Northwestern Medicine®

CMBD

CELLULAR AND MOLECULAR BASIS OF DISEASE TRAINING PROGRAM

2023 ANNUAL SYMPOSIUM

TUESDAY, JUNE 20, 2023

9AM-5PM

Northwestern University - Simpson Querrey Auditorium

303 E Superior St., Chicago

EPIGENETICS IN DEVELOPMENT AND DISEASE

TODAY'S SCHEDULE

EPIGENETICS IN DEVELOPMENT AND DISEASE

9AM	**Introductory Remarks**
9:15AM	**Dr. Feng Yue** Northwestern Medicine "Epigenetic and 3D genome alteration in Cancer "
10:05AM	**Coffee Break** Ryan Atrium
10:25AM	**Dr. Bing Yao** Emory University School of Medicine "TDP-43-mediated R-loops and 5hmC crosstalk contributes to neurodegenerative disorders "
11:15AM	**Dr. Hanna Salman** University of Pittsburgh "Quantitative measurement of epigenetic memory in bacteria through its restraint of cell-to-cell variation "
12:00PM	**Lunch and Poster Session** Ryan Atrium
2:05PM	**Dr. Danesh Moazed** Harvard Medical School "How genes and transposons are heritably silenced"
2:55PM	**Dr. Megan King** Yale School of Medicine "Nuclear mechanotransduction as a driver of cell fate and phenotype "
3:40PM	**Closing Remarks**
3:55PM-5:00PM	**Networking Reception** Ryan Atrium

Dr. Megan King

YALE SCHOOL OF MEDICINE

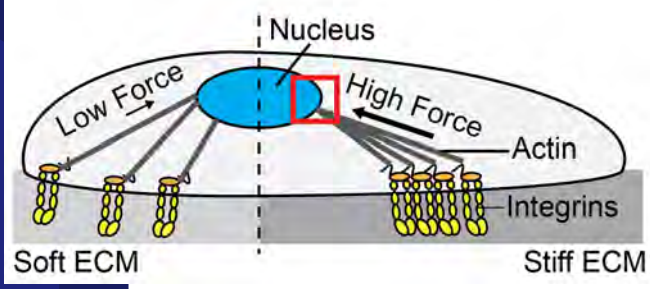
Associate Professor of Cell Biology and of Molecular, Cellular and Development Biology

Megan received her B.A. in Biochemistry from Brandeis University working with Dr. Susan Lowey and her Ph.D. in Biochemistry and Molecular Biophysics from the University of Pennsylvania working with Dr. Mark Lemmon. During her postdoctoral training with Dr. Günter Blobel at Rockefeller University, she discovered new mechanisms for the targeting and function of integral inner nuclear membrane proteins. Since founding her own group in 2009, Megan has continued to investigate the broad array of biological functions that are integrated at the nuclear envelope, from impacts on DNA repair to nuclear and cellular mechanics. Megan was named a Searle Scholar in 2011, is a recipient of the NIH New Innovator Award and is currently an Allen Distinguished Investigator.

Nuclear mechanotransduction as a driver of cell fate and phenotype

Over recent decades, numerous exquisite molecular mechanisms by which chemical and biochemical signals control cell fate have been delineated. In contrast, our understanding of how mechanical inputs impact cell fate decisions are more poorly understood. Our team has employed several model systems to define whether and how direct force transmission to the nucleus impacts cell fate, including keratinocyte differentiation in the skin and fibroblasts during the response to tissue injury. Using a molecular biosensor, we find that tension on the nucleus through Linker of Nucleoskeleton and Cytoskeleton (LINC) complexes is high when integrins are engaged. In undifferentiated epidermal stem cells, this mechanical signal promotes maintenance of the progenitor fate; release of integrin engagement as cells populate the suprabasal layers of the skin prompts epidermal differentiation. Consistent with this model, LINC complexes are required to repress epidermal differentiation in vivo in mice. More broadly, we observe that LINC complexes contribute to the force sensing mechanism by which cell substrate properties influence extracellular matrix (ECM) deposition, which manifests through an uncoupling of cytoskeletal contractility and the expression of ECM genes. As a consequence, ablation of LINC complexes promotes tissue repair without the induction of fibrosis. Taken as a whole, our work reveals a direct mechanotransduction pathway capable of relaying adhesion-specific signals to regulate cell fate.

LusKing Lab



Dr. Danesh Moazed

HARVARD MEDICAL SCHOOL

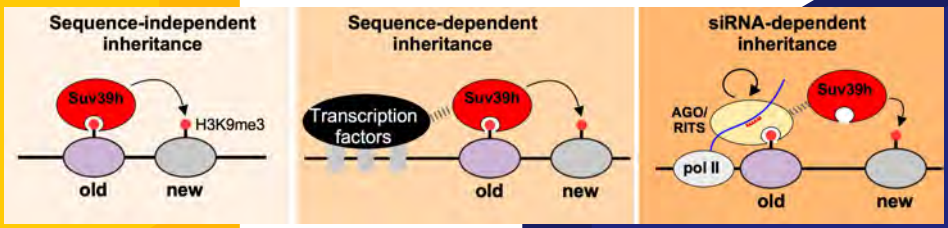
Professor and Howard Hughes Medical Institute Investigator in the Department of Cell Biology

Danesh is a member of the Harvard Biophysics Program and the Harvard Initiative for RNA Medicine (HIRM). He received his undergraduate and Ph.D. degrees from the University of California in Santa Cruz and performed postdoctoral studies at the University of California in San Francisco.

The Moazed lab studies how genes are silenced and how silencing is epigenetically inherited across generations. The lab's interests revolve around diverse pathways of heterochromatin-mediated gene silencing in yeast and mammalian cells. Work in budding yeast focuses on the structure and function of a diverged and relatively simple form of heterochromatin, which requires only three Silent information regulator ("Sir") proteins that form a histone deacetylase and chromatin-binding complex. Work in fission yeast focuses on a conserved example of heterochromatin that requires the nuclear RNA interference (RNAi) machinery, other RNA processing pathways, Heterochromatin protein 1 (HP1) homologs, and histone-modifying enzymes. In mammalian cells, the work is focused on HP1-mediated and other heterochromatin formation pathways. The lab uses approaches ranging from genetics and genomics, biochemical purification and reconstitution, and structural biology for their studies. Ultimately, the lab seeks to understand the conserved fundamental principles that govern the assembly, function, and epigenetic propagation of heterochromatin.

How genes and transposons are heritably silenced

The formation of heterochromatin and its faithful propagation during development and adult life play fundamental roles in silencing of transposons and lineage-specific cell identity genes. We have shown that heterochromatic histone H3K9 methylation and silencing can be epigenetically inherited during multiple cell divisions independent of DNA sequence. However, DNA sequence-independent epigenetic inheritance is intrinsically unstable and requires input from specific DNA sequences, or small RNAs associated with nuclear RNAi, to be stably propagated. I will discuss our recent findings on how specificity factors collaborate with histone methylation to stabilize epigenetic states.



Moazed Lab

Dr. Hanna Salman

UNIVERSITY OF PITTSBURGH

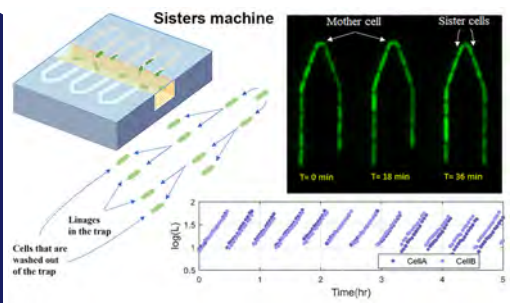
Associate Professor, Department of Physics & Astronomy

Hanna's research aims to understand the mechanisms of collective behavior and variability in bacterial cultures and their effect on the response of bacteria to changes in the environment. The continuous interaction between the environment and living organisms is one of the main effectors of evolution. There are many known strategies of responding to environmental changes, e.g. by changing the swimming pattern or the gene expression profile. And although many strategies are single-cell based, we often see cooperative behavior arising among members of the colony under certain conditions. By studying the changes in the behavior of bacteria as a function of their concentration, I am able to detect some of the collective mechanisms that govern the bacterial behavior and allow them to better endure environmental stress. Environmental changes that interest me are temperature and chemical. I utilize various optical microscopy techniques to observe the swimming pattern of bacteria under different conditions. As for the expression level of proteins, proteins of interest are labeled with fluorescent markers and the expression level is measured using fluorescence microscopy or flow cytometry.

Quantitative measurement of epigenetic memory in bacteria through its restraint of cell-to-cell variation

Epigenetic inheritance plays a fundamental role in determining cellular properties in future generations and in restraining the proliferation of non-genetic cell-to-cell variation over time. It is, therefore, important to be able to measure it and quantitatively characterize it reliably. In this talk I will present our newly developed method that allows measuring and characterizing epigenetic inheritance (or cellular memory) in the simple bacterial model organism E. coli. The method utilizes a novel microfluidic device, coined "sisters machine", that enables us to track and measure how two sister cells become different from each other over time. Our measurements reveal how epigenetic inheritance contributes to regulating the various cellular properties (e.g. size, growth rate, etc.) in future generations. We find that epigenetic cellular memory is property specific, and can last up to ~10 generations, but decreases under stress. The results obtained from this study can help uncover mechanisms of non-genetic inheritance and adaptation to stress, which can contribute to developing future treatments to bacterial infections.

Salman Lab



Dr. Bing Yao

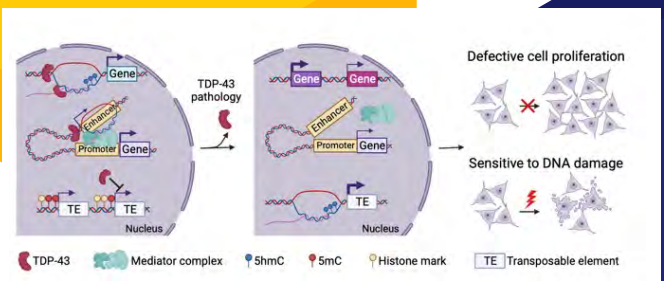
EMORY UNIVERSITY SCHOOL OF MEDICINE

Assistant Professor, Department of Human Genetics

Dr. Yao's research interests are to understand the pivotal roles of epigenetic regulation, especially various forms of covalent DNA and RNA modifications, in normal brain functions, as well as how dysregulation of these processes may contribute to human brain disorders. He received his PhD from University of Florida in Gainesville, Florida. His postdoctoral training was completed at Emory University in Atlanta, Georgia.

TDP-43-mediated R-loops and 5hmC crosstalk contributes to neurodegenerative disorders

TAR DNA-binding protein 43 (TDP-43) is an RNA/DNA-binding protein involved in many aspects of RNA metabolism, but its molecular roles in regulating gene and transposable elements (TEs) transcription is not extensively explored. TDP-43 loss-of-function due to progressive cytoplasmic aggregations serves as a pathological hallmark and potential causality for several neurodegenerative diseases. Recent evidence suggest acute knockdown of TDP-43 affects the formation of R-loops, nuclear DNA:RNA hybrid structures implicated in transcription regulation. However, how stable and chronic functional perturbation of TDP-43, which more closely resembles age-related neurodegeneration, impact global transcriptome via R-loop dysregulation remains unclear. Here we demonstrate that stable and prolonged TDP-43 loss-of-function results in impaired cell proliferation and DNA damage response. At the molecular level, TDP-43 stable knockdown impacts key gene expression through concomitantly altering intragenic R-loop dynamics and the DNA covalent modification 5-hydroxymethylcytosine (5hmC) in cis, as well as long-range R-loop-mediated enhancer-promoter interactions in trans. Furthermore, we find TDP-43 knockdown induces massive disease-related TE activation via influencing R-loop and 5hmC homeostasis in many of these loci. Our results highlight previous underdeveloped transcriptional roles of TDP-43 via R-loops regulation in coding genes, distal regulatory elements and TEs, and suggest that TDP-43 proteinopathies transcriptionally contributes to the etiology of neurodegenerative disorders.



Yao Lab

Dr. Feng Yue

NORTHWESTERN MEDICINE
Professor of Biochemistry and Molecular Genetics and Pathology

Dr. Yue is the founding director of the Center for Cancer Genomics at the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, director of the Center for Advanced Molecular Analysis at Northwestern Institute for Augmented Intelligence in Medicine, the Duane and Susan Burnham Professor of Molecular Medicine, Professor of Biochemistry and Molecular Genetics, and Professor of Pathology. He serves in the editorial board of Genome Research and Science Advances.

The main research area for Dr. Yue's group is to use modern genomic technologies, machine learning, and CRISPR genome editing to identify biomarkers and investigate the impact of genetic variants in human cancers. Dr. Yue has been an active member of several large NIH funded consortia, including the ENCODE, Roadmap/Epigenomics, and 4D Nucleome projects. During the period, he lead the integrative analysis effort for the mouse ENCODE consortium. Currently, he is serving as co-chair for the Integrative Analysis Workgroup in the 4D Nucleome Project. He also Co-chairs the Steering Committee of the NIH Impact of Genomic Variation on Function Consortium (IGVF).

Epigenetic and 3D genome alteration in Cancer

The two-meter-long DNAs need be folded accurately inside the nucleus for proper cell function, and alteration of chromatin folding has been associated with different types of human diseases. In this seminar, I will introduce our latest work in the study of 3D genome organization in cancer. We charted the first genome-wide chromatin interaction landscape in multiple cancer types, including bladder cancer, pediatric brain tumor, and acute myeloid leukemia. We demonstrated that different cancer types and subtypes have unique chromatin loops, connecting distal regulatory elements (such as enhancers and silencers) to essential cancer genes. Furthermore, our group was among the first to demonstrate that structural variants (SVs) can juxtapose enhancers from their original position to another part of the genome and activate the wrong target gene (enhancer-hijacking). Our work suggests that enhancer hijacking is a novel regulation mechanism in human diseases and is prevalent in almost all cancers. Finally, we discovered that the epigenetic drug treatment could change genome folding and reverse the cancer-specific chromatin loops, suggesting that 3D genome could serve as potential drug-targets for personalized therapy.

Yue Lab

Student Poster Presentations

Madison Spratt

Lane Lab

Single-Cell Analysis of Salmonella SPI-2 Induction Reveals Environment-Tunable Heterogeneity

During infection of a host, Salmonella must sense and process a diverse range of environmental cues to elicit virulence responses in contexts where they are required and repress them when not advantageous. One example of this is the activation of Salmonella Pathogenicity Island 2 (SPI-2) upon entry into a host cell. SPI-2 encodes a type III secretion system and secreted effectors required for intracellular proliferation. Numerous signals have been found to induce SPI-2 upon entry into the intracellular environment, including pH, magnesium concentration, and nutrient levels. However, the extent to which individual bacteria are able to sense changes in these signals to induce SPI-2 has not been explored, nor is it clear how homogenous these responses are across a clonal population. To investigate this, we used smFISH and live-cell promoter based reporters to measure SPI-2 transcriptional induction at the single-cell level. We have identified bimodal expression of Salmonella SPI-2 in in vitro conditions, and demonstrate that the timing of this response can be tuned through incremental changes in pH as well as the integration of pH and magnesium signals. We propose a mechanism by which stochastic expression of the master SPI-2 regulator ssrB drives single-cell commitment into a SPI-2 expressing-state through autoregulatory feedback. These results provide insight into the information processing capacity of the SPI-2 regulon and demonstrate a mechanism by which the fraction of cells that induce virulence genes can be probabilistically tuned depending on the context of their environment.

Eliza Duvall

Braun Lab

Network reconstruction via spectral graph sparsification

A systems biology approach considers genes that work together as a collective group to perform a function. These collections of genes and their interactions are often depicted in a biological network constructed of nodes and edges, respectively. Reconstructing networks using RNA-seq data attempts to identify specific gene-gene interactions that were previously verified experimentally. However, this can result in incomplete or missing edges, and inaccuracies of existing edges. This research is devoted to developing a computation approach for biological network reconstruction that reshapes the way inferred networks are interpreted. Rather than drawing conclusions from specific gene-to-gene connectivity, consider the network wholistically by analyzing emergent properties. Emergent properties, such as the flow of information, can only be detected at a network level. Networks will be reconstructed by first deriving a complete network in which every pair of genes has an edge weight equal the correlation of gene expression. Using a spectral graph sparsification method, edges not part of the backbone of the complete graph will be removed. The reconstructed network will be validated by comparing the flow of information between gene pairs with the flow from the complete network. This new method aims to reconstruct networks using transcriptome data and gene sets while adjusting our perspective on how networks are interpreted.

Student Poster Presentations cont'd

Kyle Krueger

Petersen Lab

A CELF/Bruno homolog regulates self-renewal of Acoel pluripotent adult stem cells

Piwi + pluripotent adult stem cells support whole-body regeneration and adult homeostasis in phylogenetically diverse species, for example neoblasts from flatworms and acoels, interstitial cells from cnidarians, and archeocytes from sponges. Such cells are known to rely on post-transcriptional regulatory mechanisms mediated by conserved RNA regulatory factors including Piwi, Vasa, Nanos, and Tudor protein families. However, the conservation of functional requirements for these factors for specific purposes in adult pluripotent stem cells is not yet fully understood. We used RNAi screening to identify factors required for neoblast self-renewal in the 3-banded panther worm, Hofstenia miamia, which diverged from planarians over 500 mya, to try to uncover conserved regulators of neoblast self-renewal. We identified hmi-bruli as a homolog of the RNA regulatory family CELF/Bruno homolog required for Hofstenia neoblast maintenance. CELF/Bruli homologs can regulate translation and alternative splicing, but whether they have specific conserved roles in adult pluripotent stem cells across divergent organisms is not known. hmi-bruli expression is expressed in a neoblast pattern in the animal, and was strongly depleted by lethal doses of ionizing radiation, similar to Piwi homologs. Double-FISH suggests hmi-bruli is expressed in a subpopulation of piwi1 + cells. RNAi of hmi-bruli caused depletion of piwi + cells, failed regeneration, and ultimately death from failed homeostatic tissue maintenance. Given the known role of planarian bruli as a key regulator of neoblast self-renewal in that organism, these results suggest a central and ancient role for CELF-mediated RNA regulation in the self-renewal of adult pluripotent stem cells.

Jesus Ortega

Gottwein Lab

Optimization of Target Directed miRNA Decay-based Inhibitors

microRNAs (miRNAs) are approximately ~22 nucleotide long non-coding RNAs that post-transcriptionally repress gene expression following base pairing to the 3' untranslated region of target mRNAs. Typically, miRNAs pair to target genes through the "seed sequence", i.e. nts 2-7 from the miRNA's 5' end, without extensive 3'-base pairing. Recent structural studies and genome-wide screens showed that following miRNA 3'-base pairing, miRNA induced silencing complexes are polyubiquitinated and degraded in a process known as target directed miRNA decay (TDMD). So far, few eukaryotic, viral, and synthetically designed RNA transcripts have been shown to induce degradation of miRNAs. In this study, we explore how to improve miRNA loss-of-function approaches by retargeting several natural and synthetic miRNA-RNA TDMD pairing architectures to two model miRNAs: miR-K11, which is encoded by Kaposi's sarcoma-associated herpesvirus (KSHV), and miR-122, a liver-specific miRNA associated with hepatocellular carcinoma. Our findings suggest that TDMD pairing architectures based on specific natural pairings induced the most robust miRNA repression compared to prior synthetic transcripts. Leveraging these findings, I am now using this approach to silence viral miRNA expression in primary effusion lymphoma cells, a B-cell lymphoma caused by latent KSHV infection. Our system shows promising applicability in inducing miRNA inhibition in systems where knockdown is required.

Student Poster Presentations cont'd

Annika Schroder

Guemez-Gamboa Lab

Identifying the molecular mechanism of PACS1 Syndrome pathogenesis

PACS1 Syndrome is a NDD hallmarked by craniofacial abnormalities and intellectual disability. Patients with PACS1 Syndrome have a single de novo missense mutation at c.607C>T of the Phosphofurin Acidic Cluster Sorting 1 (PACS1) protein, which causes an Arginine to Tryptophan substitution (R203W). PACS1 is a multifunctional sorting protein, with key roles in regulating trafficking of target proteins to and from the trans-Golgi Network (tGN). We have previously shown that PACS1 (+/R203W) forebrain organoids develop mature glutamatergic neurons with impaired expression of synaptic signaling genes when compared to isogenic controls. Additionally, PACS1 (+/R203W) neurons have prolonged network bursts, which has implications for circuit formation. While these results highlight the impact that the R203W variant of PACS1 may play in the broader context of neuronal development, it remains unknown how the R203W variant alters the function of PACS1 in the cell. Using a combination of biochemistry and live cell imaging, we are now identifying how the R203W variant impacts the function of PACS1. The R203W variant is in the Furin Binding Region (FBR) of PACS1, which is the region responsible for cargo protein interactions dependent upon acidic-cluster motifs. We hypothesize that the R203W variant will impact the trafficking dynamics of PACS1-associated cargo in between the tGN and plasma membrane through its interactions with membrane specific adaptor proteins for localization. Our data indicates there is no difference between the localization of PACS1 WT and R203W. This, however, does not provide any information regarding the dynamics of PACS1-associated trafficking, thus we are currently performing live imaging of PACS1 and its associated cargo. Given the localization of the R203W variant to the FBR, we are also performing BioID to identify how the R203W variant impacts PACS1 interactome, with the hypothesis that multiple binding interactions will be disrupted and/or gained as a consequence of this R203W substitution.

Eve Suva

Mitchell Lab

The Role of Tension in Radial Intercalation of Multicilated Cells

Epithelial tissue encompasses a diverse range of tissue that is classified as a communal sheet of cells that serve as a barrier. It is crucial that this barrier is dynamic to maintain homeostasis, due in part to regulated intercalation, cellular rearrangement, enabling tissue maintenance, repair, and growth. Radial intercalation describes a penetrative process by which a cell can move vertically to enter into epithelial tissue, allowing for distinctive epithelial subtypes. Multiciliated cells (MCCs) are a post-mitotic cell type that radially intercalates to embed into the epithelial tissue. MCCs are found in the epithelial lining of the upper respiratory tract and Fallopian tubes. MCCs have over a hundred hair-like cilia protrusions that coordinately beat to direct fluid flow; pushing mucus out of the respiratory tract and guiding an oocyte down the fallopian tubes. Thus it is of medical interest to understand the radial intercalation of MCCs. As MCCs are known to radially intercalate into the ectoderm during early Xenopus Laevis development, we can use this classical developmental model system to study this process.

Previous studies have shown MCCs can only intercalate at cellular vertices, the connection point between three or more cells. At the start of radial intercalation, the MCC first selects a vertex as a point of entry, then inserts upward pushing the outer cells to open the vertex. Once inserted the MCC expands its apical surface to begin ciliogenesis. Previous studies have examined the tension exerted by the MCC itself as it embeds into the tissue. Here we examine the active vertex selection by an MCC and determine how tension across the epithelium itself aids in the process of radial intercalation.

Student Poster Presentations cont'd

Pranathi Vadlamani

Foltz Lab

Determining the Regulation and Consequences of for Centromere Mislocalization

Faithful chromosome segregation during mitosis is carried out by the recruitment of the constitutive centromere-associated network (CCAN) and the kinetochore via centromeres. Centromeres are unique chromatin domains characterized by a unique nucleosome containing the histone H3 variant CENP-A (Palmer et al. 1987, Earnshaw et al. 2013). While a majority of centromeric DNA is composed of alpha-satellite DNA repeats, naturally occurring neocentromeres can form at genomic loci that lack alpha-satellite DNA repeats (Depinet et al. 1997, Tyler-Smith et al. 1999), indicating that centromeres are epigenetically determined. The epigenetic determinant of centromeres, CENP-A, is recruited by histone chaperone HJURP via the Mis18 complex to centromeres (Barnhart et al. 2011, Dunleavy et al. 2009, Foltz et al. 2009, Fujita et al. 2007). CENP-A is overexpressed in many types of cancer (Sun et al. 2016, Zhang et al. 2016) and upon overexpression, is deposited into chromosome arms by co-opting the H3.3 chaperone DAXX (Lacoste et al. 2014). This disruption of CENP-A deposition leads to weakened native kinetochores resulting in mis-segregation of chromosomes also known as Chromosomal Instability (CIN) (Shrestha et al. 2017). Aneuploidy is a hallmark of aggressive cancer and results from periods of CIN (Giam & Rancati 2015). Moreover, mis-segregated chromosomes lead to micronuclei formation leading to complex rearrangements via chromothripsis, which is frequently found in many types of cancer (Shrestha et al. 2017). Overexpressed CENP-A has also been shown to occlude CTCF binding, suggesting that CENP-A plays a role in chromatin structure and accessibility when mislocalized (Lacoste et al. 2014). As such, understanding the rules of centromere deposition and maintenance and effects on chromatin structure is pertinent for recognizing why misregulation occurs. This work will be important for understanding the mechanisms surrounding misregulation of centromeres which leads to genome instability and cancer.

Claire Chaikin

Peek Lab

Circadian Clock Regulation of Glucose Disposal through a HIF1α-dependent Mechanism during Diet-induced Obesity (DIO)

Circadian rhythms govern animal physiology, metabolism, and behavior and allow organisms to synchronize internal processes with their environment. Indeed, disruptions of circadian rhythms, which are prevalent in modern society, lead to accelerated and worsened symptoms of metabolic syndrome. Studies indicate that core clock factor, BMAL1, is required in skeletal muscle for proper insulin-dependent glucose uptake in healthy lean mice. However, the role of the molecular clock in skeletal muscle glucose metabolism during conditions of metabolic stress, such as diet-induced obesity (DIO), is unclear. Recent work has uncovered a link between the molecular clock and the hypoxia inducible factor (HIF) response pathway, which is known to control the induction of glycolytic metabolism in skeletal muscle under conditions of nutrient stress. Thus, we hypothesized that the molecular clock may mediate glucose utilization during DIO via control of HIF activity. Consistent with this hypothesis, induction of DIO by high-fat diet (HFD) feeding induced HIF1α target gene expression in muscle tissue compared to regular chow (RC) fed controls, indicating enhanced HIF1α activity. Furthermore, muscle-specific BMAL1-deficient mice showed impaired glucose tolerance ($p=0.09$) and reduced levels of HIF1α target genes compared to controls during DIO. However, HIF1α target genes were not reduced in RC fed mice, suggesting that BMAL1 regulates HIF1α target gene expression during HFD but not RC. Finally, we found that muscle-specific loss of VHL, a negative regulator of HIF activity, can restore glucose disposal and utilization in muscle-specific BMAL1-deficient mice during DIO. These data provide evidence of an interaction between the molecular clock and HIF response pathways during DIO. However, what causes HIF1α stabilization in skeletal muscle during obesity and the mechanism linking BMAL1 and HIF1α remains unknown and will be investigated in future studies.

Student Poster Presentations cont'd

Brent Groubert

Brickner Lab

Defining the molecular mechanism of epigenetic inheritance of H3K4me2 during transcriptional memory

Some inducible genes become primed for faster re-activation and this phenomenon can be inherited through mitosis. This phenomenon can persist for 4-15 cell divisions and is termed epigenetic transcriptional memory. INO1 memory in budding yeast is one of the best understood models for epigenetic transcriptional memory. INO1 encodes the inositol-3-phosphate synthase enzyme and is induced when cells are starved for inositol. Upon induction, the INO1 gene repositions to the nuclear periphery where it interacts with the Nuclear Pore Complex (NPC). However, when INO1 is repressed, the gene remains at the nuclear periphery for four cell divisions, poised for transcription. Previous studies have identified mechanistic differences between the molecular requirements for INO1 induction and INO1 memory. INO1 memory specifically requires binding of the transcription factor Sfl to a cis-acting Memory Recruitment Sequence (MRS) element, interaction with the nuclear pore protein Nup100, dimethylation of H3K4 (H3K4me2), and incorporation of the histone variant H2A.Z. These chromatin changes are required for recruitment of poised RNA polymerase II (RNAPII) preinitiation complex. Although Sfl1 is essential for H3K4me2 during memory, once established, H3K4me2 is inherited for four generations in the absence of Sfl1. This suggests that this mark is epigenetically heritable and that it controls the persistence of memory. The SET3C deacetylase complex and the Set1/COMPASS methyltransferase complex are essential for the persistence and inheritance of memory. Because SET3C possesses a PHD finger that is specific for H3K4me2, this suggests that these complexes serve as a reader and a writer of these marks, respectively. Indeed, core subunits of the SET3C and COMPASS complex co-immunoprecipitate (coIP). Using coIP, combined with molecular genetics, my project will test the hypothesis that H3K4me2 is inherited through mitosis via a read-write mechanism between the SET3C reader and the COMPASS writer.

Irena Gushterova

Eichner Lab

Defining the Class IIa HDACs as Transcriptional Regulators in Lung Cancer

The Class IIa Histone Deacetylases (HDAC4, -5, -7, and -9) are direct substrates of the LKB1-SIK signaling pathway. The SIKs directly phosphorylate the Class IIa HDACs to mediate their nuclear exclusion where they either co-activate or inhibit their targets. Our preliminary data confirms that LKB1 status dictates the phosphorylation and subcellular localization of Class IIa HDACs in human lung tumor cells. By genetically deleting the Class IIa HDACs in a GEMM of LKB1-mutant lung cancer, we have shown that the Class IIa HDACs regulate the expression of ~25% of the entire LKB1-dependent transcriptome in lung tumors. We have also identified the Class IIa HDACs as potent regulators of in vivo tumor growth control, H3K27ac acetylation, and global chromatin access. Furthermore, we have bioinformatics evidence that the impact of the Class IIa HDACs on both H3K27ac and chromatin access are related to MEF2 function. Therefore, we hypothesize that Class IIa HDACs are master regulators of LKB1-dependent chromatin access and are therefore a key contributor to LKB1-mutant lung tumor transcriptional identity. The goal of this project is to (1) define how Class IIa HDACs contribute to the LKB1 transcriptional signature and (2) define how MEF2 contributes to the transcriptional program regulated by the LKB1-Class IIa HDAC molecular axis and provide clarity whether MEF2 contributes to LKB-mutant lung tumor identity.

Student Poster Presentations cont'd

Zhuoling He

Bozza Lab

Cell Type Specification and Gene Choice Bias in The Mouse Main Olfactory System

Organismal function relies on the correct elaboration of myriad cell types with distinct identities. Despite the fact that errors in cell types specification underlie many disease states, our understanding of this process is incomplete. The mouse olfactory system encompasses more than 1,000 distinct populations of olfactory sensory neuron (OSN) residing in the olfactory epithelium. Each OSN expresses one odorant receptor (OR) from over 2,000 alleles in a random monoallelic fashion. The singular expressed OR imparts both chemical sensitivity and a unique axon guidance identity to the OSNs, which allows axons from OSNs that express the same OR to project to defined glomeruli in the olfactory bulb of the brain. In addition to this OR dependent mapping, our previous work suggested the presence of distinct OSN cell-type lineages that impart an OR independent identity and also influence OR gene choice. However, the precise mechanism for OR gene choice bias and OSN cell type specification still remain elusive. To uncover the identity of these putative cell types, we are analyzing an extensive single cell RNA sequencing data set. The data reveal for the first time that there are clear developmental lineages in the olfactory epithelium that give rise to our hypothesized cell types. We have identified several lineage specific genes that may play roles in cell type specification and OR gene choice. The upcoming stage of my project will examine their potential functions by genetically manipulating these candidates during early olfactory neurogenesis in mice. My work will shed light on mechanisms of biased random monoallelic expression and cell type specification in the olfactory system that may be broadly applicable to other neuronal and non-neuronal cells.

Austin Klein

Mendillo Lab

Delineating the cellular proteostasis network using a comprehensive chemical-genetic approach

The proteostasis network (PN) comprises molecular chaperones, co-chaperones, and a variety of additional support proteins that are integral for the proper synthesis, folding, stability and degradation of the cellular proteome. Dysfunction of the PN has been implicated in numerous diseases ranging from cancer to Alzheimer's to Parkinson's. As such, targeting components of the proteostasis network (PN) is a strategy for the development of novel treatments of these diseases. Thus, understanding the regulation and function of the PN is critically important in understanding its role in health and disease, and in improving therapeutic strategies. In this regard, we have recently developed FIREWORKS, a computational pipeline to analyze genome scale fitness screening data from a large set of cancer cell lines to identify a network of genes with vital functions spanning diverse stress contexts. While this network contained both known and novel factors with critical roles in proteostasis regulation, it remains incomplete. As an example, our network surprisingly contained only a small subset of chaperones known to play an important role in cellular stress response. This has been attributed to the intricate feedback mechanisms built into the PN. Here, we pursue an integrated approach combining our mammalian chemical-genetic screening platform with our FIREWORKS computational pipeline to identify components, function, and regulatory nodes within the PN. We assembled a comprehensive library of sgRNAs that target 1,500 PN genes including canonical chaperones and co-chaperones as well as upstream regulators and downstream effectors. Immortalized mammalian cells were infected with our sgRNA PN library and individually treated with a diverse collection of compounds that broadly modulate the proteostasis milieu (e.g. proteasome inhibitors, ERAD inhibitors, heat shock protein inhibitors) to identify genes critical for growth in these stressed cells. Our data reveal canonical functions of well-studied components within the PN along with functions of those whose activity were previously unknown. In addition, we define functional relationships between chaperones and their regulators, along with implications for disease-relevant contexts in which their function becomes critical to cell fitness.

Student Poster Presentations cont'd

Jordy Martinez

Wignall Lab

Investigating the role of the Ring Complex in lateral microtubule organization and spindle architecture using C. elegans oocytes

During mitosis, cells rely on microtubule nucleation by centrosomes to stabilize the bipolar spindle and carry out faithful chromosome partitioning. However, in oocyte meiosis centrosomes are degraded prior to the initiation of cell division yet the bipolar spindle still forms in their absence. Interestingly, instead of forming end-on attachments to chromosomes, microtubules run laterally alongside chromosomes in C. elegans oocytes and a ring complex (RC), composed of at least 15 proteins, encircles the middle region of each meiotic chromosome pair (bivalent). The RC contains a plus end-directed microtubule motor, which assists in aligning chromosomes to the middle of the spindle during a process called congression. More recently, our lab found that when the RC formation is entirely prevented, bipolar spindles fail to form. Therefore, in addition to mediating congression, ring proteins potentially have secondary roles that aid proper spindle organization.

We are investigating the role of the RC in organizing and stabilizing lateral microtubule bundles. We used genetic mutants, where chromosomes fail to condense into 6 bivalents and instead form 12 univalents. In one mutant, spo-11, we found that a subset of univalents recruited RCs while the other subset did not. Interestingly, univalents with RCs also had lateral microtubule bundles associated with them suggesting that the RC influences their organization. Additionally, we have used other mutants where oocytes generate 12 univalents with either no RCs or all with RCs to analyze microtubule bundling in these conditions. Additionally, our lab has adapted a cold-stable assay to assess microtubule stability and revealed stabilized microtubule populations in the vicinity of chromosomes. Using this technique, we will expose our mutant oocytes to cold conditions and investigate if univalents with RCs also stabilize lateral microtubule bundles. Lastly, we will further assess how essential the RC is to establish spindle bipolarity by manipulating chromosome pairing in early prophase. These experiments will test our hypothesis that RCs contribute to lateral microtubule bundle organization and the spindle's unique architecture.

Brian Miller

Akhtar Lab

Neonatal HSV-2 Isolates Show Differential Expression of Neurovirulence Factor ICP34.5 Isoforms

Approximately 14,000 newborns are infected with herpes simplex virus (HSV) each year. In stark contrast to adult HSV infections, which are typically limited to recurrent lesions at mucosal surfaces, nearly half of infected newborns experience an invasive infection of the central nervous system. The reasons underlying the extreme variability of neonatal HSV disease outcomes remain poorly understood. Recently, we performed viral whole genome sequencing on a cohort of neonatal HSV-2 isolates which revealed substantial intra- and interhost viral genomic variation. This analysis highlighted variation of interest in the HSV-2 34.5 gene, which encodes the known neurovirulence factor infected cell protein (ICP)34.5. Using Illumina and Nanopore sequencing data, we have identified a sequence pattern in the 34.5 intron that is unique to isolates that cause the most severe neurological disease both in human newborns and following direct intracranial infection of mice. Our preliminary data suggest a correlation between this intron sequence pattern and expression of the truncated "beta" ICP34.5 isoform in infected cell cultures. While several specific functions of HSV-1 ICP34.5 are well defined, comparatively little is known about the HSV-2 homolog aside from its requirement for neurovirulence. Determining how HSV-2 ICP34.5 expression is regulated during infection, the function of each isoform, and the contribution to neurovirulence will provide important insights into how HSV-2 genomic variation may contribute to disease outcomes.

Student Poster Presentations cont'd

Jori Mills

Satchell Lab

Actin Crosslinking Domain of Vibrio cholerae MARTX Toxin is Proinflammatory and Activates Host MAP Kinases

The Gram-negative bacteria Vibrio cholerae causes the watery diarrheal disease cholera and infects millions each year, resulting in tens of thousands of deaths. V. cholera has a repertoire of toxins that promote infection, including the Multifunctional Autoprocessing Repeats in Toxin (MARTX). This large, pore-forming toxin delivers an "effector cassette" to the host cytosol. Within the cell, these effectors are auto-processed by the MARTX cysteine protease domain into individual proteins that remodel host signaling and disrupt cytoskeletal integrity to promote infection. One effector is the Actin crosslinking domain (ACD) which introduces covalent bonds between g-actin monomers causing actin aggregation and cytoskeletal collapse. Our lab previously demonstrated that treating T84 intestinal epithelial cells with V. cholerae expressing a MARTX toxin with ACD as the only effector caused increased phosphorylation of MAP kinases ERK, P38, and JNK and the release of IL-8. However, it was unclear whether ACD was sufficient for this response independent of bacterial factors. To determine if the effector alone is responsible for this signaling, we used an anthrax toxin lethal factor N-terminus/ACD chimera (LFN-ACD) plus protective antigen intoxication. Intoxication of T84 cells revealed similar IL-8 release but only significant increases in phosphorylation for JNK and its downstream target c-JUN. These results support that ACD is sufficient to trigger proinflammatory MAP kinase signaling without a bacterium and that actin crosslinking is a damage-associated molecular pattern.

Brooke Simonton

Gate Lab

Investigating hypothalamic pathology and immune infiltration in Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia and is projected to affect approximately 14 million people in the US by 2060. Pathological hallmarks of AD include extracellular amyloid-β (Aβ) plaques, intracellular neurofibrillary tau tangles (NFTs), and neuroinflammation which ultimately result in brain atrophy due to neuronal death. Clinical symptoms of AD include progressive cognitive impairment, sleep disturbance, and body weight fluctuations. Nearly two-thirds of all AD cases are in females, suggesting sex differences in both prevalence and presentation. Yet, the mechanisms underlying sex disparities in AD remain unknown. The hypothalamus plays a key role in regulating numerous physiological processes that are disrupted in AD, such as sleep-wake cycle, appetite, and sex hormone production. The hypothalamus is also uniquely positioned as a hub for peripheral signals in the brain due to its leaky blood-cerebrospinal fluid-barrier (BCSFB), position in the neuroendocrine axis, and bi-directional neuronal circuits. Recent evidence suggests tanycytes, specialized ependymal cells that comprise the hypothalamic BCSFB, transport tau out of the cerebrospinal fluid (CSF) and may be dysfunctional in AD. However, hypothalamic involvement and mechanisms of immune cell infiltration in this region remain critically understudied in AD. Here, I hypothesize that BCSFB dysfunction in the hypothalamus results in increased peripheral immune infiltration and subsequent neuroinflammation in AD. I propose to test this hypothesis using spatial transcriptomics and single-cell fixed RNA profiling. Interrogating this link between peripheral immune infiltration and hypothalamic pathology will aid understanding of clinical symptoms underlying sex disparities in AD.

Student Poster Presentations cont'd

Yiren Tu

Wang Lab

An optogenetic approach for studying native chromatin accessibility

Physical access of the genetic material is essential for all DNA-dependent cellular processes. The accessibility of chromatin is regulated at multiple levels. Recently, increasing evidence has suggested that chromatin is organized into sub-megabase packing domains that exhibit some characteristics of liquid condensates and modify the molecular crowding environment in the nucleus. This dynamic higher-order organization and the resulting accessibility play key roles in regulation of the gene expression at any given cell state. Traditional methods such as ATAC-seq and MNase-seq measure the accessibility by exposing exogenous molecular probes (e.g. nuclease) to genomic DNA in detergent-permeabilized cells, thus unsuitable for investigating additional layers of regulatory information imposed by higher order chromatin architecture in living cells.

To overcome this technical limitation, our lab has developed a new technical platform that combines optogenetic apparatuses with genomics to quantitatively measure chromatin accessibility in live cells. The technique relies on an engineered MNase that can be rapidly recruited to a modified histone H2B through light induced dimerization to probe the physical accessibility of higher order chromatin in living cells.