and oocyte quality. Further investigations will clarify the significance of SIRT7-dependent H3K36 deacetylation in preserving oocyte integrity during reproductive aging.