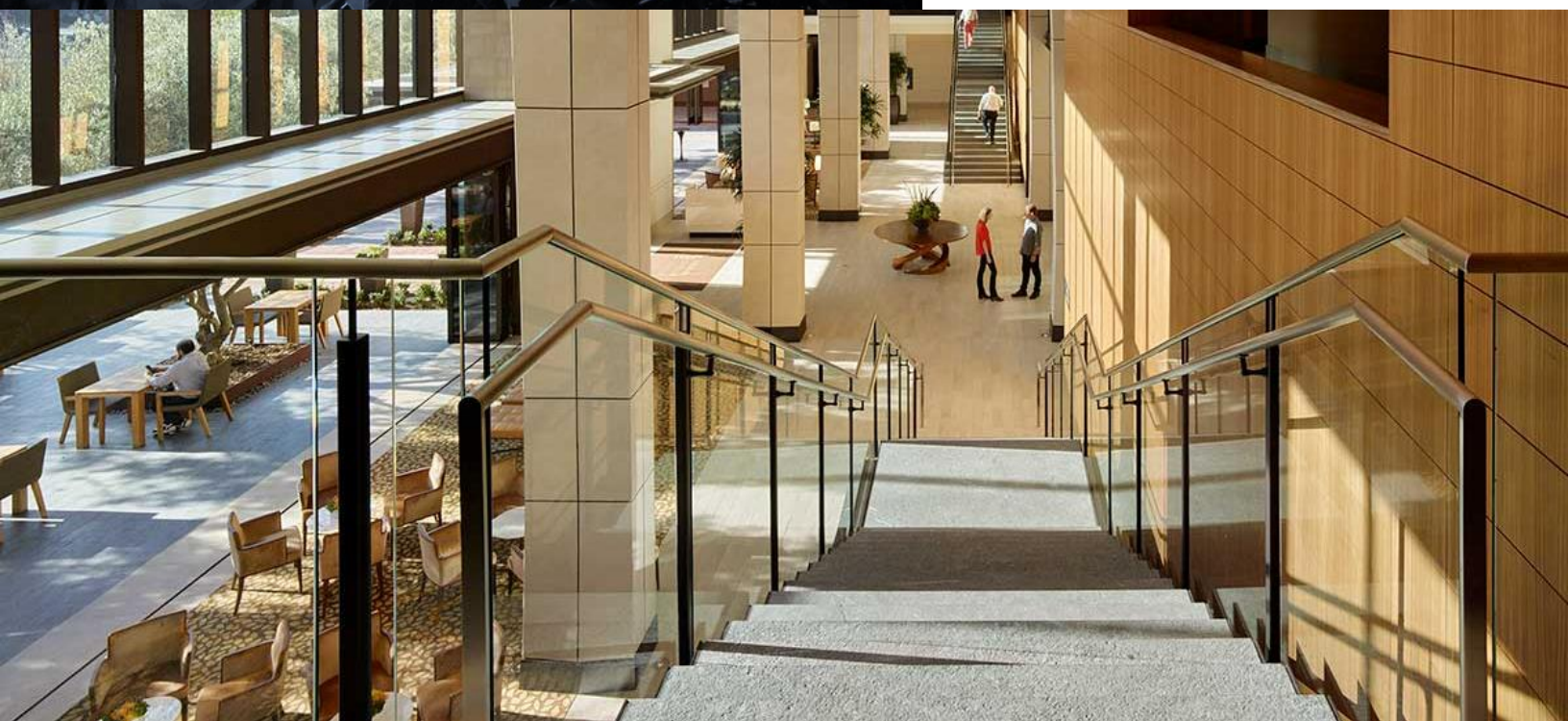
MBI 2021 Annual Retreat & Research Conference

8 –10 September
Program Guide

Hybrid Meeting
Virtual & In-person

Luskin Conference Center



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2021 MBI RETREAT PLANNING COMMITTEE

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2021 MBI RETREAT PROGRAM SCHEDULE

WEDNESDAY, 8 SEPTEMBER (ZOOM)

1:00 – 1:20	**Welcome Remarks** **Presented by Hilary A. Coller, PhD** Professor, Molecular, Cell and Developmental Biology Interim MBI Director	Zoom
1:30 – 2:45	**SESSION I: STUDENT AND FACULTY SPEAKERS** **Chair: Claudio J. Villanueva, PhD** Associate Professor, Integrative Biology & Physiology	Zoom
1:30 – 1:45	**Kristie Yu** Graduate student, IMMP, Whitcome Fellow	
1:45 – 2:00	**Andrew Hildreth** Graduate student, IMMP, Whitcome Fellow	
2:00 – 2:15	**Jeff S. Abramson, PhD** Professor, Physiology	
2:15 – 2:30	**Pavak Shah, PhD** Assistant Professor, Molecular, Cell and Developmental Biology	
2:30 – 2:45	**Ambre M. Berthole, PhD** Assistant Professor, Physiology	
3:00 – 4:30	**DEI WORKSHOP** Hosted by the Center for Education and Innovation & Learning in the Sciences (CEILS) An interactive workshop for students, faculty, and postdocs on inclusive and equitable classroom environments.	Zoom
5:00 – 7:00	**MEET THE FACULTY** 1st & 2nd year MBIDP students only. Food and drinks provided.	Room 159, Boyer Hall

THURSDAY, 9 SEPTEMBER (ZOOM)

1:00 – 2:15	**SESSION II: STUDENT AND FACULTY SPEAKERS** **Chair: Pavak Shah, PhD** Assistant Professor, Molecular, Cell and Developmental Biology	Zoom
1:00 – 1:15	**Jenna Giafaglione** Graduate student, CDB	
1:15 – 1:30	**Luis Sanchez** Graduate student, CDB	
1:30 – 1:45	**Matt Lowe** Graduate student, CDB	
1:45 – 2:00	**Devin Gibbs** Graduate Student, CDB	
2:00 – 2:15	**Hua Linda Cai, PhD** Professor, Anesthesiology and Perioperative Medicine	
2:30 – 3:30	**CONCURRENT CAREER PANEL** Panel I – Industry research careers *Moderator: Luke Riggan*	Panel I
	Panel II – Non-traditional research careers *Moderator: Emily Peluso*	Panel II
3:45 – 4:45	**Poster session** Session I Session II	Discord
6:00	**Junior Researcher Social & Trivia Night** All students welcome. Food and drinks provided.	Room 159, Boyer Hall

2021 MBI RETREAT PROGRAM SCHEDULE

FRIDAY, 10 SEPTEMBER (Luskin and Livestreaming)

10:00 – 10:45	**SESSION III: STUDENT AND FACULTY SPEAKERS** **Chair: Aparna Bhaduri, PhD** Assistant Professor, Biological Chemistry	Ballroom, Luskin Center
10:00 – 10:15	**Claudio J. Villanueva, PhD** Associate Professor, Integrative Biology & Physiology	
10:15 – 10:30	**Peter Back** Graduate student, IMMP, Whitcome Fellow	
10:30 – 10:45	**Anthony J. Covarrubias, PhD** Assistant Professor, Microbiology, Immunology and Molecular Genetics	
11:00 – 12:00	**SESSION IV: STUDENT AND FACULTY SPEAKERS** **Chair: Elissa Hallem, PhD** Professor, Microbiology, Immunology and Molecular Genetics	Ballroom, Luskin Center
11:00 – 11:15	**David (Alex) Salisbury** Graduate Student, GREAT	
11:15 – 11:30	**Ian Ford** Graduate Student, IMMP	
11:30 – 11:45	**Sean Jiang** Graduate Student, BBSB, Whitcome Fellow	
11:45 – 12:00	**Aparna Bhaduri, PhD** Assistant Professor, Biological Chemistry	
	LUNCH	
1:30 – 2:30	**KEYNOTE ADDRESS** **HOW DO GROWTH FACTORS ACTIVATE THEIR RECEPTORS?** **Presented by Natalia Jura, PhD** University of California San Francisco Cardiovascular Research Institute Department of Cellular and Molecular Pharmacology	Ballroom, Luskin Center
2:45 – 4:00	**SESSION V: STUDENT AND FACULTY SPEAKERS** **Chair: Anthony J. Covarrubias, PhD** Assistant Professor, Microbiology, Immunology and Molecular Genetics	Ballroom, Luskin Center
2:45 – 3:00	**Luke Riggan** Graduate Student, CDB	
3:00 – 3:15	**Lucia Ichino** Graduate Student, GREAT, Whitcome Fellow	
3:15 – 3:30	**Elissa Hallem, PhD** Professor, Microbiology, Immunology and Molecular Genetics	
3:30 – 3:45	**Pavlo Nesterenko** Graduate Student, CDB	
3:45 – 4:00	**Rachel Hodge** Graduate Student, CDB	
4:30 – 5:30	**PRESENTATION OF AWARDS** Poster Awards Teaching Awards DEI Award Dissertation Award Closing Remarks	Ballroom Patio, Luskin Center
5:30	**CLOSING RECEPTION**	Ballroom Patio, Luskin Center

MEET THE FACULTY SOCIAL NIGHT

WEDNESDAY, 8 SEPTEMBER
5:00 – 7:00 PM
Venue: Boyer Hall, Room 159

New MBIDP students are invited to meet with MBI faculty over a casual dinner and social night. This relaxed networking event allows for new young researchers to actively discuss topics of common interest with peers and faculty in our community.

(This event is for 1st& 2nd year MBIDP students and MBI faculty only.)

JUNIOR RESEARCHER SOCIAL & TRIVIA NIGHT

THURSDAY, 9 SEPTEMBER
5:00 PM
Venue: Boyer Hall, Room 159

This relaxed event allows for students to meet and mingle for the first time in over a year as a community. The night will include a trivia competition between MBIDP home areas. Bring all your odd knowledge and show once and for all which home area is on top!

(This event is for students only.)

DEI WORKSHOP

WEDNESDAY, 8 SEPTEMBER
3:00 – 4:30 PM
Virtual Session, Zoom

It will start with a reflection on identity to navigate young researchers and faculty through conversations about equity issues, building communication, navigating power dynamics, and creating collegiate, welcoming, and inclusive spaces both in the lab and in the classroom.

Hosted by the Center for Education and Innovation & Learning in the Sciences (CEILS)

CAREER PANEL

THURSDAY, 9 SEPTEMBER
2:30 – 3:30 PM
Concurrent Virtual Sessions, Zoom

Panel I – Industry research careers
Panel II – Non-traditional research careers

Junior researchers are invited to join a virtual panel for an in-depth discussion about different career paths. Ask the questions you have been wondering, to find what the right future job looks like for you. Do you want to work in research? Considering your options between academia, industry, non-traditional careers? Worried about the challenges of a “non-linear” career path? Come listen to our panelist and ask about their stories and how they found success. Have your questions answered to move your own future forward!

POSTER SESSION

THURSDAY, 9 SEPTEMBER
3:45 – 4:45 pm
Virtual Session, Discord

Junior researchers presenting new and exciting research findings within the MBI community. Browse through many posters on a virtual platform and interact with researchers that share topics of interest.

Poster awards will be announced at the end of the retreat.

FEATURED SPEAKER

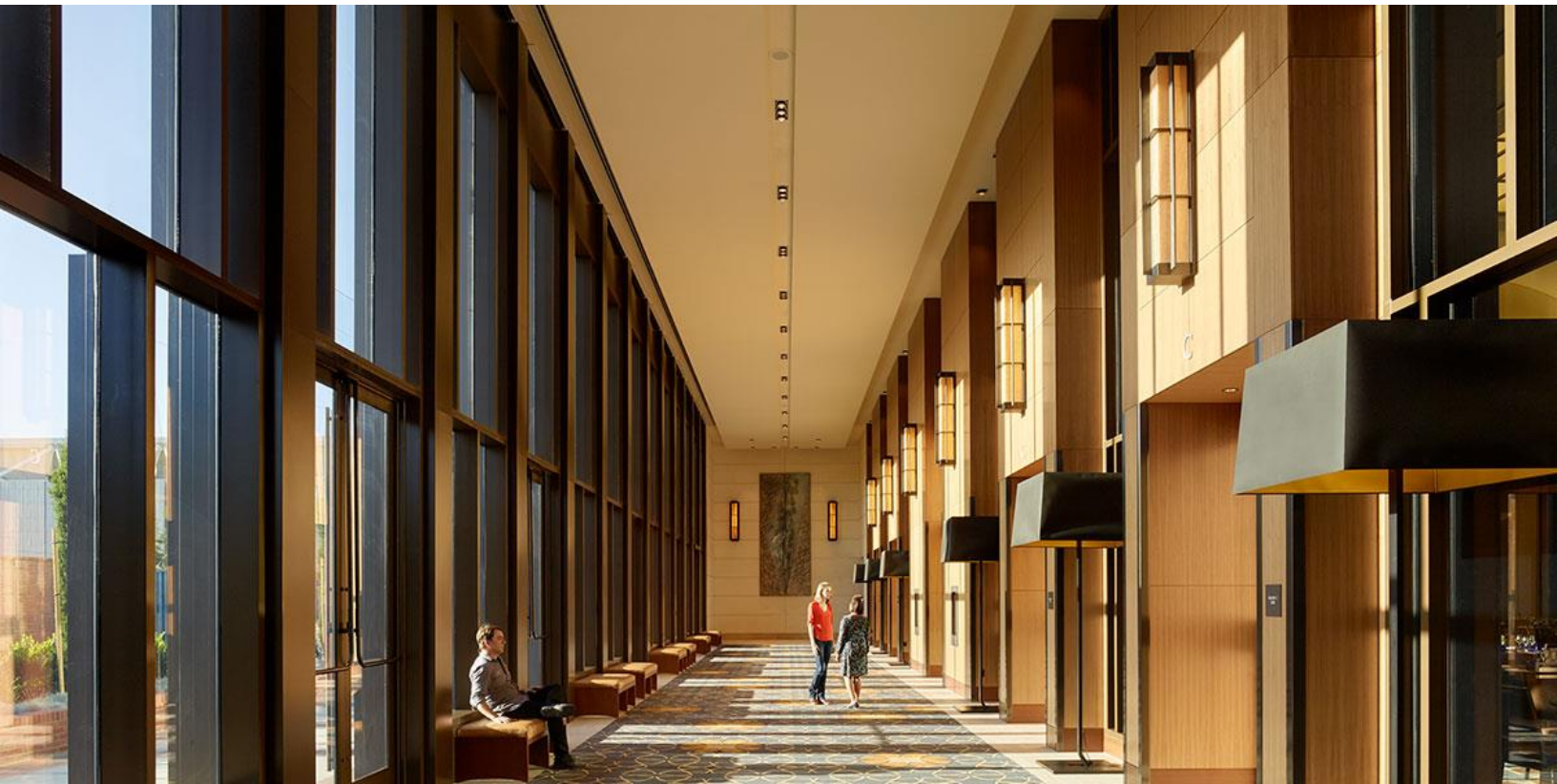
KEYNOTE ADDRESS

FRIDAY, 10 SEPTEMBER
1:30 – 2:30 PM
Ballroom, Luskin Center and Livestreaming

How Do Growth Factors Activate Their Receptors?

Natalia Jura, PhD
University of California San Francisco
Cardiovascular Research Institute
Department of Cellular and Molecular Pharmacology

The Jura laboratory uses structural and cell biology, to study how membrane-associated kinases assemble into functional complexes and regulate signaling. The long-term goal of our group is to develop an atomic-scale understanding of signaling transduction that will allow for its precise manipulation for effective and tolerable disease treatments.



CAREER PANEL I

THURSDAY, 9 SEPTEMBER

1:30 – 3:30 PM

Virtual Session, Zoom

Moderated by: Luke Riggan

Omar Barnaby, Ph.D.

After a PhD and post-doc, Omar began his Pharma career in discovery bioanalysis at BMS and subsequently moved to Amgen. Omar has built a unique combination of experience in LC–MS bioanalysis of proteins, LM and SM drugs, biomarkers and regulatory bioanalysis. Omar's work has included the qualification and bioanalysis of multiplexed biomarker panels, oligonucleotide analysis, development of challenging tissue protein biomarker analyses and more recently application of hybrid LC–MS methods for GLP and clinical studies, as well as development of high-sensitivity HRMS techniques for bioanalysis of intact protein drugs in biofluids. Omar has a full grasp of the rigor required for FDA bioanalytical validations and GLP/GCP study support, including leading the first Amgen application of hybrid LC–MS methods to GLP studies as a Principal Investigator, while continuing to investigate novel methods such as HRMS for proteins. Omar has collaborated closely with project team colleagues to develop novel drug and biomarker assays to enable program objectives. In the future, Omar is set to continue to develop novel LC–MS methods for proteins and LM drugs to enable rapid progress of drug development candidates while meeting the high standards for fully validated GLP and clinical bioanalytical methods set by the FDA. He has 15 publications, 1 patent, received the Distinguished Young Alumni Award from Warren Wilson College (2017), and was recently nominated as a finalist for the Bioanalysis Rising Star Award (Bioanalysis Zone, 2020).

Mariana F. Uchoa, PhD

Mariana Uchoa is a senior scientist at Karma Biotechnologies. Her broad research focus is on identifying the underlying mechanism of diseases and detecting targetable components leading to the development of immunotherapies. Her scientific career began with elucidating imbalanced pathways in neurodegenerative diseases, which led her to exploring harnessing the power of the innate immune system to ameliorate Alzheimer's disease. Mariana joined Karma in April of 2020 and began working on developing Xavines TM , Karma's lipid nanoparticles engineered to induce immune tolerance in antigen presenting cells. She earned her Bachelor of Science in Biology (2009-2014) at the Federal University of Santa Catarina (Brazil), during which she went to UCLA for one year, where she studied Parkinson's disease. Mariana developed her doctoral thesis on neuroimmunology and immune tolerance of Alzheimer's disease at USC, which granted her Doctor of Philosophy in Neuroscience (2014-2020).

Rishi Masalia, PhD

Rishi R. Masalia is the director of data science and communications for LeafWorks, a botanical startup focusing on Cannabis genomics. With an aim to increase label fidelity and mitigate fraud, Rishi helped launch LeafWorks in 2019, and has been working to develop R&D pipelines and build products to help the cannabis community (growers, distributors, consumers, cultivators) know what they are buying, selling, growing, and consuming. Rishi received a PhD in Plant Biology from the University of Georgia in 2018, and a Bachelors in Science from the University of Arizona in 2012. He has also spent much of his career advocating for early career researchers, as well as practicing and promoting science communication endeavors through the creation of organizations and his work with national scientific societies.

Dara Burdette, PhD

Dara is a senior research scientist at Gilead Sciences. She currently works for Gilead's Virology department making advances on HBV therapy. She obtained her PhD in 2009 at UT Southwestern Medical Center in the lab of Kim Orth. She then did a Postdoctoral fellowship at UC Berkley before transitioning her research career from academia to industry. She continues to give presentations at national and international conferences of her team's new research and advances.

Neil Bajpayee, PhD

Neil currently works as Associate Director at Kite Pharma. He earned his Ph.D. in Molecular & Medical Pharmacology from UCLA and his B.S. in Microbiology from Pennsylvania State University. Neil is registered to practice before the United States Patent & Trademark Office. He has extensive experience on the marketing and financial side of the research biomedical industry. He has worked for UChicagoTech and UCLA focusing on patents and licensing biomedical technologies. His responsibilities covered evaluations of novel discoveries, securing patents where appropriate and licensing between academia and industry.

CAREER PANEL II

THURSDAY, 9 SEPTEMBER
1:30 – 3:30 PM
Virtual Session, Zoom
Moderated by: Emily Peluso

Lynnea Waters, PhD

Lynnea Waters is currently a Medical Communications Senior Manager at Amgen supporting the prostate cancer pipeline. Lynnea first joined Amgen in 2018 and has supported multiple pipeline and marketed products in both hematology and solid tumors. Most recently, Lynnea served as the US Medical Communications lead for the launch of a first-in-class small molecule inhibitor of KRAS G12C in non-small cell lung cancer. Prior to joining Amgen, Lynnea earned her Ph.D. at UCLA studying B cell signaling and metabolism under Dr. Michael Teitell. Lynnea also completed a consulting internship at Acsel Health where she assisted in developing commercial strategies for drug targets in inflammation.

Wen Wang, Ph.D.

Wen received her PhD in the Mikkola lab at UCLA MBI exploring DNA repair and stress responses in the B cell progenitors. While doing cool scientific work in the lab, she explored a number of opportunities on bridging business to science during her PhD, which led her on a path to consulting. She joined McKinsey as a general consultant in 2016, continuing her passion for healthcare and driving real world impact. Wen is now an engagement manager at the Silicon Valley office of McKinsey, primarily working with pharmaceutical and MedTech clients on commercial topics.

Maryam Zaringhalam, Ph.D.

Maryam Zaringhalam, PhD is the Data Science and Open Science Officer in the National Library of Medicine's Office of Strategic Initiatives. In this role, Zaringhalam works to enhance capacity in the biomedical research community for data science and open science, as well as promoting diversity, equity, and inclusion among the research workforce. Prior to her current position, she was an AAAS Science & Technology Policy Fellow at NLM from 2017 to 2019. Maryam received her PhD in molecular biology from the Rockefeller University in 2017. She is also a long-time science communicator and advocate for diversity, equity, and inclusion in the sciences, working as a Senior Producer for the science-inspired storytelling series The Story Collider.

Erin Wall, Ph.D.

Dr. Erin A. Wall is a reviewer in the Office of Pharmaceutical Manufacturing Assessment under the Office of Pharmaceutical Quality at the Center for Drug Evaluation and Research in the Food and Drug Administration. Her role is to evaluate manufacturing facilities and production processes for sterile drug products that will be distributed to the American public. In the United States, drug quality is seen as a given; this is only due to the efforts of many scientists and other professionals dedicated to monitoring the drug supply for quality assurance. Her position entails assessing risks to drug quality by examining the readiness of the drug manufacturing facility and determining whether the drug manufacturing process is designed to produce sterile, pure drug products every time. Prior to joining the FDA, Dr. Wall was a postdoctoral fellow in the lab of Susan Gottesman at the National Cancer Institute and obtained her Ph.D. in microbiology from Virginia Commonwealth University, assessing a new antimicrobial target in Staphylococcus aureus under the mentorship of Gail Christie.

Wei Wu, Ph.D.

Wei received her Ph.D. from UCLA in Biological Chemistry and completed her postdoctoral research in the Department of Pathology at Stanford University. She is currently a Principal of Johnson&Johnson Innovation- JJDC, the corporate venture group of J&J. She initiates and manages equity investments in biopharma, medical device, and consumer health, to drive innovation and fuel new and sustainable businesses. Prior to joining JJDC, Wei was a Senior Associate at Illumina Ventures with a focus on genomics-enabled precision medicine including therapeutics, diagnostics, and life science tools. Prior to that, she was the Director of Healthcare Investment and Business Development of BOE Ventures where she focused on VC fund investment, direct investment, and business development opportunities at the intersection of technology and life sciences. Before becoming an investor, Wei was a R&D project lead at NuGEN Technologies (later acquired to become TECAN Genomics).

WEDNESDAY, 8 SEPTEMBER, 1:30 – 2:45

SESSION I: STUDENT AND FACULTY SPEAKERS

Virtual Session, On Zoom

1:30 – 1:45

ELUCIDATING THE EFFECT OF GUT BACTERIAL CARBOHYDRATE UTILIZATION ON HOST DIETARY PREFERENCE

Kristie Yu[1], Jorge Paramo, Anisha Chandra, Kelly Jameson, Laurent Vergnes, Karen Reue, and Elaine Y. Hsiao

[1]*Immunity, Microbes, and Molecular Pathogenesis (IMMP)*

Obesity is a rapidly growing global epidemic. Since one contributing factor is the availability and consumption of high-calorie foods, interventions to alter preferences towards healthier foods may prevent obesity. This requires an understanding the biological determinants of food preference. The gut microbiota regulates host metabolism by interacting with dietary nutrients, particularly non-digestible complex carbohydrates, and liberating metabolites to the host, such as short chain fatty acids (SCFAs) which can reduce host appetite. Whether microbial fermentation impacts host food choice in mammals remains unknown. We hypothesize that nutrient preferences of select gut bacteria will condition host dietary preferences, via bacterial metabolites signaling to neuronal circuits underlying feeding. Bacteroides species, which comprise ~25% of the human gut microbiota, digest fibers via genetically-encoded polysaccharide utilization loci (PUL). Bacteroides thetaiotaomicron (theta) and B. ovatus express PUL variants that restrict their metabolism to fructans with different linkages: B. theta ferments levan with beta2-6 linkages, whereas B. ovatus ferments inulin with beta2-1 linkages. Here we show that colonization of germ-free mice with B. theta or B. ovatus modifies host preference for a levan- or inulin-restricted diet towards the non-fermentable fructan: mice colonized with B. theta prefer inulin diet, while mice colonized with B. ovatus prefer levan diet in food choice assays. To elucidate underlying metabolic and neural mechanisms, we show that bacterial fructan utilization impacts mitochondrial metabolism, and that SCFAs can impact vagal nerve signaling. A mechanistic understanding of how gut microbes influence feeding behavior will raise the potential of using microbial manipulation to ameliorate obesity.

1:45 – 2:00

SINGLE CELL SEQUENCING OF HUMAN WHITE ADIPOSE TISSUE IDENTIFIES NOVEL CELL STATES IN HEALTH AND OBESITY

Andrew D. Hildreth [1], Feiyang Ma, Yung Yu Wong, Ryan Sun, Matteo Pellegrini, and Timothy E. O'Sullivan

[1]*Immunity, Microbes, and Molecular Pathogenesis (IMMP)*

White adipose tissue (WAT) is an essential regulator of energy storage and systemic metabolic homeostasis. Regulatory networks consisting of immune and structural cells are necessary to maintain WAT metabolism, which can become impaired during obesity in mammals. Using single-cell transcriptomics and flow cytometry, we unveil a large-scale comprehensive cellular census of the stromal and obese human WAT. We report novel subsets and developmental trajectories of adipose-resident innate lymphoid cells (ILCs), dendritic cells (DCs) and monocyte-derived macrophage populations that accumulate in obese WAT. Analysis of cell-cell ligand receptor interactions and obesity-enriched signaling pathways reveals a switch from immunoregulatory communication hubs in lean WAT to structural cell, DC, ILC and macrophage-mediated inflammatory networks in obese WAT. These results provide a detailed and unbiased cellular landscape of homeostatic and inflammatory circuits in healthy human WAT.

THURSDAY, 9 SEPTEMBER, 1:00 – 2:15

SESSION II: STUDENT AND FACULTY SPEAKERS

Virtual Session, On Zoom

1:00 – 1:15

METABOLIC MODULATION OF CELL FATE TO COMBAT TREATMENT-RESISTANT PROSTATE CANCER

Jenna Giafaglione[1], Preston Crowell, Amelie Delcourt, Matthew Bernard, Takao Hashimoto, Daniel Ha, Nicholas Nunley, Andrew Goldstein

[1]*Cell & Developmental Biology (CDB)*

In 2021, over 34,000 men will die from treatment-resistant prostate cancer in the United States. Prostate lineage plasticity enables the transition from a luminal lineage to a lineage with basal- and stem-like features that is therapy-resistant. Preventing or reversing plasticity in prostate cancer has the potential to increase sensitivity to therapy and limit disease progression. Surprisingly, key regulators of cell identity in the prostate remain largely undefined. Metabolic alterations can drive differentiation in several tissues; however, the metabolic signaling associated with lineage transitions in the prostate remain unknown. We developed an approach to perform metabolic profiling and nutrient tracing on primary basal and luminal cells from freshly-isolated mouse prostate. Profiling experiments reveal basal cells have increased abundance of glycolytic metabolites while luminal cells have elevated levels of several TCA cycle metabolites. Gene set enrichment analysis also identified enrichment of Myc targets in basal cells and enrichment of pyruvate metabolism in luminal cells. Furthermore, using an ex vivo model of basal to luminal differentiation, we demonstrate that the induction of luminal markers correlates with increased pyruvate oxidation. Importantly, we used pharmacological and genetic approaches to functionally implicate mitochondrial pyruvate oxidation in the regulation of luminal identity in both the benign prostate epithelium and prostate cancer. Ongoing work aims to elucidate the mechanism by which mitochondrial pyruvate oxidation is antagonistic towards luminal identity. Our study identifies a metabolic pathway that regulates cell fate in the prostate and has the clinical potential to improve therapeutic strategies by preventing or reversing epithelial plasticity.

1:15 – 1:30

TIME-COURSE ANALYSIS OF HUMAN TRABECULAR MESHWORK SINGLE CELL CONTRACTION AFTER A 5-DAY DEXAMETHASONE TREATMENT

Luis Sanchez[1]

[1]*Cell & Developmental Biology (CDB)*

Glucocorticoids, such as dexamethasone (Dex), are speculated to alter the contractile properties of human trabecular meshwork (HTM) cells. However, there are no definitive studies showing that dexamethasone treatment modulates the contractile forces exerted by HTM cells in vitro. Because cellular contraction is a dynamic process, it is important to quantify changes in contractile force generation over time. Additionally, observed heterogeneity in primary HTM cell cultures call for single cell contraction measurements. In order to measure HTM cell contraction at the single cell level, primary HTM cells from 4 different donors were cultured for 5 days in the presence of DMSO vehicle, or 0.1µM Dex. After 5 days, primary HTM cells from each condition were harvested, dissociated to single cells, and seeded into the wells of the fluorescently labelled elastomeric contractible surfaces (FLECS) assay for single cell contraction measurements. Data was collected every 2 hours over a period of 16 hours and analyzed using computational algorithms. Interestingly, a single large population of weakly contractile cells and smaller subpopulations of strongly contractile cells were identified across both conditions. The strongly contractile subpopulations remained smaller compared to the single weakly contractile cell population throughout the course of 16 hrs. Furthermore, the strongly contractile cell subpopulations became increasingly prominent in the DMSO vehicle condition throughout the course of 16 hrs. Dex treated cells also displayed small subpopulations of strongly contractile cells. However, these subpopulations were smaller compared to DMSO vehicle, and remained mostly unchanged throughout the course of 16 hrs. Based on our findings, Dex treatment appeared to reduce the ability of a population of primary HTM cells to exert strong contractile forces.

1:30 – 1:45

EED/H3K27me3 REGULATES THE TIMING OF MOUSE PRIMORDIAL GERM CELL DIFFERENTIATION

Matthew Lowe[1], Amander Clark

[1]*Cell & Developmental Biology (CDB)*

Primordial germ cells (PGCs) are the founding cells of the entire germline and are required for reproduction. During mammalian PGC development, major epigenetic remodeling occurs to establish an epigenetic landscape for gametogenesis. In both human and mouse PGCs, the global depletion of DNA methylation (5mC) across the genome and the nuclear enrichment of Histone H3 lysine 27 trimethylation (H3K27me3) are among the most well conserved epigenetic changes. While it has been hypothesized that the enrichment of H3K27me3 may play a compensatory repressive role following loss of 5mC, this has yet to be fully explored. To address this, we used the mouse model to create a PGC specific conditional knockout mouse for Embryonic Ectoderm Development (EED), an essential component of polycomb repressive complex 2 which trimethylates H3K27. Through imaging, FACS and RNA sequencing we show that EED/H3K27me3 regulates the timing of PGC differentiation in male and female embryos as well as X chromosome dosage decompensation in male PGCs. Additionally, we identified a subset of gametogenesis promoters in the mouse pluripotent epiblast that exhibit a unique dual H3K27me3 and 5mC repressive signature and provide evidence that EED and DNA methyltransferase 1 (DNMT1) may interact in pluripotent mouse cells. Taken together, we propose that EED/H3K27me3 joins DNMT1/5mC as a major regulator of PGC differentiation timing which is wired into the epiblast prior to PGC specification. This work presents a critical advancement in our understanding of mammalian PGC epigenetic regulation with implications upon ways to improve PGC differentiation in vitro from stem cells.

1:45 – 2:00

CHARACTERIZING HUMAN SKELETAL MUSCLE EXTRACELLULAR MATRIX AND INVESTIGATING ITS ROLE IN MODULATING SATELLITE CELL FATE ACROSS HUMAN DEVELOPMENT

Devin Gibbs[1]

[1]*Cell & Developmental Biology (CDB)*

Endogenous skeletal muscle stem cells (MuSC), known as satellite cells (SCs) in adult and skeletal muscle progenitor cells (SMPCs) in fetal development, are responsible for skeletal muscle development and repair. In healthy adult muscle, SCs reside in a quiescent state, in the satellite cell niche. These cells activate in response to exercise or injury and differentiate into muscle progenitor cells that fuse at the site of damage. Duchenne muscular dystrophy (DMD), the most common lethal genetic disease in children, is a degenerative skeletal muscle disease caused by a mutation in dystrophin. There is currently no cure for DMD. Human induced pluripotent stem cells (hiPSCs) have enormous potential for use in regenerative medicine as they are pluripotent and provide a resource for developing disease models in vitro. SMPCs derived from hiPSCs are immature and engraft poorly. In contrast, SCs acquired from adult human biopsies engraft, fuse to form functional myofibers, and are capable of residing in the niche in vivo. To make hiPSC-SMPCs a viable means of therapy, they need to be matured to a more SC like state. Using scRNA-seq, mass spectrometry, and QCONCATS we identified the extracellular matrix (ECM) as a potential driver of SMPC to SC maturation. The ECM contains products secreted by cells which provide both a scaffolding for the cells as well as other signaling cues that affect all cell activity. To further explore how the different ECM conditions affect SMPC maturation and differentiation we have developed several models that more closely mimic in vivo development conditions.

Friday, 10 September, 10:00 – 10:45

SESSION III: STUDENT AND FACULTY SPEAKERS

Ballroom, Luskin Conference Center

10:15 – 10:30

AN ESSENTIAL IMC PROTEIN COMPLEX GOVERNS TOXOPLASMA INVASION AND EGRESS

Peter S. Back[1], William O'Shaughnessy, Andy S. Moon, Xiaoyu Hu, Pravin Dewangan, Michael L. Reese, Peter J. Bradley

[1]*Immunity, Microbes, and Molecular Pathogenesis (IMMP)*

Toxoplasma gondii is an opportunistic intracellular pathogen that uses specialized organelles to invade their host cells and ultimately cause disease. One organelle is the inner membrane complex (IMC), a membrane system composed of flattened vesicles that underlie the plasma membrane, coupled to a cytoskeletal meshwork of intermediate filament-like proteins. IMC proteins localize to three subcompartments – the base, the body, and the apical cap. Recently, we demonstrated that the apical cap plays a critical role in forming the apical complex, a group of cytoskeletal structures that functions in regulated secretion and parasite motility. Specifically, the conditional depletion of any of the three essential apical cap proteins AC10, AC9, and ERK7 eliminates the apical complex, blocks organelle secretion, and completely inhibits invasion and egress. AC9 accomplishes this by recruiting the MAP kinase ERK7 to the apical cap and regulating its kinase activity. However, how the three elements interact together and are organized in the apical cap remains unknown. In this study, we use a combination of deletion analyses and yeast two-hybrid experiments to explore the organization of this protein complex and identify functionally critical interactions. We show that AC10 is a foundational component of the AC10/AC9/ERK7 complex and demonstrate that all regions of AC10 are important for interacting with AC9 and ERK7. Importantly, we also show that AC10 is phosphorylated in an ERK7-dependent manner. Together, we propose a regulatory mechanism that involves a dynamic interplay between AC9-based inhibition and phosphorylation of AC10 to coordinate proper biogenesis and stability of the apical complex.

FRIDAY, 10 SEPTEMBER, 11:00 – 12:00

SESSION IV: STUDENT AND FACULTY SPEAKERS

Ballroom, Luskin Conference Center

11:00 – 11:15

DYNAMIC REGULATION OF DE NOVO LIPOGENESIS BY INTERNAL RNA MODIFICATIONS

David Salisbury[1]

[1]*Gene Regulation, Epigenomics and Transcriptomics (GREAT)*

N6-Methyladenosine (m6A) is the most common chemical modification present on eukaryotic RNA. Multiple lines of evidence have shown that m6A plays a critical role in cell fate determination including stem cell renewal, however, the contributions of internal RNA modifications on metabolic control is less well explored. Here, we mapped the m6A landscape in mouse liver showing that RNA modifications are dynamically regulated in response to diet, segregate lipid rich versus control livers and strongly enrich lipogenic genes. Liver-specific deletion of m6A installing machinery resulted in an increase in fatty acid biosynthetic proteins and hepatic triglyceride levels. Our studies reveal insights into the mode of regulation of m6A and physiologic contributions in lipid homeostasis. In summary, we find that the epitranscriptome is dynamically regulated in response to diet and is essential for proper maintenance of metabolic homeostasis.

11:15 – 11:30

A MECHANISTIC STUDY OF THE REGULATION OF STING BY STEROLS

Ian Ford[1],

[1]*Immunity, Microbes, and Molecular Pathogenesis (IMMP)*

Recent investigations have shown surprising links between STING signaling and lipid metabolism. Previous studies from our lab and others have demonstrated that disruption of cholesterol biosynthetic programs in macrophages results in heightened STING signaling. Importantly, replenishing cholesterol normalizes STING signaling within these cells. However, the molecular mechanism underlying the regulation of STING by lipids has not been clear. In this study, we first show that addition of exogenous cholesterol inhibits STING translocation from the endoplasmic reticulum to perinuclear puncta even in the presence of its activating ligand, thus limiting the ability of STING to induce TBK1-IRF3 signaling and Type I IFN expression. Conversely, genetic or pharmacological depletion of cellular cholesterol results in spontaneous STING activation and translocation. Using a cholesterol-mimetic probe, trans-sterol, we demonstrate that cholesterol binds directly to STING, and in silico analysis of STING reveals two putative cholesterol binding motifs, termed CRAC and CARC. We show that disruptions in the CARC and/or CRAC domains within STING renders its activity insensitive to changes in cellular cholesterol and eliminates binding to trans-sterol, supporting a direct binding mechanism for regulation of STING by cholesterol. The results of this study demonstrate a complex crosstalk between immune signaling and cholesterol metabolism in the context of acute infection.

11:30 – 11:45

INVOLVEMENT OF TMEM106B AMYLOID FIBRILS IN FRONTOTEMPORAL LOBAR DEGENERATION REVEALED BY CRYO-EM

Yi Xiao (Sean) Jiang[1], Qin Cao, Michael R. Sawaya, Romany Abskharon, Peng Ge, Michael DeTure, Dennis W. Dickson, David S. Eisenberg

[1]*Biochemistry, Biophysics & Structural Biology (**BBSB**)*

Frontotemporal lobar degeneration (FTLD) is the second most common cause of presenile dementia, behind Alzheimer's disease. FTLD presents clinically in 45-64 year-olds as disorders of social behavior and language skills. The major subtype of FTLD is characterized by neuronal inclusions containing TAR DNA-binding protein (TDP-43), termed FTLD-TDP. Here, we extracted amyloid fibrils from autopsied brains of four patients, all classified clinically and pathologically as FTLD-TDP, and determined 12 near atomic-resolution structures by cryo-EM. Surprisingly, all fibrils examined are formed by transmembrane protein 106B (TMEM106B), a protein previously identified as a genetic risk factor for FTLD-TDP. Our observations suggest that amyloid involvement in FTLD-TDP is primarily or possibly exclusively of protein TMEM106B rather than of TDP-43.

FRIDAY, 10 SEPTEMBER, 2:45 – 4:00

SESSION V: STUDENT AND FACULTY SPEAKERS

Ballroom, Luskin Conference Center

2:45 – 3:00

Fli1 DRIVES THE FORMATION OF A MEMORY PRECURSOR-LIKE NK CELL STATE DURING VIRAL INFECTION

Luke Riggan[1], Tim O'Sullivan

[1]*Cell & Developmental Biology (CDB)*

Natural killer (NK) cells are innate lymphocytes that possess traits of adaptive immunity, such as clonal expansion, contraction, and generation of long-lived memory cells following viral infection. However, the molecular mechanisms by which clonally expanded Ly49H+ effector NK (NKeff) cells persist to form memory cells is not well understood. Utilizing single cell RNA sequencing, we identify two NKeff cell populations following mouse cytomegalovirus (MCMV) infection defined by the cell surface protein Ly6C. Ly6C– NKeff cells displayed enhanced survival during the contraction phase in a Bcl2-dependent manner. Ly6C– NKeff cells displayed distinct transcriptional and epigenetic signatures compared to Ly6C+ NKeff cells, with a core epigenetic signature shared with memory precursor (MP) CD8+ T cells enriched in Fli1 DNA-binding motifs. Fli1 controlled memory NK cell fate by promoting terminal maturation and restricting formation of MP-like NK cells. Our results suggest that memory NK cells, similar to memory T cells, are generated by a subset of epigenetically distinct MP cell states that preferentially survive during the contraction phase of the response to viral infection.

3:30 – 3:45

TCR CLONING BASED ON INTRACELLULAR CYTOKINE PROFILES VIA CLInt-Seq

Pavlo Nesterenko[1], Owen N. Witte

[1]*Cell & Developmental Biology (CDB)*

Cell therapy is an emerging field that combines advances in engineering and synthetic biology to address previously unmet medical need. A bottle neck in the field is the paucity of known T cell antigens (less than 200 well validated human T cell targets). T cell receptors (TCRs) are generated by somatic recombination of V/D/J segments to produce up to 1015 unique sequences. Highly sensitive and specific techniques are required to isolate and identify the rare TCR sequences that respond to antigens of interest. We describe the use of mRNA sequencing via cross-linker regulated intracellular phenotype (CLInt-Seq) for efficient recovery of antigen-specific TCRs in cells stained for combinations of intracellular proteins such as cytokines or transcription factors. This method enables high-throughput identification and isolation of low-frequency TCRs specific for any antigen. Regulatory T cells were profiled based on intracellular FOXP3 staining, demonstrating the ability to examine phenotypes based on transcription factors. We applied this technology to identify TCRs that can target viral disease and cancer

3:45 – 4:00

THE SEPTATE JUNCTION PROTEIN BARK BEETLE IS REQUIRED FOR DROSOPHILA INTESTINAL BARRIER FUNCTION AND HOMEOSTASIS

Rachel Hodge[1], Emma Edmond, Fernando de la Torre, Martin Resnik-Docampo, Leanne Jones

[1]*Cell & Developmental Biology (CDB)*

The intestinal epithelial barrier is maintained by tight junctions (TJs) in mammals and septate junctions (SJs) in insects. Age-related loss of intestinal barrier function has been found across multiple species, including Drosophila melanogaster and humans, and its causes remain unknown. The tricellular junction (TCJ) is a specialized SJ region where three adjacent cells meet. Previous studies by our lab indicated that mis-localization of the TCJ protein Gliotactin (Gli) correlates with aging. Depletion of Gli in young flies leads to loss of intestinal homeostasis, including increased intestinal stem cell (ISC) proliferation. In the embryonic epithelium, the TCJ protein Bark beetle (Bark) is required to recruit Gli to the TCJ. We hypothesized that Bark would be required for maintenance of intestinal homeostasis and barrier function. Indeed, depletion of Bark from the TCJ of enterocytes in a young fly posterior midgut increased ISC proliferation, accelerated intestinal barrier loss, and shortened lifespan. Simple overexpression of Bark does not rescue age-associated loss of barrier function. In addition, our data show that Bark becomes mis-localized with age, similar to Gli. Antibody staining for Bark shows a decrease in intensity at the TCJ in intestines from aged flies, with a modest, but significant, increase in Bark staining at the BCJ and in the cytoplasm. In summary, the TCJ protein Bark is required at the TCJ to maintain intestinal homeostasis and barrier integrity in Drosophila. Our work on the mechanisms leading to loss of the intestinal barrier will provide insight into strategies to treat age-related gastrointestinal diseases.

POSTER ABSTRACTS

POSTER SESSION I

THURSDAY, 9 SEPTEMBER
3:45 – 4:15

1. Raquel Aragon
2. Kaiser Atai
3. Nivedita Damodaren
4. Weixian Deng
5. Jonathan DiRusso
6. Kaitlin Hartung
7. Evan Hurlow
8. Grace Kunkel
9. Matthew McVeigh
10. Joshua Misa
11. Hope Pan
12. Rebecca Pasquarelli
13. Carina Sandoval
14. Angela Sun
15. Amara Thind
16. Lauren Thurlow
17. Miranda Villanueva
18. Emily Yang

POSTER SESSION II

THURSDAY, 9 SEPTEMBER
4:15 – 4:45

1. Brandon Boone
2. Amanda Creech
3. Allison Daly
4. Michael Emami
5. Angelina Flores
6. Cody Gillman
7. Kristofer Gonzalez-DeWhitt
8. Nina Harpell
9. Ashley Julio
10. Alexander Kim
11. Michael Kronenberg
12. Yan Li
13. Gregory Lum
14. Brandon Nadres
15. LeAnn Nguyen
16. Eric Pang
17. Jess Soule
18. Dylan Valencia
19. Daniel Velez-Ramirez
20. Sining Wang

Discord instructions will be sent via email.

THURSDAY, 9 SEPTEMBER, 3:45 – 4:15

POSTER SESSION I

Virtual Session, On Discord

Determining the influence of cell-specific Spp1 on cell-cell interactions in the dystrophic muscle niche

Raquel Aragón, Chino Cresse, Feiyang Ma, April Pyle, Jackie McCourt, Rachelle H. Crosbie, Matteo Pellegrini, Irina Kramerova, Melissa J. Spencer

In DMD, chronic cycles of degeneration and regeneration lead to aberrant inflammation and accumulation of fibrosis in the muscle. Previous work in the Spencer lab showed that Spp1 promotes muscle fibrosis and shifts macrophages to a pro-inflammatory phenotype. However, how Spp1 influences cellular dynamics, and thus phenotype, in dystrophic muscle is not known. In this study, Spp1 floxed mice were crossed to muscle stem cell (MuSC)-specific (Pax7 cKO) and macrophage-specific Cre (MΦ cKO) drivers to understand how Spp1 derived from these cell types affects disease processes and to dissect impact of local sources of Spp1 on the dystrophic cellular milieu. To dissect the source of Spp1, we conducted functional testing to assess how Spp1 ablation affects muscle performance. We used scRNAseq to characterize how loss of cell-specific Spp1 dynamically impacts phenotypes of cellular targets in the dystrophic niche. Functional testing showed a mild, but not statistically significant effect of MuSC-specific (Pax7-Cre) Spp1 conditional knockout. Bulk RNAseq of sorted Pax7(+) cells revealed autocrine effects of inducing pro-myogenic genes and inhibiting inflammatory and fibrotic genes. scRNAseq analysis of MΦ cKO mice showed increased pro-regenerative macrophages. Functional testing showed a mild improvement in open field (durance and stamina) and a trend in improved pulmonary function compared to Lyz2 Cre negative controls (Mdx). scRNAseq shows a population of Pdgfra(+) stromal cells with upregulated ApoD expression that disappears in the Spp1 conditional knockout. Future analysis will determine the functional effects of these fibroblast populations on the dystrophic phenotype. These findings suggest that both muscle stem cell and macrophage-derived Spp1 uniquely regulate cell-cell interactions that impact proper muscle regeneration and fibrosis.

Functional characterization of genome-related changes during reversible cell cycle exit in human dermal fibroblasts

Kaiser Atai, Mithun Mitra, Kirthana Sarathy, Alex Dunkwu, Kenya Bonitto, Hilary A. Coller

Quiescence is the reversible arrest of cell proliferation that is important across biological processes like stem cell maintenance and cancer cell dormancy. We, and others, have shown that entering quiescence leads to specific upregulation of H4K20me3 (by ~10-fold) and global changes in chromatin organization. However, how epigenetic states and chromatin architecture influence quiescence is not known in detail. To answer these outstanding questions, we use a quiescence cell-culture model based on the human dermal fibroblast. In these fibroblasts, H4K20me3, a repressive heterochromatin histone mark, is strongly upregulated in quiescence. Transcriptional profiling of quiescent cells by RNA-seq across different systems has shown that transcriptional repression is a key hallmark of quiescence, thus providing a potential role for H4K20me3 in quiescence programing. To better understand how this mark is influencing the transcription and nucleus of quiescence cells, we are performing Cut&Tag to identify where H4K20me3 is enriched in the genome and whether it specifically influences cell-cycle related genes. Further, we find that nuclei from quiescent cells are 22% smaller on average when compared to nuclei from proliferating cells with the same amount of DNA—further suggesting that the chromatin in quiescent cells is more compact. We are supplementing these experiments with cas9-mediated fluorescent in-situ hybridization to visualize how important genomic features, like telomeres and centromeres, change in the quiescent nucleus. Specifically, we observe clustering of telomeres in quiescent cells and a dispersal of centromere foci in MEFs lacking H4K20me3. We've also found that mice born lacking H4K20me3 are larger than their littermates, a phenotype often indicative of hyperproliferation.

Short vs long term effects from depletion of a splicing regulator: identification of early splicing changes after rapid depletion of the RNA-binding protein Matrin-3.

Nivedita Damodaren, Chia-Ho Lin, Harry Yang, Elsie Jacobson, Kathrin Plath & Douglas Black

Splicing programs are controlled by multiple RNA Binding Proteins (RBPs) acting in concert to create multilayered regulatory networks impacting sets of alternative exons. RBPs are often cross-regulated by other RBPs, and perturbation of one factor can alter the concentrations of other splicing regulators. The multi-day time courses of standard protein depletion strategies do not identify which targets are directly bound by the protein. We adopted the auxin-based degron system to investigate the short-term vs long-term effects on splicing and gene expression resulting from loss of the splicing regulator Matrin-3 (MATR3) in mouse embryonic stem cells (ESC). We found progressive changes in alternative splicing events over time after protein depletion, with a large number of changes in gene expression and splicing manifesting at later time points from 12 to 24h. Notably, we found a significant number of alternative exons that exhibit increased exon inclusion as early as 3h. These initial splicing targets are enriched for transcripts encoding RNA processing factors including the Clk kinases. By allowing us to identify fast and slow developing changes after loss of MATR3, our results indicate that MATR3 depletion reorganizes the RBP landscape, and likely leads to downstream effects on splicing and gene regulation with a larger impact on the splicing regulatory network than simply the directly bound targets.

Carboxylate modified magnetic bead (CMMB)-based peptide fractionation enables rapid and robust off-line peptide mixture simplification in bottom-up proteomics

Weixian Deng

Introduction: Previously, strong cation exchange (SCX) chromatography and high-pH reverse phase (RP) are the most commonly used peptide fractionation method. However, both of them lead to a quite prominent material loss during the fractionation procedure, and hence they are not suitable for low input peptide fractionation. Recently, hydrophilic interaction chromatography (HIC) was reported to be used in proteomics

sample preparation procedures for both protein and peptide clean-up and it was shown only has minimal amount of material loss and high binding capability. But only limited success has been made in using HIC for peptide fractionation, because with the decrease of organic solvent concentration, peptides are eluted from the carboxylate modified magnetic bead (CMMB) collectively within a very narrow concentration range.

Methods: Complex peptide mixture is repelled to the surface of carboxylate coated beads in high organic phase solution, then cleanup can be achieved by washing with organic solvents. For peptide fractionation, we designed a series of elution solutions and they make relatively equivalent number of peptides get eluted into different fractions. Eluted fractions are then measured by LC-MS label-free quantification. This method can be applied to be a second dimension to high-pH reverse phase fractionation for high complexity samples, and it is also used for fractionating streptavidin affinity purification analytes to get around dominant digested streptavidin peptides from this kind of on-bead digested samples.

Results: First of all, using specifically designed elution solutions, in a descent width of concentration range, peptides can be eluted into different fractions linearly. Each fraction contains different sets of peptides and they are more evenly distributed on the LC acidic acetonitrile gradient comparing to commonly used off-line high-pH reverse phase fractionation method. Secondly, by doing CMMB-based peptide fractionation, with same machine time, it identified and quantified ~50% more peptides than evenly splitting the analyte, and averagely it gives significant higher number (~8%) of identified peptides than doing reverse phase fractionation. Third, with reverse phase and CMMB 2-dimension fractionation, in same number of fractions, 2D gains ~6% more identification than doing reverse phase 1D fractionation. Then, we applied this method to APEX-based approximate labeling experiment, using transcriptional factor Oct4 as bait to characterize it interacting partner in nucleolus. With doing fractionation, we identified 698 proteins enriched in the bait sample over negative control and over ~60% are nuclear proteins, however without fractionation, only 21 proteins enriched over negative control, which suggests by doing CMMB-based fractionation we can successfully alleviate signal suppression from streptavidin peptides in the on-bead digested samples. Finally, we explored the mechanism of the elution profile by fitting the elution profile and peptide's physical chemistry properties into a multi-variable linear regression, with r equals 0.8, we found the elution profile is mainly determined by both peptides charge and hydrophobicity. In all, we developed a new peptide fractionation method which is compatible with low material loss cleanup method and low input samples, meanwhile, with similar reproducibility to reverse phase fractionation, it costs significantly less in terms of both time and money.

Novel aspect: We developed a new off-line peptide fractionation method which has significantly low material loss and low cost.

Transposon Control by TRIM28 is Necessary for Proper PGC Development in Mice

DiRusso, J., Tao, Y., Wang, A., Clark, A.

Germline genomic integrity is of paramount importance to organismal fitness. While most Transposable Elements (TEs) are no longer mobile, those capable of translocation present a threat to genomic integrity. Mouse Primordial Germ Cells (mPGCs), which give rise to the adult germline, must maintain a chromatin environment in which transposition competent transposons are suppressed throughout waves of epigenetic reprogramming, including global DNA demethylation. Here, we examine the role of tripartite-motif containing-28 (TRIM28) in silencing young, mobile TEs during mPGC development. TRIM28 acts as an epigenetic scaffold protein, binding sequence specific Kruppel-assocated box (KRAB)-zinc fingers and recruiting epigenetic modifiers to durably repress targeted loci. Employing a PGC-specific knockout strategy, we show TRIM28 loss results in a reduction of mPGCs beginning at E12.5, concomitant with mPGC exit from latent pluripotency and acquisition of sex-specific transcriptomes. Using RNA-seq at E11.5, E12.5 and E13.5, we show that young, mobile Intracisternal A-particle (IAP)-, Early Transposon (ETn)-, and MMERV-elements are derepressed in mPGCs at E11.5 and becomes more pronounced by E12.5. While loss of epigenetic modifiers such as DNMT1 result in a precocious mPGC entry into meiosis (females) and differentiation (males), loss of TRIM28 results instead in a transcriptomic shift away from entry into oogonia and pro-spermatocyte transcriptomes at E13.5. Collectively, we show TRIM28 is necessary for repression of young retrotransposons and proper acquisition of the early, sex-specific transcriptomes. However, it remains to be seen if TE derepression results in gene misregulation or if the two phenomena are independent.

Design and synthesis of bioorthogonal host-guest pairs

Kaitlin M. Hartung and Ellen M. Sletten

The bioorthogonal chemical reporter strategy has proven useful for labeling biomolecules in their native environments and studying natural processes with minimal perturbation. However, this approach faces drawbacks arising from an inability to achieve fast reactivity in dilute cellular media without compromising the size and stability of the reactive partners. Bioorthogonal complexation offers an alternative approach that may circumvent these challenges, employing the principles of host-guest chemistry to study living systems. Recent work in our group focusing on known host cucurbituril[7] has shown that this method can be used for efficient cell labeling. Other recent work in our lab has revealed the strength of noncovalent interactions between perfluoroaromatics and other pi systems in aqueous media, leading us to propose these structures as useful bioorthogonal guests. The current work is focused on designing and synthesizing host structures for these fluorinated guests. We seek to engineer these new host-guest systems to participate in noncovalent interactions that are rarely found in biology.

Towards a method for scaffold-directed RNA-small molecule cocrystallization.

Evan Hurlow

MicroRNAs (miRs) are an important layer of post-transcriptional regulation within the cell. Their dysregulation is associated with many diseases, including cancer. Disruption of miR biosynthesis can lead to tumor regression and apoptosis, painting certain miR precursors (pre-miRs) as valuable therapeutic targets. Targeting structured RNAs with small molecules is a new, challenging field; lead optimization is made difficult by limitations of existing structure-based methods. I am developing a novel scaffold-directed crystallography method to obtain X-ray structures of prodiginine small molecules interacting with their target pre-miR.

This will enable rapid structure-based optimization of the prodiginine scaffold, allowing design of potent disruptors of miR biosynthesis. My initial experiments have characterized the pre-miR-21 binding activity of several prodiginines and I have elucidated their binding site. Using this information, I designed and synthesized scaffolds bearing the binding site and have produced several promising crystals. Completion of this project will lay the groundwork for a new method in RNA–small molecule structural biology, yield a valuable crystal structure of a modulatory small molecule interacting with its pre-miR target, and facilitate development of a potentially therapeutic small molecule.

Efficient Post-polymerization Access to Block Copolymers via Organometallic Gold (III) Reagents

Kunkel, G.; Montgomery, H.; Doud, E.; Spokoyny, A.; Maynard, H.

The vast applications realized through polymer chemistry are in part due to the numerous possible polymeric architectures that contribute to distinct structure-property relationships. Despite innovations in controlled polymerizations techniques, conjugation between existing polymer chains is critical towards achieving many interesting architectures, particularly when the polymer target contains monomers requiring disparate polymerization techniques. The conjugation of existing polymer chains to form block copolymers requires highly efficient chemistry due to the low concentration of reactive units and the steric hinderance caused by polymer chains. To this end, bench-stable (Me-DalPhos)Au(III)Aryl reagents have been applied to post-polymerization, “graft-to” block copolymer synthesis. The thiophilicity of Au(III) allows this S-arylation approach to mitigate the issue of low reactive unit concentration. Reversible addition fragmentation chain-transfer (RAFT) polymerization is used to prepare the thiol-capped polymers via aminolysis of trithiocarbonate chain transfer agents (CTA). Synthesis of aryl iodide-capped polymers is accomplished by modifying small molecule initiators and termination moieties with aryl iodides for polymers prepared by ring opening polymerizations (ROP), ring opening metathesis polymerizations (ROMP), and atom transfer radical polymerization (ATRP). The accessible polymer scope highlights the chemoselectivity, versatility, and tolerability of the Au(III) block copolymer synthesis strategy.

Palladium-Catalyzed Annulations of Strained Cyclic Allenes

Matthew S. McVeigh, Andrew V. Kelleghan, Dominick C. Witkowski, and Neil K. Garg

Transient strained cyclic molecules, such as strained cyclic alkynes and allenes, are valuable building blocks in the synthesis of bioactive natural products and drug candidates. In this presentation, we report palladium-catalyzed chemical reactions of in situ generated strained cyclic allenes. This transformation leverages readily available reaction partners in order to generate fused heterocycles, which are important scaffolds for pharmaceutical research. The reaction proceeds via the formation of two new bonds and a stereocenter. Moreover, stereoselective transformations are accomplished, ultimately enabling the rapid enantioselective synthesis of a complex hexacyclic molecule. This study is expected to fuel the development of reactions that strategically merge transition metal catalysis and transient strained cyclic molecules for the synthesis of biologically relevant scaffolds.

Engineering a Yeast-Based Platform for Production of Novel Monoterpene Indole Alkaloids

Joshua Misa, Yi Tang

Monoterpene indole alkaloids (MIAs) are an expansive class of plant natural products, many of which have been named on the World Health Organization’s List of Essential Medicines. Their biological activities include anti-cancer, anti-malarial, anti-addiction, and more. However, MIAs are some of the costliest small-molecule drugs due to low production from native hosts. Thus, a more sustainable and reliable source of these drugs is critical to meet global demand. The model eukaryote, Saccharomyces cerevisiae (Baker’s yeast), has proven to be an effective host for production of numerous plant natural products in recent years. Here we report the development of a yeast-based platform for high-titer production of the universal MIA precursor, strictosidine, using a combination of synthetic biology and metabolic engineering techniques. Our fed-batch platform produces 50 mg/L strictosidine starting from the inexpensive commodity chemical, geraniol, and is the highest titer reported thus far. Additionally, our robust platform can produce strictosidine analogues through feeding modified substrates to our host. Our platform will enable future reconstitution of downstream biosynthetic pathways towards production of more elaborate MIAs such as the frontline cancer therapeutic vinblastine, the anti-addiction agent ibogaine, and their novel analogues.

Designing novel therapeutics for Alzheimer’s disease using structural studies of tau

Hope Pan, Paul Seidler, Romany Abshkaron, Sally Frautschy, Gregory Cole, David S Eisenberg

Alzheimer’s disease (AD) is a neurodegenerative disease that affects more than 5 million Americans. AD occurs when abnormal amounts of amyloid-β and tau accumulate as amyloid plaques and neurofibrillary tangles, resulting in neuron loss and brain atrophy. No treatments that effectively stop the progression of AD have been found so far. Here, we propose a novel therapeutic for AD designed from structural studies of tau.

Previous studies in our lab determined the structure of an aggregation-prone segment of tau with the sequence VQIVYK. We designed a fibril-capping peptide inhibitor, referred to as WIW, to target the aggregating interfaces of VQIVYK. WIW successfully blocks seeding of tau aggregates by AD patient-derived fibrils in HEK293 biosensor cells. One major obstacle to peptide therapeutics is they often cannot cross the blood-brain barrier (BBB). A peptide tag designed outside our lab, L57, binds low-density lipoprotein receptor-related protein 1 (LRP1) on the BBB and enters the brain via receptor-mediated endocytosis. When linked to WIW, L57 does not interfere with its ability to block seeding by patient-derived fibrils. We treated mice with L57-WIW and successfully visualized the peptide in brain parenchyma using immunohistochemistry.

In future studies, L57-WIW will be tested in mouse models of AD to determine whether it can decrease tau aggregation and prevent cognitive decline. L57-WIW will be administered once a week starting at seven months of age in tau P301S mice, which develop tau pathology around nine months of age. We will use behavioral tests to compare disease progression between control and treatment groups and histopathology to compare tau aggregation and neuron loss. We aim to demonstrate that our

structure-based inhibitors of tau aggregation can be used as a novel treatment for AD.

IMC53 is an essential *Toxoplasma gondii* daughter IMC protein involved in replication

Rebecca R. Pasquarelli, Peter S. Back, Jihui Sha, James A. Wohlschlegel, Peter J. Bradley

Toxoplasma gondii is an obligate intracellular parasite which causes severe disease in congenitally infected neonates and immunocompromised individuals. Its life cycle relies on a unique organelle named the inner membrane complex (IMC) which plays a crucial role in motility, host cell invasion, and replication. The IMC acts as a scaffold for developing daughter buds during replication, but the exact composition of the IMC, particularly in daughter buds, remains unknown. Using TurboID in vivo biotinylation we identified IMC53, a novel IMC protein that is predicted to be fitness-conferring by a genome-wide CRISPR/Cas9 screen. To investigate the function of IMC53, we used CRISPR/Cas9 to attach an auxin-inducible degron to the C-terminus of the protein, allowing for rapid proteasomal degradation upon treatment with indoleacetic acid. The phenotype of the conditional knockdown was assessed by IFA and plaque assay, which showed that IMC53-depleted parasites exhibit aberrant morphology, mislocalization of another key daughter protein IMC32, and an ultimately lethal defect in replication. These results demonstrate that IMC53 is an essential component of the daughter cell scaffold and suggest that IMC53 could play a key role in parasite replication by recruiting other IMC proteins to developing daughter buds. To further explore the function of IMC53, future studies will seek to determine key functional domains using deletion analyses. In addition, we will use a combination of TurboID, yeast two-hybrid analysis, and co-immunoprecipitation to identify IMC53 binding partners.

Vpr-induced DNA damage is independent of Cul4A^{DCAF1} recruitment and alters cellular transcription

Carina Sandovala, Oliver I. Fregoso

The accessory protein Vpr is conserved among primate lentiviruses and is important for viral replication in vivo. Yet, the primary conserved function of Vpr remains unclear. Some of the phenotypes described for Vpr include induction of G2/M cell cycle arrest, engagement of the Cul4A^{DCAF1} complex (CDD), modulation of nuclear factor-kB (NF-kB), and stimulation of the DNA damage response (DDR). Recently, we have shown that an additional conserved function of Vpr is induction of DNA damage. Through mutational analysis, we found that the ability of Vpr to cause DNA damage is distinct from cell cycle arrest, indicating that Vpr has two independent yet conserved phenotypes: induction of DNA damage and cell cycle arrest. While Vpr-mediated cell cycle arrest has been widely studied, how and why Vpr induces DNA damage is unclear. Moreover, how Vpr-induced DNA damage connects to other phenotypes of Vpr, such as CDD recruitment and NF-kB activation, have not been explored.

By using live cell imaging of the DNA double-strand break sensor NBS1 and the downstream signaling protein 53BP1, we have found that Vpr-induced DNA damage causes NBS1 and 53BP1 foci formation that confers damage detection and signaling, respectively. Moreover, NBS1 and 53BP1 foci follow the spatial and temporal dynamics of etoposide-induced double-strand breaks, suggesting Vpr-induced double-strand breaks activate the classical DDR response. Furthermore, DDR signaling is conserved among HIV-1 and HIV-2 Vpr orthologs. In addition, Vpr mutants that are unable to recruit DCAF1 are still able to induce NBS1 and 53BP1 foci, indicating that CDD engagement is not necessary for DDR signaling activation. Consistent with activation of the DDR, we found that HIV-1 and HIV-2 do not require CDD engagement to induce DNA damage. Together this suggests that HIV-1 and HIV-2 Vpr induce DNA breaks that activate the DDR in the absence of CDD engagement.

As both DNA damage and Vpr cause transcriptional changes that are led by NF-kB, we tested our hypothesis that Vpr-induced DNA damage activates NF-kB and causes transcriptional changes in infected cells. Our preliminary data shows that Vpr-induced DNA damage activates NF-kB as shown by p65 nuclear translocation. Furthermore, RNA-sequencing identified over 200 differentially expressed genes in cells expressing a Vpr mutant that causes DNA damage without CDD engagement and induction of cell cycle arrest. Among the top 100 upregulated genes, 30 were consistently upregulated in cells expressing HIV-1 Vpr and Vpr mutants H71R and S79A, suggesting these genes are upregulated in response to Vpr engaging the DDR rather than inducing cell cycle arrest. Together, our data supports a model where Vpr-induces DNA damage independent of Cul4A^{DCAF1} recruitment, activates NF-kB and alters cellular transcription.

Mapping CD8 T cell immune responses to CMV in kidney transplantation

Angela Sun, Harry Pickering, Subha Sen, Rajesh Parmar, Elaine Reed and CMV Systems Immunobiology Group

Human cytomegalovirus (CMV) establishes mostly asymptomatic but lifelong infections, with a global seroprevalence of 83%. Primary infection or reactivation of CMV can be disease-causing in immunosuppressed individuals, including solid organ transplant (SOT) recipients, leading to increased risk of chronic graft injury, graft rejection, and mortality. CMV infection induces specific and unspecific alterations in the composition and function of immune cells, marked by sustained CD8 effector memory T cell expansion, termed ‘memory T cell inflation.’ Inflationary CD8 T cells influence the ability of the immune system to mediate heterologous graft injury, predispose to opportunistic infections, and respond to vaccines post-transplantation. This study characterized longitudinal CD8 T cell immune responses to CMV in a cohort of 31 CMV seropositive and seronegative kidney transplant (KTX) recipients with CMV viremia and 31 matched, non-viremic KTX recipients. Using multi-color flow cytometry and single-cell RNA sequencing, we characterized longitudinal immunophenotypes of CD8 T cells and observed heterogeneity and advanced differentiation signatures potentiated by prior CMV exposures. We also reconstructed in silico developmental trajectories of CD8 T cells with corresponding transcriptomic profiles.This work aims to elucidate CD8 memory T cell phenotypic and transcriptional programs associated with CMV infection, and for future studies we will map longitudinal epigenetic network, clonotypic heterogeneity and functional capabilities of CD8 T cells to CMV antigen specificity and immunodominance.

Novel secreted dense granule proteins of Toxoplasma gondii bradyzoites revealed by proximity biotinylation

Amara C. Thind, Santhosh M. Nadipuram, Jihui Sha, Caroline M. Mota, James A. Wohlschelgel, Tiago P. Mineo and Peter J. Bradley

Toxoplasma gondii is an intracellular eukaryotic parasite that causes serious disease in immunocompromised patients and congenitally infected neonates. During the acute infection, T. gondii tachyzoites invade their host cells where they form a parasitophorous vacuole (PV) that is necessary for intracellular survival. The parasites switch to slow-growing bradyzoites in the chronic infection, and a cyst wall forms around the PV allowing the parasite to persist for the life of the host. To maintain its intracellular niche, T. gondii secretes dense granule proteins (GRAs) into the PV space and cyst. Few bradyzoite GRAs have been discovered, and their roles in establishing and maintaining the chronic infection are largely unknown.

To identify bradyzoite GRA candidates, we implemented the BioID approach on bradyzoites using biotin ligases fused to MAG1 or TgME49_297900, bradyzoite-upregulated GRAs. Using this approach, we identified eleven new GRAs that were confirmed by endogenous gene tagging and immunofluorescence. We functionally characterized several of these novel GRAs by gene deletion. Disruption of one of these proteins demonstrated that they play important roles in parasite replication in vitro, and its absence results in lower brain cyst burden in vivo. Disruption of another GRA produced normally replicating tachyzoites but resulted in fewer cysts in vivo. Finally, disruption of a third GRA resulted in a dramatic increase in cyst burden and appears to be involved in modulating the host immune response. Together, this study identifies key proteins involved in maintenance of the chronic infection and promises to provide lead for developing novel therapeutics for toxoplasmosis.

Regulation of the chromatin remodeler Snf2 in S. cerevisiae

Lauren Thurlow and Tracy Johnson

Eukaryotic organisms have evolved complex gene regulatory networks to launch coordinated responses to external conditions and stimuli. Under environmental stress, such as nutrient starvation, these responses involve reallocation of cellular resources to stress-induced genes. In addition to transcriptional control, this occurs through several mechanisms including repression of ribosome biogenesis factors, altered translation initiation, and RNA features which modulate translation efficiency and transcript stability. Upon nutrient starvation in Saccharomyces cerevisiae, diploid cells undergo meiosis (sporulation in yeast). We have previously shown that the catalytic component of the SWI/SNF chromatin remodeling complex, Snf2, is responsible for shifting gene expression away from intron-rich ribosomal protein genes (RPGs) to enhance splicing of meiotic intron-containing genes (ICGs) during sporulation. Although critical to its activity, the mechanism by which Snf2 protein levels change remains unknown. Here we describe temporally-regulated SNF2 mRNA isoform switching under batch growth and sporulation conditions, which we hypothesize is involved in tuning the translation propensity of individual SNF2 transcripts. Specifically, a long isoform of SNF2 contains three upstream open reading frames (uORFs), which we hypothesize control translation of the downstream ORF. To address how each of these regulatory features impact Snf2 expression, we designed a reporter system in which the uORF start codons are endogenously mutated to prevent translation. In light of the conservation of Snf2, investigating its dynamic regulation in response to environmental changes in S. cerevisiae may carry important implications for Snf2 chromatin remodeling activity in higher eukaryotes.

A proteomics approach to identify metabolic regulatory mechanisms in fatty liver disease

Miranda Villanueva

Fatty liver disease is a common liver disorder characterized by accumulation of lipids and altered redox biology. However, the underlying molecular mechanisms remain poorly characterized. We aim to utilize novel photoaffinity sterol probes to identify unknown sterol interacting protein partners and identify redox sensitive cysteines to determine how alcohol induced oxidation of cysteines impact fatty liver disease. We plan to utilize diazirine photo-affinity probes to covalently capture cholesterol interactions after UV irradiation. Then, the alkyne handle will be captured and enriched for downstream analysis by bottom-up mass spectrometry to identify the site of binding. We expect to identify novel interacting partners and identify specific binding sites. Separately, we will also apply our heavy and light iodoacetamide alkyne probes to identify redox sensitive cysteines. We first label reduced cysteines with the heavy probe. Then we reduce the entire sample and capture the remaining cysteines. The heavy to light ratio informs us about the redox state and sensitivity of each cysteine. These two approaches will provide information on the mechanisms of lipid accumulation and inform us on the role of cysteine oxidation during fatty liver disease to help inform future therapeutic interventions.

The requirement for ubiquitination in TRIM25-mediated ZAP antiviral activity

Emily Yang, Yasaman Jami-Alahmadi, James A. Wohlschlegel, and Melody M.H. Li

Type I interferon signaling upregulates hundreds of interferon-stimulated genes with potential antiviral function, such as zinc finger antiviral protein (ZAP), which inhibits replication of multiple RNA and DNA viruses. We study ZAP by investigating how it blocks viral translation in the context of alphavirus infection. We previously discovered the ubiquitin E3 ligase tripartite motif-containing protein 25 (TRIM25) to be indispensable for ZAP inhibition of alphavirus translation. Though it has been shown that TRIM25 ligase activity is required for its synergy with ZAP, its ubiquitinated substrates that function in the antiviral response are unknown. We hypothesize that TRIM25 mediates ZAP antiviral effects by ubiquitinating and modulating host factors critical for viral translation.

Here we show successful development of a “substrate trapping” approach to identify TRIM25 substrates. We identified a residue critical for TRIM25 E2 binding and thus its catalytic activity; mutation of this residue cripples TRIM25 autoubiquitination and antiviral activity, and enriches for TRIM25 substrates. We co-immunoprecipitated this mutant TRIM25 in the presence of ZAP and viral infection to identify by mass spectrometry TRIM25 interactors required for antiviral response. We uncovered stress granule proteins and RNA helicases with known roles in viral infection and additional host factors in translation and RNA processes to be potential TRIM25 substrates, and will validate and characterize their impact on alphavirus translation . Our

study may reveal novel insights as to how this catalytically dead protein inhibits viruses by ubiquitination of other substrates via recruitment of the E3 ligase TRIM25.

Exploring the Biochemical Mechanism of DNA Methylation Mediated Gene Silencing by the MBD5/6 Complex

Brandon A. Boone, Steve Jacobsen

DNA methylation is a well-studied and conserved epigenetic mark, but it is unclear how plants use DNA methylation to regulate gene expression. Recently, two methyl-CpG-binding domain (MBD) proteins (MBD5 and MBD6) were published to redundantly silence genes and transposons in a complex with three heat shock proteins (HSPs): ACD21, ACD15, and SILENZIO (SLN). Currently, there is no mechanism to explain how these HSPs work with MBD5 and MBD6 to regulate genes. Our hypothesis is that MBD5/6 contain an inherent ability to form phase separated condensates at sites of methylated DNA which are regulated by HSPs to maintain a proper biophysical state needed for silencing genes. Using a combination of in vitro and in vivo approaches, our preliminary data suggests that MBD6 can phase separate on its own, with methylated DNA, and can colocalize with SLN condensates. Further, dynamics of MBD6 puncta in root cells of *SLN* mutant plants changes compared to WT. These preliminary data begin to support my phase separation hypothesis while future experiments are needed to understand the mechanism of gene silencing. Providing evidence that HSPs act as chaperones which regulate chromatin related, phase separated compartments without a stress is a novel finding and will broaden the functions of HSPs while providing a novel perspective on MBD protein mediated gene silencing.

Alteration of Antigen Presentation in Pancreatic Ductal Adenocarcinoma with MAPK Inhibition

Amanda L. Creech, Thuc M. Le, Joseph R. Capri, Evan R. Abt, Hailey R. Lee, Khalid Rashid, Timothy R. Donahue, Caius G. Radu

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with an increasing rate of incidence in the United States and 5-year survival rate of less than 10%, highlighting the need for new therapeutic options. Hallmarks of this aggressive disease include constitutive activation of the MAPK signaling pathway via mutant KRAS (mKRAS) and an immunosuppressive tumor microenvironment (TME). Non-specific immunostimulatory treatments such as checkpoint blockade inhibition as a single agent or combined with conventional chemotherapy in PDAC have been marginal at best. Significant gaps in knowledge remain in understanding how alterations in MAPK signaling contribute to immunosuppression in PDAC, and how such intersectionality can be targeted to increase treatment response. Here we present preliminary data demonstrating that direct inhibition of mKRASG12C, a major oncogenic driver in a subset of pancreatic cancers, decreased mRNA abundance of several immunosuppressive chemokines such as CXCL1 and CXCL8 and increased several genes involved in the antigen presentation pathway. Additional proteomic analysis showed an upregulation in several tumor-associated antigens (TAAs) including prostate stem cell antigen (PSCA) and mesothelin (MSLN). Furthermore, initial profiling of ligands bound to MHC-I molecules (termed immunopeptidome) of murine PDAC cells identified epitopes derived from MSLN and PSCA, among other immunopeptidome alterations. These findings demonstrate a method to identify antigens that can be targeted with a specific, cell-mediated immunotherapy approach, and the potential for MAPK and other signaling inhibition to increase the potential immunogenicity of PDAC cells.

Mechanistically defining the role for each NFkB family member in pro-inflammatory macrophage activation

Allison Daly

The p50 subunit of NFkB does not contain a transactivation domain (TAD) and thus, cannot act alone to activate gene expression. Preliminary RNA-seq analysis, reveals p50 is responsible for the activation of key inflammatory cytokines. Since other NFkB family members cannot compensate for the loss of p50, I hypothesize that p50 associates with a protein outside of the NFkB family to activate gene expression. Preliminary data suggest that p50 may associate with a member of the nuclear IkB family, IkBz which contains a TAD. To explore the overlap in expression dependencies between p50 and IkBz, I will analyze p50-/- and IkBz-/- ChAR-seq data. To investigate the mechanisms underlying p50 gene regulation, I will begin by analyzing p50 and IkBz ChIP-seq data. Further analysis of IkBz ChIP-seq performed in p50-/- BMDMs will reveal if p50 is necessary for IkBz binding genome-wide.

Identifying new AAV serotypes with muscle stem cell tropism

Michael Emami

Duchenne muscular dystrophy (DMD) is a monogenic disease caused by out-of-frame mutations in the DMD gene. We have developed a CRISPR/Cas9 gene-editing platform as a therapeutic strategy for DMD. Systemic delivery of CRISPR/Cas9 can be achieved using adeno-associated virus (AAV); however, to achieve a long lasting therapy, muscle stem cell (MuSC) must be targeted by AAV. We observed that AAV9 can transduce MuSCs and edit the DMD gene in neonates but we do not detect editing in adult mice with our CRISPR platform. To improve MuSC targeting strategies, we developed and screened an AAV library to identify new AAV serotypes with the ability to transduce MuSCs. Randomized 21 nucleotide oligo sequences were inserted into the variable surface region of the capsid sequence, which encode a 7-mer peptide displayed on the surface of the viral capsid. Adult mice were injected with the library, and tissues were screened by PCR for the library insert. We identified unique AAV variants targeting MuSCs as well as the heart and differentiated skeletal muscle. A second round of screening of tissue-enriched AAV variants is ongoing to select AAV candidates for further validation. Identification of MuSC-tropic AAVs will provide a long-lasting supply of CRISPR-corrected MuSCs and offer a sustainable therapy for DMD.

THURSDAY, 9 SEPTEMBER, 4:15 – 4:45

POSTER SESSION II

Virtual Session, On Discord

DEFINING THE ROLE OF NUDIX5 IN THE MECHANOBIOLOGY OF OVARIAN CANCER

Angelina Flores

Cancer is a complex disease that involves significant changes in the genetic makeup and physical properties of cells and tissues to promote tumorigenesis. The ability of cancer cells to resist and survive mechanical challenges by regulating their mechanical properties is critical for cancer progression. To discover novel mechanoregulators, the Rowat lab invented a deformability screening platform using high throughput filtration. Nudix5 was identified as a potential regulator of cellular mechanical behaviors. Interestingly, Nudix5 is highly expressed across various types of tumors compared to normal tissues. Nudix5 is a member of the Nudix (nucleoside diphosphate linked moiety X) hydrolase superfamily, which has been shown to mediate chromatin remodeling and transcriptional changes in breast cancer cells through its role in synthesizing nuclear ATP. However, knowledge of the role of Nudix5 in regulating the mechanical behaviors of cells that contribute to cancer progression and its potential as a therapeutic target is lacking. Here, we show that Nudix5 regulates the deformability of human ovarian cancer cells: both pharmacologic inhibition and siRNA knockdown of Nudix5 cause cancer cells to be more deformable. In future work, I will determine mechanistically the role of Nudix5 as a mechanical regulator by testing the hypothesis that Nudix5 regulates the deformability and migratory behavior of cells through its role in promoting nuclear ATP production. Developing a mechanistic understanding of the interplay between metabolism and mechanobiology should advance our ability to manage the complex progression of cancer and design effective treatments.

Analysis of a voltage-gated sodium channel in lipids by MicroED

Cody Gillman

Voltage-gated sodium channels in humans (hNaVs) are responsible for the transmission of electrical signals in the heart, central and peripheral nervous systems, and muscle tissues. All hNaVs feature a sodium-specific pore that opens in response to depolarizing shifts in membrane potential, thereby generating the rising phase of action potential. Genetically inherited hNaV mutations cause dysregulation of channel gating and lead to cardiac arrhythmia, epilepsy, and pain disorders. Investigating the molecular structure of hNaVs allows us to better understand the molecular basis of these diseases and develop effective treatments. Recent breakthroughs in CryoEM technology in the past 5 years have finally enabled structural biologists to characterize the first 3D molecular structures of hNaVs. However, none of the structures published to date represent hNaVs within a lipid bilayer to mimic the native plasma membrane environment. The use of detergents to solubilize hNaVs in these studies may have allowed the protein to adopt low-energy (i.e. non-native) conformations. This project aims to capture the first structures of a hNaV isoform while stabilized by a lipid bilayer. I anticipate these structures to represent the ion channel in native-like conformations and to recapitulate the interactions that take place with annular lipids in the native plasma membrane. Lipid-based crystallization techniques will be utilized to produce lipid-embedded microcrystals, which will be subjected to Microcrystal Electron Diffraction (MicroED) to determine high-resolution crystal structures.

Structural investigation of a type III polyketide synthase involved in cannabinoid biosynthesis

Kristofer Gonzalez-DeWhitt

Phytocannabinoids from Cannabis sativa L. are natural plant products with therapeutic potential in humans. Rapidly changing Cannabis legislation has fostered a burgeoning pharmaceutical cannabinoid industry, which is projected to be worth USD 5.7 billion by 2027. To keep pace with rising demand, research groups are working to develop heterologous expression methods for cannabinoid production. Current strategies show promise but suffer from low product yields. In this biosynthetic pathway, a plant type III polyketide synthase referred to as tetraketide synthase (TKS) catalyzes a highly modular reaction to generate essential precursors in the biogenesis of more than 113 phytocannabinoids, such as cannabidiol and Δ9-tetrahydrocannabinol. The TKS-catalyzed reaction is prone to forming premature hydrolysis by-products that derail phytocannabinoid biosynthesis. Preliminary experiments employing X-ray protein crystallography, structure-guided mutagenesis, and high-performance liquid chromatography provide critical insight into this highly modular reaction. Crystal structures of catalytically active TKS provide the first mechanistically relevant view of the TKS substrate-binding pocket. Mutations of specific residues within this pocket dramatically alter the relative ratios of polyketides produced during the TKS-catalyzed reaction. These preliminary experiments have enabled us to identify key residues controlling polyketide formation. Future experiments will continue to interrogate the TKS substrate-binding pocket to identify combinations of residues that will improve reaction yields for specific cannabinoids. These results will have broad implications for enabling biosynthetic approaches to cannabinoid production.

The Effects of Chemical Conjugation on CCMV Virus-Like Particle Assembly

Nina Harpell, Jerrell Tisnado, Anilu Duran-Meza, Cheylene Tanimoto, William Gelbart, Jose Rodriguez

In vitro reconstituted Virus-Like Particles (VLPs) provide an effective and versatile platform for packaging, protecting, and delivering RNA. In particular, the capsid proteins (CPs) of plant RNA viruses such as Cowpea Chlorotic Mottle Virus (CCMV) have been shown to self-assemble with heterologous RNA into perfect RNAse-resistant T=3 icosahedral VLP structures. While these VLPs can enter the cell through various non-specific mechanisms, conjugating them to targeting ligands would enable therapeutic mRNAs, for example, to be delivered to specific organs or cell types. In my research I seek to study the assembly competence of CCMV VLPs when a select number of cysteinylated CP is introduced into the VLP for maleimide chemical conjugation. First, I will introduce a cysteine mutant into the CCMV CP followed by VLP assembly using mixtures of WT and cysteinylated CP. Next, I will use Cryo-EM for high resolution reconstructions of these “dilution” VLPs to identify structural changes in the capsid compared to the WT virus. Finally, I will assess whether fluorescent dye-conjugate CP incorporated into the VLP assembly mix results in VLP formation. This research will identify the extent to which in vitro reconstituted VLPs can be chemically modified while retaining their original function of RNA protection and delivery.

Chemical degradation of the SARS-CoV-2 protein nsp14

Ashley R. Julio, Flowreen Shikwana, Nikolas R. Burton, Jian Cao, Keriann M. Backus

SARS-CoV-2, the virus responsible for COVID-19, has caused millions of deaths worldwide. The long term effectiveness of currently available COVID-19 vaccines is unknown given the rapid emergence of new variants, making alternative antiviral strategies highly desirable. Non-structural protein 14 (nsp14) of SARS-CoV-2 represents a promising and novel target for such therapies. Nsp14 harbors an exonuclease domain that functions to proofread viral RNA during replication, and is thus essential for high fidelity RNA synthesis and removal of nucleotide analogues. By employing a small chemoproteomic screen, we have identified a small molecule, JC19, that induces rapid degradation of nsp14. Our studies reveal that JC19 likely targets nsp14 and a host protein, which leads to a subsequent decrease in intracellular levels of nsp14. Preliminary data suggests that protein disulfide isomerase 1 (PDIA1), which facilitates the folding of proteins in the endoplasmic reticulum, is a putative target of JC19, and is likely involved in activating the unfolded protein response (UPR) upon JC19 treatment. Through medicinal chemistry efforts, we have developed an even more effective analogue of JC19, demonstrating that the small molecule can be optimized for increased potency. Collectively, our studies reveal that JC19 may serve as a starting point for the development of alternative antiviral agents which function by degrading viral proteins.

Subcellular localization of neurotransmitter receptors during circuit assembly is a cell-type specific process

Alexander J. Kim, Piero Sanfilippo, Juyoun Yoo, Harry Bevir, Pegah Mirshahidi, S. Lawrence Zipursky

During circuit assembly in the developing brain, neurons undergo dynamic expression of many neurotransmitter receptors and subunits, which they use to establish a series of postsynaptic domains. To achieve precise localization of these receptors, neurons are thought to rely on specialized trafficking using a series of adaptor and scaffolding proteins, which are regulated by activity-dependent processes. However, our understanding of the molecular logic that determines synapse composition between classes of inputs remains limited. The Drosophila central nervous system demonstrates particularly striking specificity of synapse types, and brain wiring is accomplished through a combination of genetic programs and patterned neural activity in a highly stereotyped fashion. New tools have been developed to study the localization of endogenously expressed neurotransmitter receptors at single-cell resolution, providing a window into the process of synaptogenesis and the establishment of variously defined postsynaptic sites in the brain. Our studies demonstrate that neurotransmitter receptors are precisely localized to distinct dendritic domains within neurons that can be mapped to connectome data. We also find that localization can vary between receptors and across cell types, occurring at either an early stage of synapse formation, or during a late period of widespread spontaneous neural activity. This process varies between cell and receptor types. We speculate that neurotransmitter receptor localization at later stages of development is dependent upon spontaneous activity, whereas early localization plays an instructive role in synapse formation. Future studies will provide insight into the mechanisms regulating neurotransmitter receptor localization and their participation in circuit assembly.

INO80 and MOT1 silence transcription at replication origins and prevent DNA damage in Non-Small Cell Lung cancer

Michael Kronenberg, Fei Sun, Xianglong Tan, Michael Carey

The chromatin remodeler INO80 functions as a molecular swiss army knife, capable of regulating various DNA metabolic processes such as transcription, replication, and DNA repair.

Recent work has revealed that INO80 is upregulated across Non-Small Cell Lung Cancer (NSCLC) samples and is critical for NSCLC proliferation. However, it remains unclear how INO80 mechanistically facilitates lung cancer growth. Interestingly, emerging evidence has demonstrated that INO80 functions broadly to prevent DNA damage during DNA replication across a spectrum of cancer types. Furthermore, work in our own lab has revealed a novel function of INO80 and the transcriptional regulator MOT1 in preventing transcription-replication collisions (TRCs) and subsequent DNA breaks through silencing pervasive transcription at yeast replication origins. We hypothesized that INO80 binds at replication origins in NSCLC and silences transcription, thus preventing TRCs and toxic DNA breaks in tumor cells. Our findings show that INO80, along with MOT1, binds at putative replication origins in the A549 NSCLC cell line and silences transcription at these locations. Moreover, INO80 depletion in A549 cells induces replication stress, stabilizes p53, upregulates yH2Ax, and increases the expression of apoptotic genes, suggesting activation of the DNA damage response. Our findings additionally show that DNA damage induced by INO80 depletion is replication-dependent, and that MOT1 depletion in tandem with INO80 inhibition amplifies induced genotoxicity in tumor cells. Fascinatingly, depletion of INO80 in a normal lung epithelial cell line shows no growth inhibitory or genotoxic effects, suggesting INO80 inhibition selectively induces DNA damage and cytotoxicity in NSCLC cells. Collectively, our work demonstrates that INO80 functions as a genome protectant in NSCLC, potentially through preventing transcription-replication collisions at replication origins.

Structure-based design of antisense oligonucleotides that inhibit SARS-CoV-2 replication

Yan Li

Antisense oligonucleotides (ASOs) are an emerging class of drugs that target RNAs. Current ASO designs strictly follow the rule of Watson-Crick base pairing along target sequences. However, RNAs often fold into structures that interfere with ASO hybridization. Here we developed a structure-based ASO design method and applied it to target severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Our method makes sure that ASO binding is compatible with target structures in three-dimensional (3D) space by employing structural design templates. These 3D-ASOs recognize the shapes and hydrogen bonding patterns of targets via tertiary interactions, achieving enhanced affinity and specificity. We designed 3D-ASOs that bind to the frameshift stimulation element and transcription regulatory sequence of SARS-CoV-2 and identified lead ASOs that strongly inhibit viral replication in human cells. We further optimized the lead sequences and characterized structure-activity relationship. The 3D-ASO technology helps fight coronavirus disease-2019 and is broadly applicable to ASO drug development.

Uncovering roles for the human gut microbiome in protecting against pediatric refractory epilepsy

Gregory R. Lum, Christine A. Olson, Beck Reyes, Joyce H. Matsumoto, Jorge R. Paramo, and Elaine Y. Hsiao

The ketogenic diet (KD) is a mainstay clinical treatment for children with refractory epilepsy; however, it is difficult to implement, maintain and manage. Exactly how the KD reduces seizure symptoms when other anti-epileptic drugs are ineffective is poorly understood. The microbiome is a key intermediary between diet and host metabolism, neural activity, and behavior. Recent studies report that the KD modifies the gut microbiome and mediates the anti-seizure effects of the KD in mice. Whether these pre-clinical findings are relevant to human pediatric epilepsy and the clinical KD remains unclear. This study aims to determine effects of the human gut microbiome from pediatric epilepsy patients in mediating the anti-seizure effects of the clinical KD. Matched fecal samples from pediatric epilepsy patients were collected before starting clinical KD treatment (pre-KD) and at 1-month after initiation of KD therapy (post-KD) and each transplanted into cohorts of germ-free (GF) mice, and resultant humanized mice were tested for seizure susceptibility. Remarkably, mice colonized with the post-KD samples exhibited increased seizure thresholds compared to those colonized with matching pre-KD samples. Metagenomic profiling of patient donor and recipient mouse fecal material revealed key differences in functional potential between pre-KD and post-KD microbiomes that correlate with seizure protection. These preliminary findings suggest that diet-induced changes in the human gut microbiome may contribute to seizure protection in response to the clinical KD.

OXPHOS activity classifies metabolic dependencies in Osimertinib naïve and resistant *EGFR*-mutant lung tumors

Brandon Nadres and David B. Shackelford

Lung cancer is the deadliest cancer worldwide, with non-small cell lung cancers (NSCLC) accounting for 85% of these cases. One of the most common subtypes of NSCLC is lung adenocarcinoma (LUAD), with around 30% of LUADs being driven by oncogenic mutations in EGFR. The best therapeutic option for LUAD patients that carry an *EGFR*-activating mutation is targeted therapy, such as the current 1st line FDA approved EGFR inhibitor, Osimertinib. Osimertinib provides patients with an average of 18 months of progression-free survival, however, many of these patients will relapse because of acquired resistance. Previous studies have focused on utilizing these small molecule inhibitors to treat EGFR-mutant LUADs, but none have looked at their metabolic dependencies as a point of vulnerability. We hypothesized that the OXPHOS and glycolytic signatures in *EGFR*-mutant LUADs would determine sensitivities to metabolic treatments. Since these treatments most likely be given to patients that have already received Osimertinib treatment, we generated *EGFR*-mutant cell lines that were either naïve or resistant to Osimertinib treatment. We then functionally profiled the OXPHOS and glycolytic signatures of these *EGFR*-mutant cell lines and matched their profiles with cell viability data after complex I, complex II, glycolysis or glutaminolysis inhibition. We are further testing this strategy in mouse models, by utilizing PET imaging to classify glucose (18F-FDG) and OXPHOS (18F-BnTP) dependencies to determine the metabolic treatment for the mice to receive. This study will help the field rethink therapeutic strategies for *EGFR*-mutant lung cancer.

Elucidating mechanisms of alphaviral evasion from zinc finger antiviral protein (ZAP)

LeAnn P. Nguyen, Emily Yang, Kelly S. Aldana, Melody M.H. Li

The type I interferon response forms the crucial first line of defense against viral infection by stimulating expression of antiviral genes. One such gene is zinc finger antiviral protein (ZAP), which inhibits a broad spectrum of viruses. ZAP recognizes viral CG dinucleotide motifs, recruits mRNA degradation machineries, and/or inhibits viral mRNA translation, but the exact viral determinants that sensitize a virus to ZAP inhibition remain unknown. We are particularly interested in ZAP's translational inhibition of alphaviruses, a group of arboviruses that can cause fever, arthritis, and encephalitis in humans. Alphaviruses exhibit a diversity of sensitivity to ZAP: Sindbis virus (SINV) and Ross River virus are more sensitive to ZAP inhibition than o'nyong'nyong virus (ONNV) and the highly pathogenic chikungunya virus. Although ZAP is a CG dinucleotide sensor, the CG contents of these viral genomes do not correlate with ZAP inhibition, suggesting additional viral determinants of sensitivity. We hypothesize that resistant alphaviruses A) contain sequence elements that escape detection by ZAP or B) encode viral antagonist(s) of ZAP. We are generating chimeras between SINV (ZAP-sensitive) and ONNV (ZAP-resistant) to identify viral non-structural gene region(s) that contain ZAP sensitivity determinant(s). To address parts A and B of our hypothesis, respectively, we are also assaying ZAP binding to different alphaviral RNA regions, particularly those with CG hotspots, and evaluating potential ZAP antagonism by alphaviral non-structural proteins. Our work will uncover novel alphaviral strategies of virulence, illuminate mechanisms of host pathogen recognition, and provide new targets for the development of antiviral therapies.

Recognizing covalently damaged proteins for proteolysis through PCMTD1, an isoaspartyl-specific E3 ligase

Eric Z. Pang, Rebeccah A. Warmack, Esther Peluso, Joseph Ong, Jonathan D. Lowenson, Jorge Torres, Jose Rodriguez, and Steven G. Clarke

Cellular proteostasis is regulated in large part by the ubiquitination-proteasomal pathway. Here, E3 ligases control the proteolytic fate of proteins through targeted ubiquitination. No E3 ligases characterized to date appear to recognize age-damaged proteins. However, work in the Clarke lab suggests that the human protein carboxyl methyltransferase domain-containing protein 1 (PCMTD1) is a potential E3 ligase that ubiquitinates proteins damaged by spontaneous asparagine and aspartic acid isomerizations for proteolysis. PCMTD1 contains an N-terminal domain with L-isoaspartate and AdoMet binding motifs that are homologous to PCMT1, an L-isoaspartyl methyltransferase repair enzyme. This protein also contains a unique C-terminal domain with SOCS box ubiquitin ligase recruitment motifs found in substrate receptor proteins belonging to Cullin family E3 ligases. We found PCMTD1 interacts specifically with AdoMet and components of the E3 Cullin-RING ligase complex but does not exhibit L-isoaspartyl methyltransferase activity necessary for protein repair. However, the protein, in combination with Cullin-RING ligase

proteins, can reduce levels of L-isoaspartate substrates detected by PCMT1 in vitro.

PCMTD1 may therefore provide an alternative L-isoaspartate maintenance pathway by acting as an L-isoaspartyl specific E3 ligase. We aim to fully characterize PCMTD1's interactions, validate its molecular activity, and determine its structure with contemporary structural biology techniques. Studying PCMTD1 is vital because accumulations of damaged proteins coincide with proteostatic perturbations and aging-related health decline. These damages also enhance the formation and stability of amyloid fibrils which are hallmarks of neurodegenerative diseases. Fully understanding PCMTD1's role at the intersection of protein damage and proteolysis will lead to developing therapeutic strategies towards the restoration of cellular proteostasis and overall well-being.

Insight into how pathogenic Streptococcus pyogenes scavenges heme iron from human hemoglobin

Jess Soule, Ramsay A. MacDonald, Román Aguirre, Sunny H. Wu, Jose A. Rodriguez, Robert T. Clubb

The Gram-positive obligate pathogen Streptococcus pyogenes causes an estimated 7 million infections and 500,000 deaths annually, and rising reports of antibiotic resistant strains necessitate novel therapeutics for combatting infections. These bacteria require the redox-active nutrient iron for vital cellular processes, but the availability of free iron is limited at the host pathogen interface, mandating a means of obtaining this essential metal from alternate iron-containing molecules. Gram-positive pathogenic bacteria have evolved systems to acquire iron from host hemoglobin (Hb), which houses ~75-80% of the total iron in the human body within its heme prosthetic groups. In S. pyogenes, the membrane-anchored receptor Shr captures Hb at the cell surface using two Hb-interacting domains (HIDs) in its N-terminal region. The Hb receptor additionally contains two near iron transporter (NEAT) domains within its C-terminal region. While NEAT domains in other bacterial species have been demonstrated to have both Hb binding and heme scavenging capabilities, the roles of the NEAT domains in S. pyogenes Shr have yet to be defined. Here we present biochemical studies toward determing the function of the Shr NEAT domains. Stopped-flow kinetic data has been acquired to report on the minimum domain requirement for capturing heme from Hb and to analyze the function of individual domains in the context of the Shr receptor's role in iron acquisition.

Determining the Role of Mammalian Formin FHOD3 in Cardiomyocytes

Dylan Valencia, Margot Quinlan, & Atsushi "Austin" Nakano

A highly organized structure in striated muscle cells, known as the sarcomere, is responsible for contractions. The mammalian formin Formin HOmology Domain containing 3 (FHOD3) is important for sarcomere development and maintenance in cardiomyocytes. However, the specific role of FHOD3 in sarcomere formation and maintenance remains unclear. The objective of this research is to better understand which biochemical activities of FHOD3 are necessary or sufficient for sarcomere formation and maintenance in cardiomyocytes. Using bulk actin assembly assays and seeded elongation assays, we found that FHOD3 is a strong nucleator and weak elongator in vitro. We also performed a proof-of-principle rescue experiment in cardiomyocytes, in which endogenous FHOD3 is knocked down with siRNA, followed by adenoviral infection to drive exogenous expression of wild-type FHOD3. This rescue experiment paves the way for future rescue experiments using function-separating mutants of FHOD3 to understand whether nucleation strength or elongation activity is more important for proper sarcomere formation in cardiomyocytes. This work will help us better understand the mechanisms by which many formins build specific structures in vivo.

cAMP signaling during the intracellular infection cycle of Trypanosoma cruzi

Daniel Velez-Ramirez

Trypanosoma cruzi is the causative agent of Chagas disease, a vector-borne disease distributed along the American continent. Chagas disease manifests clinically as cardiovascular disease, characterized by hypertrophy of heart, esophagus and colon. Congestive heart failure is the main cause of death (58%) in Chagas patients, whereas cardiac arrhythmias and unexpected deaths add another 36%. A major cause of heart pathology in Chagas disease damage is caused by the host immune system, as it attacks chronically infected tissue. Therefore, pathology of the disease is a direct consequence of the ability of the parasite to invade host cells, so it can establish chronic infection. To achieve this, T. cruzi must sense and adapt to the host environment, but the underlying mechanisms are poorly understood. In particular, parasite signaling pathways used to sense and transduce signals from the host environment are most completely unknown. Several lines of evidence suggest T. cruzi cAMP signaling is important for host cell invasion, differentiation and persistent infection, which in turn underlies heart tissue pathology of Chagas disease. A transcriptome analysis revealed that mRNA of proteins involved in cAMP metabolism, i.e. adenylate cyclases and phosphodiesterases, are either upregulated or downregulated during the intracellular infection cycle. In fact, the phosphodiesterases have flagellar homologs with known cAMP signaling functions in a related parasite. This suggests that cAMP might fluctuate during as T. cruzi invades, differentiates, and multiplies inside the host cells.

Computational analysis and biochemical characterization of *COQ5* single nucleotide variants

Sining Wang, Eric Z. Pang, Steven G. Clarke, Catherine F. Clarke

The *COQ5* gene encodes an *S*-adenosylmethionine (AdoMet)-dependent enzyme responsible for catalyzing the C5 methylation of the coenzyme Q (CoQ) precursor, 2-methoxy-6-polyprenyl-1,4-benzoquinol ($DDMQH_2$), in humans and in the yeast *Saccharomyces cerevisiae*. Coq5-deficient yeast lack CoQ and exhibit respiratory deficiency. While it is known that primary CoQ deficiency in humans can cause mitochondrial, cardiovascular, kidney, and neurodegenerative disorders, clinical data on the *COQ5*-associated CoQ deficiency in humans have been limited. Here, we identify conserved and functional regions in human COQ5 based on multiple sequence alignment and available crystal structures in yeast. We examine human missense single nucleotide variants (SNV) reported in Missense3D-DB, most of which have unknown clinical significance, and locate potentially deleterious SNVs. Next, we will analyze the structural and functional effect of these SNVs using homology modeling and a wider variety of missense mutation classifiers. Top-ranking SNVs will be selected for *in vitro* and *in vivo* biochemical characterization to study their

catalytic activity and ability to stabilize other members of the CoQ synthome. Our results will help overcome the limitations in structural data availability and shed light on the relationship between the structure and function of the polypeptides encoded by yeast and human COQ5.