SEPTEMBER 13-14, 2023

2023 MBI ANNUAL RETREAT & RESEARCH CONFERENCE

UCLA & THE HUNTINGTON, PASADENA

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RETREAT COMMITTEE

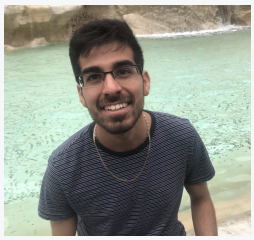
Jocelyn Rodriguez
Co-chair

Emily Smith
Co-chair

Martin Alcaraz Jr.
Subcommittee Lead

Dana Franklin
Subcommittee Lead

Carlos Galvan
Subcommittee Lead



Benancio Rodriguez
Subcommittee Lead

Gabriella Rubert
Subcommittee Lead

Znala Williams
Subcommittee Lead

Jessica Carstens-Kass

Nicolas Coral

Lindsey Dudley

Patricia Mendez

Allison Schiffman

Aniketa Sinha

Angela Sun

Sari Terrazas

Cindy Wang

Kailee Yoshimura

MBI STAFF

Helen Houldsworth

Stephanie Cuellar

Nadia Avila

2023 MBI RETREAT AGENDA

WEDNESDAY, SEPTEMBER 13 (UCLA)

8:30-9:00am **Coffee/Snacks** Northwest Campus Auditorium

9:00am **Welcome by Hilary Coller, PhD**

9:15-10:45am **Session 1 Talks: Mitochondria/Metabolism/Age-Related Diseases**
Session Chair: Andrew Goldstein, PhD

9:15-9:30am	Tara TeSlaa, PhD
9:30-9:45am	Jessica Carstens-Kass
9:45-10:00am	Kelsey E. Jarrett, PhD
10:00-10:15am	Jordan Tibbs
10:15-10:30am	Grigor Varuzhanyan, PhD
10:30-10:45am	Chao Peng, PhD

10:45-11:00am **Break**

11:00-12:00pm **Session 2 Talks: Gene Regulation and Computational Biology**
Session Chair: Thomas Vondriska, PhD

11:00-11:15am	Pavak Shah, PhD
11:15-11:30am	Brandon Boone
11:30-11:45am	Gabriella Rubert
11:45-12:00pm	Mykel Barrett

12:15-1:00pm **Lunch (RSVP Required)** Boyer 159
Pre-ordered t-shirts will be distributed during lunch.

1:00-2:30pm **Equity Pathways: Navigating Intersectional Identities, Inclusion, and Collaboration in Academia** Boyer 159

2:45-3:45pm **Career Panels**
Panel 1: Industry Focus Boyer 159
Moderated by Gabriella Rubert
Panel 2: Alternative Careers BSRB 154
Moderated by Allison Schiffman

4:00-6:00pm **Poster Session** Hershey Hall Hallway and South Courtyard

6:30pm **Social Night** Boyer 159

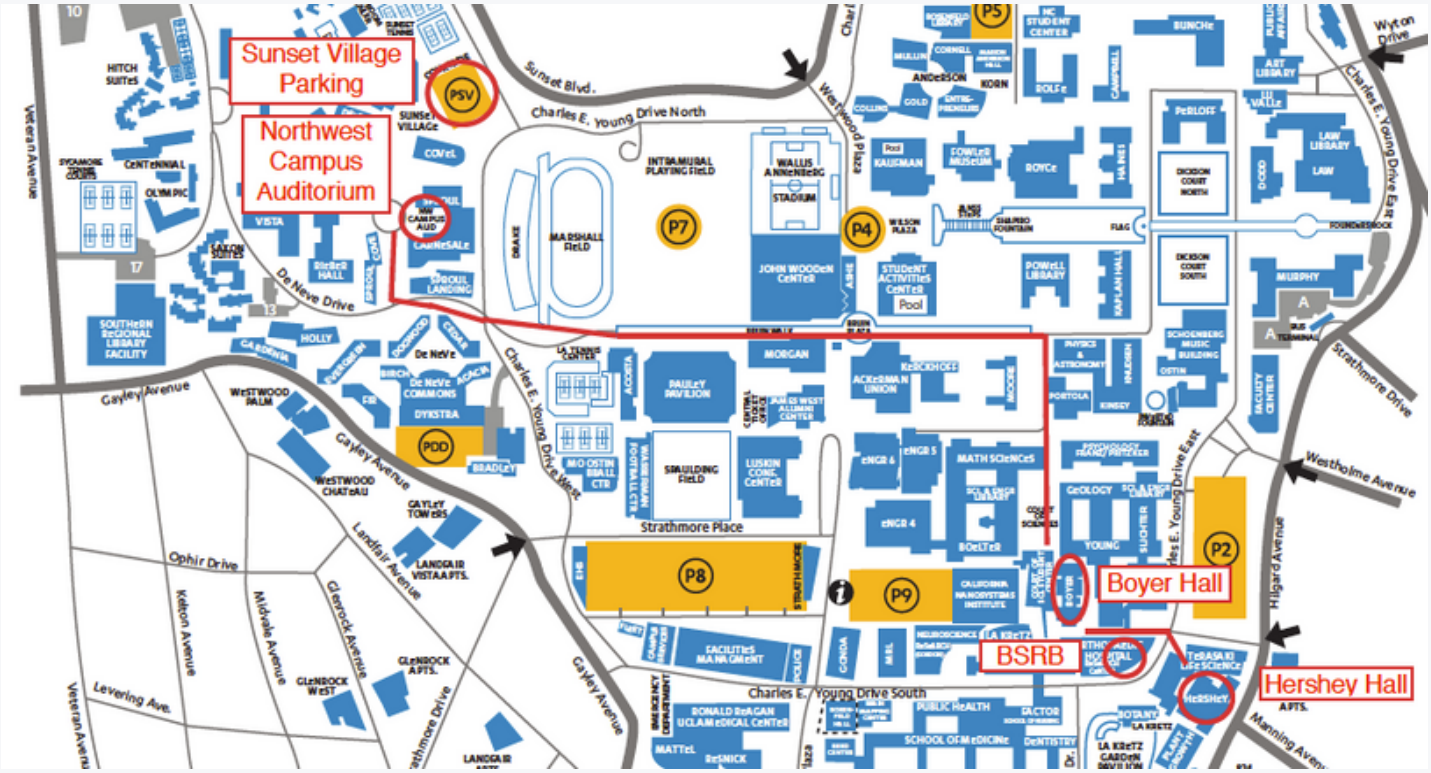
LOCATIONS

WEDNESDAY, SEPTEMBER 13 (UCLA)

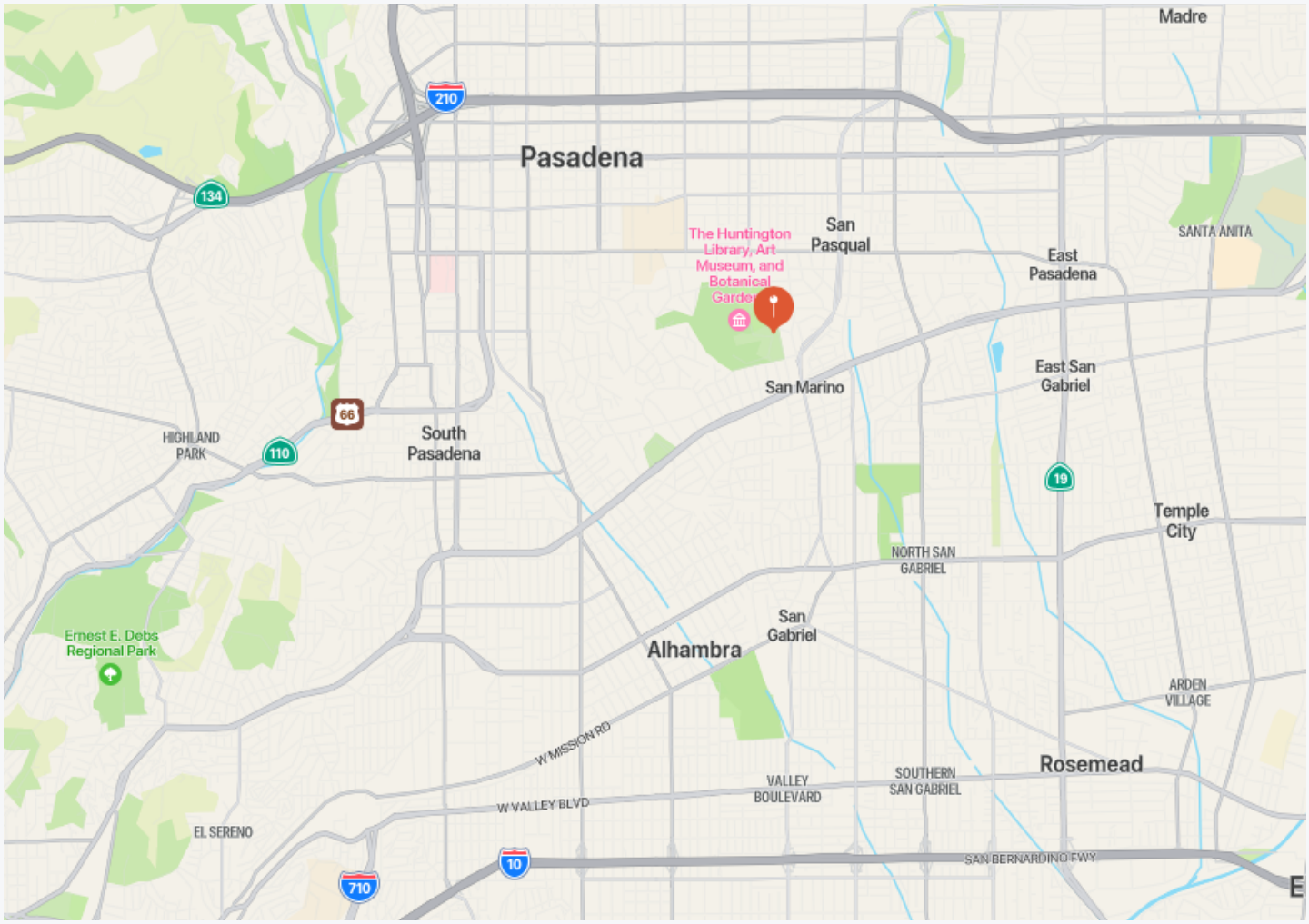
SESSION 1 AND 2 TALKS + WELCOME
9:00AM-NOON
NORTHWEST CAMPUS AUDITORIUM
350 DE NEVE DR, LOS ANGELES, CA 90024
PARKING AVAILABLE AT SUNSET VILLAGE (200 DE NEVE DR)

CAREER PANEL 2: ALTERNATIVE CAREERS
2:45-3:45PM
BSRB 154
615 CHARLES E YOUNG DR S, LOS ANGELES, CA 90095

POSTER SESSION
4:00-6:00PM
HERSHEY HALL HALLWAY/SOUTH COURTYARD
612 CHARLES E YOUNG DR E, LOS ANGELES, CA 90095



THURSDAY, SEPTEMBER 14 (THE HUNTINGTON, PASADENA)
1151 OXFORD RD, SAN MARINO, CA 91108
PARKING IS FREE



7:00am	**Meet Buses. Departure by 7:15am**	Outside P. Lot 2
8:00-9:00am	**Breakfast**	
9:00-10:30am	**Session 3 Talks: Immunity and Cell Development**	
	Session Chair: Anthony Covarrubias, PhD	
	9:00-9:15am	Isaias Roberson
	9:15-9:30am	Jonathan DiRusso
	9:30-9:45am	Joey Li
	9:45-10:00am	Julia Gensheimer
	10:00-10:15am	Angela Sun
	10:15-10:30am	Grasiela Torres
10:30-10:45am	**Break**	

THURSDAY, SEPTEMBER 14 (THE HUNTINGTON, PASADENA)

10:45-11:45am **Keynote Lecture**

Introduction by Jessica Carstens-Kass

"Red Queen rules: genetic conflicts shape biology"

Harmit Malik, PhD

Professor, Fred Hutchinson Cancer Research Center

Howard Hughes Medical Investigator

12:00-1:20pm **Lunch & Garden Tour**

1:30-2:45pm **Session 4 Talks: Infectious Diseases and Microbes**

Session Chair: Hung Ton-That, PhD

1:30-1:45pm Damia Akimori
1:45-2:00pm Breanna Walsh
2:00-2:15pm Ivan Salladay-Perez
2:15-2:30pm Karly Nisson
2:30-2:45pm Vivian Yang

2:45-3:00pm **Break**

3:00-4:00pm **Session 5 Talks: Structure/Chem Bio**

Session Chair: Catherine Clarke, PhD

3:00-3:15pm AJ Addae
3:15-3:30pm Alek Lotuzas
3:30-3:45pm Roger Castells Graells, PhD
3:45-4:00pm Jonathan Jih

4:00-4:15pm **Break**

4:15-5:00pm **Awards and Introduction of First Year Students**

DEI Awards
Poster Awards
Best WQE Award
Dissertation Award
Faculty Teaching Awards
Teaching Excellence Awards

5:00-6:30pm **Reception**

6:30pm **Buses Return to UCLA**

NETWORKING & EVENTS

EQUITY PATHWAYS: NAVIGATING INTERSECTIONAL IDENTITIES, INCLUSION, AND COLLABORATION IN ACADEMIA

Wednesday, September 13
Lunch: 12:15-1:00pm
Discussion Panel: 1:00-2:30pm
Boyer 159

Engage in an open forum to exchange ideas and explore solutions with both trainees and faculty, as we collectively work towards a more equitable academic landscape. Our panelists will delve into the intricate interplay between identity, inclusion and collaboration in academic spaces. As a community, we will share insights and strategies on fostering a diverse, thriving academic community and championing a meaningful mentorship.

Scan QR code or click here to join via Zoom

Coordinated by MBIDP students Martín Alcaraz Jr. and Patricia Mendez
DEI panel and lunch sponsored by the HHMI Gilliam Fellowships for Advanced Study (Bensinger/Kennewick)

CAREER PANEL

Wednesday, September 13
2:45-3:45pm
Concurrent Livestreaming Sessions

1: Industry Focus: Boyer 159
Moderated by Gabriella Rubert
2: Alternative Careers: BSRB 154
Moderated by Allison Schiffman

Junior researchers are invited to attend an insightful career panel with experts in industry and alternative careers. Our panelists will have answers to your questions about career paths in research, industry or unconventional careers.

Scan QR code or click here to join Panel 1 via Zoom

Scan QR code or click here to join Panel 2 via Zoom

POSTER SESSION

Wednesday, September 13
4:00-6:00pm
Hershey Hall Hallway and South Courtyard

Junior researchers will showcase novel research findings. Explore a variety of posters and connect with researchers across the fields of molecular biology.

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

PANELISTS

EQUITY PATHWAYS: NAVIGATING INTERSECTIONAL IDENTITIES, INCLUSION, AND COLLABORATION IN ACADEMIA

Stephanie Correa, PhD (she/her)

Former MBIDP DEI Advisor

2022 LS Excellence in Promoting Diversity & Inclusion Award Recipient

I am an Associate Professor in the Department of Integrative Biology at UCLA. I grew up and attended public schools in Bell Gardens, a city in southeast Los Angeles. My parents are immigrants from Ecuador and Colombia and I am a first generation college student. I earned a BA in Biology from Pomona College and a PhD in Neurobiology and Behavior from Cornell University. My dissertation research with Elizabeth Adkins-Regan and Patricia Johnson tested the effects of ovarian steroids on sex determination in birds. My postdoctoral research at Boston University Medical Center identified strain differences in the testis determination pathway in mice. My postdoctoral research with Holly Ingraham at UCSF identified neurons in the hypothalamus that regulate physical activity and body weight in female mice. In my lab at UCLA, I lead a diverse and integrative research team focused on understanding the effects of estrogens on the neural circuits that regulate temperature and energy balance. As a mentor, I am dedicated to helping students and postdocs forge their paths in academia, using a combination of challenge, support, and strategy. My roles across campus include our departmental Anti-Racism Taskforce, STEM related initiatives of the Latina Futures 2050 Lab, and advisory committees for the Division of Life Sciences and the Chicano Studies Resource Center. I run a joint lab with my husband and we have twin 7-year-old daughters.

Ketema Nnamdi Paul, PhD

2023 LS Excellence in Promoting Diversity & Inclusion Award Recipient

Dr. Ketema Paul, Professor in the Department of Integrative Biology and Physiology at UCLA, studies the genetic, molecular, and neural underpinnings of sleep. His primary interests consist of uncovering the mechanisms responsible for the negative effects of sleep deprivation. He also probes the origins of gender/sex differences in the ability to recover from sleep loss in order to develop therapeutic targets for sleep disorders that disproportionately affect women. After receiving his Bachelor of Science from Howard University, Dr. Paul studied neurobiology and circadian biology at Georgia State University in Atlanta, Georgia, where he received his doctorate. He completed a postdoctoral fellowship at Northwestern University in Evanston, Illinois, in 2006 at the Center for Sleep and Circadian Biology, after which he spent 10 years as a faculty member of the Neuroscience Institute at Morehouse School of Medicine. He joined the faculty at UCLA in the Division of Life Sciences in 2016.

Dr. Paul's current research involves applying a forward genetics approach to uncover the core genes responsible for sleep-wake regulation. Effective treatments for common sleep- wake disorders are elusive. Dr. Paul conducts a forward genetics approach to facilitate gene identification, taking advantage of natural variation in sleep-replete and sleep-deprived mice. These studies are expected to identify novel sleep regulatory genes and lead to the development of new therapeutic targets and improved treatments for sleep disorders.

PANELISTS

EQUITY PATHWAYS: NAVIGATING INTERSECTIONAL IDENTITIES, INCLUSION, AND COLLABORATION IN ACADEMIA

Edgar Perez

MBIDP-IMMP PhD Candidate
Dubinett Lab

Edgar Perez is a rising 5 th year graduate student in the MBI Department. Edgar is currently in Dr. Steven Dubinett's lab in the Department of Medicine, where his research project focuses on investigating immune signatures and their associations with metastatic mutational landscape and neoantigens. Since he started UCLA, Edgar has been in many leadership positions in varies different orgs such as SACNAS, DEI committee, Buddy System, and currently holding the position of MBIDP Student Rep. Before coming to UCLA, Edgar received his BS in Biology and his MS in Chemistry emphasis in Biochemistry from California State University Los Angeles. He started at UCLA as a research associate where he began his interest in immunology. His passion for DEI efforts is rooted in his past experiences in navigating life and academia while being a first-generation student, part of the LGBTQ community, and a DACA recipient. He is also passionate about mental and physical health and leads a workout group that meets every Monday throughout the year. His hobbies are weight training, swimming, hiking, and considers himself a big foodie.

JoAnn Roberts, PhD (she/her)

Program Director, Antiracist Educator Initiative, CEILS

Dr. Roberts is a Bermuda-born Jamaican and identifies as a Black Caribbean woman, a scientist, a vocal artist, and an educator. She is a Molecular & Cellular Biologist by training and has done research on both host-pathogen interactions in the oral cavity and stem cell research for corneal regeneration in the eye. She completed her postdoc as an IRACDA Scholar here at UCLA in the Department of Ophthalmology, but has since devoted her career to STEM Education & Research reform in Higher Ed. Therefore, she serves at UCLA as the Associate Director of JEDI in Teaching at CEILS (the STEM Teaching & Learning Center for Life & Physical Sciences). In this role she facilitates conversations on topics of equity, inclusion, justice, and antiracism in STEM teaching and research and builds programming and partnerships to support JEDI work.

Dr. Roberts is also part of a national initiative, NSF INCLUDES, that is driving institutional change in increasing diversity in STEM and enhancing the professional success of BIPOC STEM Faculty. She is the Founder & Director of a summer research program in vision science for HBCU students here at UCLA and is also facilitator of research mentoring training for faculty and postdocs here at UCLA through graduate programs in bioscience.

Ultimately, Dr. Roberts endeavors to be intentional in both her professional and personal life to elevate JEDI work in STEM and support others to do so as well.

PANELISTS

EQUITY PATHWAYS: NAVIGATING INTERSECTIONAL IDENTITIES, INCLUSION, AND COLLABORATION IN ACADEMIA

Claudio Villanueva, PhD

MBIDP DEI Advisor

Dr. Villanueva studies transcriptional pathways involved in nutrient sensing, gene expression, and lipid metabolism. His research aims to understand the role of the adipose tissue in the physiological and pathophysiological adaptation to nutrient overload. The adipose tissue can undergo dramatic restructuring in obesity, where there is cellular infiltration of various immune cell types and depletion of progenitor cells that give rise to new adipocytes. The recruitment of new adipocytes to the adipose tissue is essential to maintain metabolic homeostasis. His lab aims to understand how metabolic programs are transcriptionally regulated to identify new strategies to treat metabolic diseases like diabetes. Dr. Villanueva's lab is in the metabolism theme space, a collaborative group of investigators that bring together clinical faculty with basic scientists studying the links between metabolism and health. The Metabolism Research Theme was recently launched at UCLA as a strategic research initiative to position investigators in a collaborative environment to confront the diabetes epidemic with new treatments and knowledge for prevention. He completed his PhD with Bob Farese at UCSF and postdoctoral training at UCLA with Peter Tontonoz. Dr. Villanueva is involved in several efforts that support institutional transformation to have a long-lasting impact on equity, diversity and inclusion. As an Assistant Professor at the University of Utah, he helped to start a SACNAS chapter that began with a few graduate students, and now has 50+ trainees that participate regularly. He received the Inclusive Excellence Award at the University of Utah for developing and implementing innovative recruitment practices for the Bioscience Graduate Program, which led to an increase in recruitment and enrollment of underrepresented students, from an average of 6% to 30%. Dr. Villanueva was recruited to UCLA as a Mentor Professor, a recruitment strategy to identify candidates with both an excellent research record and experience in supporting initiatives that support a diverse student body. Dr. Villanueva is co-director of the NIH funded IRACDA program that is designed to train postdocs to acquire faculty positions in biomedical research. This program trains postdoctoral fellows to use innovative pedagogical practices that can be implemented in college courses to increase retainment of underrepresented groups in the sciences. He also leads the research mentor core for basic science for junior scientists at UCLA through LIFT-UP (Leveraging Institutional Support for Talented, Underrepresented Physicians and/or Scientists). He is also the faculty advisor for the MBI program and has been involved in several faculty hiring initiatives at UCLA. Dr. Villanueva has made every effort to advocate for groups historically underrepresented in science and to develop an innovative research program.

Ruhi Patel, PhD (she/her)

Postdoctoral Researcher, Hallem lab

Dr. Ruhi Patel is a postdoctoral scholar in Dr. Elissa Hallem's lab at UCLA. Ruhi received her B. Tech in Biotechnology at Anna University in Chennai, India, where she was born and raised. She earned her Ph.D. in Dr. Alison Frand's lab at UCLA, where she characterized the genetic pathways that time the molting cycle of C. elegans. As a postdoctoral fellow in the Hallem lab, she seeks to understand how mechanosensation enables soil-transmitted helminths to locate and infect their hosts.

KEYNOTE LECTURE

Thursday, September 14
10:45-11:45am
The Huntington

"RED QUEEN RULES: GENETIC CONFLICTS SHAPE BIOLOGY"

Harmit Malik, PhD
Professor
Fred Hutchinson Cancer Research Center
Howard Hughes Medical Investigator

Harmit Malik got his Bachelors in Technology in Chemical Engineering at the Indian Institute of Technology, Mumbai, India. There, he became interested in molecular biology due to Prof. K. K. Rao and in evolution by reading Richard Dawkins' "The Selfish Gene." He then moved to the US to get his Ph.D. in Biology, at the University of Rochester, NY, under the mentorship of Prof. Thomas Eickbush, to work on the evolution of retrotransposable elements. In 1999, he moved to Seattle to the Fred Hutchinson Cancer Center (the "Hutch"), to start his postdoc with Dr. Steve Henikoff on the evolution of centromeres and centromeric proteins. In 2003, he started his own lab at the Hutch, where he has been ever since. Together with his colleague Michael Emerman, Malik and his trainees have used an evolutionary lens to dissect and discover both primate antiviral as well as viral adaptation strategies. By taking advantage of viral "fossils" in animal genomes and intense episodes of ancient host gene adaptation, his work has helped found the field of Paleovirology. In 2009, he was appointed as an Early Career Scientist of the Howard Hughes Medical Institute and as a Full Investigator in 2013. He was awarded the 2017 Eli Lilly Prize in Microbiology, the most prestigious prize awarded by the American Society of Microbiology. and the 2022 Edward Novitski Prize by the Genetics Society of America for 'an extraordinary level of creativity and intellectual ingenuity in the solution of significant problems in genetics'. He was elected as a member of the US National Academy of Sciences in 2019, as a fellow of the American Association for the Advancement of Science in 2019, and as member of the American Academy of Arts and Sciences in 2022. He served as President of the Society for Molecular Biology and Evolution in 2021 and is currently President of the Drosophila board.

Unable to attend in-person? Click here to to view on Zoom.

CAREER PANEL 1 - INDUSTRY FOCUS

Wednesday, September 13
2:45-3:45pm
Boyer 159

Bethan Clifford, PhD (bcliff02@amgen.com)
LinkedIn
Senior Scientist, Amgen
Dr. Bethan Clifford began her scientific career at the University of Nottingham, UK, where she completed her BS and PhD studying the nitric oxide system in renal aging and hypertension. In 2016 Dr. Clifford began her postdoctoral work in lipid metabolism under the mentorship of Drs Tarling and Vallim at UCLA where she focused on the regulation of cholesterol by the E3 ubiquitin ligase, RNF130 and the role of FXR agonism in treating fatty liver disease. Having been drawn to biological sciences to understand human disease, Dr. Clifford now hopes to combine her expertise in metabolism and the cardiovascular system to develop new medications to combat cardiometabolic disorders. She is currently a Senior Scientist at Amgen Inc working in Research and Discovery in the Cardiometabolic pipeline.

Andrea Johnstone, PhD (ajohnstone@epicypher.com)
LinkedIn
Senior Director, Product Development, Epicypher
Andrea Johnstone, PhD is the Senior Director of Product Development at EpiCypher, a developer and provider of innovative technologies aimed to fully realize the potential of chromatin research to improve human health. Andrea earned her PhD in Neuroscience at the University of Miami Miller School of Medicine. After completing postdoctoral studies in epigenetics and drug development, she joined EpiCypher as a Research Scientist in the early stages of the startup biotech company. In her current role, she leads a team of Product Managers and coordinates with interdisciplinary stakeholders to commercialize new technologies and expand chromatin science into untapped research and clinical applications.

Natalie Chen, PhD (chenn23@gene.com)
LinkedIn
Postdoctoral Scholar, Genentech
Natalie Chen is a postdoctoral fellow in the Discovery Oncology department at Genentech. She did her PhD in the MCIP program at UCLA under Dr. Stephen Young, and investigated nuclear membrane ruptures in the context of nuclear lamin deficiencies. After her PhD, Natalie did postdoctoral training at Caltech investigating ER membrane protein biogenesis for one year before coming to Genentech.

CAREER PANEL 2 - ALTERNATIVE CAREERS

Wednesday, September 13
2:45-3:45pm
BSRB 154

Katharina Grötsch, PhD (katharinagrotsch@gmail.com)
LinkedIn
Senior Copywriter, Area 23
Kathy is a chemist, a science communicator, and loving mother to Dobby (her 3-year old chihuahua). During her PhD she worked on organic synthesis and drug discovery, particularly for compounds targeting the CB2 receptor. She is also the former President of Biotech Connection Los Angeles, an organization committed to fostering the connection between academia and the biotech industry in LA. During this time, she launched a new Biotech podcast, Inside Biotech, and discovered her passion for communicating complicated scientific concepts to various audiences. She now works at Area 23, an advertising agency that focuses on pushing creativity and invention in healthcare marketing.

Gabriel Hassler, PhD (gabriel.w.hassler@gmail.com)
LinkedIn
Associate Statistician, RAND Corporation
Gabriel Hassler (he/him) is an associate statistician at the RAND Corporation. He is interested broadly in computational Bayesian statistics and developing flexible yet highly structured statistical models that are scalable to big data. He has applied his methodological work to study relationships between viral genetics and clinical outcomes in HIV/AIDS, the evolutionary dynamics of the SARS-CoV-2/COVID-19 epidemic in North America, and the evolution of numerous other biological organisms over time. Gabriel received his B.A. in anthropology and biology at Washington University in St. Louis and his Ph.D. in biomathematics at UCLA.

Bridgett Kohno, PhD (bkohno@wsgr.com)
LinkedIn
Patent Agent, Wilson-Sonsini Goodrich & Rosati
JD Candiate, Duke Law
Bridgett is a patent agent at Wilson Sonsini Goodrich and Rosati, and a 1L at Duke Law. Bridgett received her PhD in Chemistry from UC Irvine, where she investigated nuclear quantum effects using Monte Carlo methods. She was also a fellow at the Invention Transfer Group at UC Irvine Beall Applied Innovation, where she assessed the patentability and marketability of technology developed at the university. As a patent agent, she drafts patent applications, prosecutes patent applications, and performs freedom to operate and patentability analyses. She also started her first year at Duke Law. In her spare time, she likes to go on runs, rock climb, and play with her little dog Cooper.

SPEAKER ABSTRACTS

Wednesday, September 13
Session 1: 9:15-10:45am

Chair: Andrew Goldstein, PhD

9:15-9:30am

IN VIVO ISOTOPE TRACING REVEALS NOVEL ASPECTS OF MAMMALIAN METABOLISM

Tara TeSlaa, PhD

Molecular & Medical Pharmacology

As a lab we are interested in 1) development of methods to measure metabolism in vivo, 2) how metabolic fluxes are altered in disease, and 3) how changes in metabolic flux alters cell fate and function.

9:30-9:45am

BROWN ALGAL MICROBIOMES AND THEIR CULTIVATION THROUGH ANTIBACTERIAL PROPERTIES OF THE HOST CELL WALL

Jessica Carstens-Kass

IMMP, Braybrook lab

Brown algae (Phaeophyceae) are phylogenetically distant from green algae and red algae. After diverging around three million years ago from a single unwalled cell common ancestor, convergent evolution has given rise to cell walls in all algae and plants, but with important structural differences: brown algal cell walls are composed predominantly of unique components, called alginate and fucose-containing sulfated polysaccharides (FCSPs). The latter have exhibited antibacterial activity against both Gram-negative and Gram-positive bacteria, including Escherichia coli and Staphylococcus aureus, but this mechanism of action is unknown. Recent characterizations of algal microbiomes have revealed they are host-specific and distinct from the surrounding seawater microbial profile. We hypothesize that FCSP antibacterial activity allows brown algae to cultivate a specific microbiome which is essential for brown algal health. With a better understanding of how brown algal microbiomes are affected by and benefit their hosts, we will help seaweed farmers improve their growing techniques for these economically and environmentally significant species. This is especially relevant in the context of the United States' fast-growing aquaculture industry. To explore the ability of brown algae to cultivate a microbiome, we first collected algal tissue from a common microenvironment for whole microbiome sequencing. This revealed that the brown algal microbiome has lower α-diversity than green and red algal microbiomes. Using innovative marine agar recipes which include brown algal carbon sources (food for bacteria), we have also directly cultivated and identified 87 bacterial species from algae in natural environments on the California coast. We are currently testing the ability of these isolates to grow in the presence of FCSPs; we predict that brown algal microbiome isolates will be more resistant to FCSP antibacterial activity compared to green and red algal microbiome isolates. Finally, we are bioinformatically investigating metabolic explanations of resistance and susceptibility to FCSPs in algal microbiomes.

9:45-10:00am

MANIPULATING BILE ACID PRODUCTION USING GENOME EDITING TO MODULATE CARDIO-METABOLIC DISEASE

Kelsey E. Jarrett, PhD

Tarling/ Vallim lab

Kelsey E. Jarrett, Heidi M. Schmidt, Alvin P. Chan, Madelaine C. Brearley-Sholto, Angela Cheng, Michelle E. Steel, Rodrigo Baltazar-Nunez, Chloe A. Borja, Anne Iwata, Kevin J. Williams, Elizabeth J. Tarling, Thomas Q. de Aguiar Vallim

Cardiovascular disease (CVD) remains the leading cause of death in the United States, in part due to consumption of a high cholesterol Western diet. Absorption of dietary cholesterol is facilitated by detergents, called bile acids, which are produced in the liver. Bile acids are a functionally diverse group of molecules with

different capacities to affect lipid absorption. We hypothesize that specific changes in the bile acid pool will change cholesterol absorption and alter development of CVD. To alter the bile acid pool, we disrupted two enzymes using liver-directed AAV-CRISPR: Cyp7a1 and Cyp8b1. Cyp7a1 catalyzes the rate-limiting step of bile acid synthesis, whereas Cyp8b1 mediates production of cholic acid. Consequently, we show that loss of CYP7A1 in adult mice reduces the size of the bile acid pool while loss of CYP8B1 alters the composition. To study atherosclerosis, we co-disrupted Cyp7a1 or Cyp8b1 concurrently with Low Density Lipoprotein Receptor (Ldlr) to induce hypercholesterolemia on a Western diet for 20 weeks. Loss of these bile acid enzymes in males resulted in atheroprotection by different mechanisms. Loss of CYP7A1 resulted in modest protection from atherosclerosis and reduced cholesterol absorption. Loss of CYP8B1 caused modest reductions in plasma cholesterol and moderate atheroprotection but did not alter cholesterol absorption. We hypothesize that these differences in atherosclerosis observed with different bile acid profiles are due to systemic changes in cholesterol metabolism that are driven, in part, by changes in cholesterol absorption. Our studies suggest specific changes in bile acids have the potential to become a novel precision treatment for cardio-metabolic diseases.

10:00-10:15am

DEVELOPING SMALL MOLECULE MODULATORS FOR MITOCHONDRIAL PROTEIN TRAFFICKING

Jordan Tibbs
CBI, Koehler lab

Primary Hyperoxaluria type 1 (PH1) is an autosomal recessive metabolic disorder that is caused by the accumulation of glyoxylate in the liver. Typically, glyoxylate is detoxified by the enzyme alanine-glyoxylate aminotransferase (AGT) through conversion to glycine. However, different mutations in AGT result in the accumulation of glyoxylate, which is converted to oxalate. A large subset of PH1 cases is caused by mutations in AGT that result in mistargeting of functional AGT from the peroxisome to the liver. This disorder provides a system to investigate mechanisms of protein targeting to mitochondria and peroxisomes. I hypothesize that small modulators can be used to alter protein trafficking to mitochondria in mammalian cells. I developed a new liver cell model for AGT in HepG2 cells by engineering the cells to stably express mutant AGT and then determine whether the small molecules restored trafficking of mutant AGT to peroxisomes from mitochondria. This study will provide deeper insight into key intracellular metabolic processes and yield a better understanding of mitochondrial import that may result in therapeutics for disorders of a similar pathology.

10:15-10:30am

PGC1A-INDUCED MITOCHONDRIAL RESPIRATION AS A NOVEL THERAPEUTIC TARGET FOR SMALL CELL NEUROENDOCRINE CANCERS OF THE PROSTATE AND LUNG

Grigor Varuzhanyan, PhD
Witte lab

Small cell neuroendocrine prostate cancer (NEPC) is the deadliest form of prostate cancer and novel therapeutic strategies are needed to improve patient outcomes. To identify new therapeutic targets, we recently defined the epigenomic and transcriptomic evolution during NEPC development using a human tissue transformation system termed PARCB. Multiomics approaches in a time course analysis of PARCB transformation revealed extensive chromatin and transcriptional changes as tumors progressed towards NEPC. Tracing with single-cell resolution revealed two distinct tumor lineages with either low (Type I) or high (Type II) neuroendocrine differentiation. However, the mechanisms that drive these two tumor lineages remain unclear. Here, we show that lineage determination during NEPC transformation is regulated by PGC1a, a master regulator of mitochondrial biogenesis and function. Type II PARCB tumors with high neuroendocrine differentiation have pronounced upregulation of PGC1a expression and oxidative phosphorylation (OXPHOS) expression and activity. Reverse genetics during PARCB transformation

indicated that PGC1a is functionally required for NEPC development. Genetic inhibition of PGC1a blocked PARCB tumorigenesis, reduced prostate organoid growth, and reduced viability of cultured NEPC cell lines. Conversely, overexpression of PGC1a promoted PARCB tumorigenesis, and tumors with high PGC1a expression had high OXPHOS expression and neuroendocrine differentiation. Pharmacological inhibition of OXPHOS had a similar anti proliferation effect in cultured NEPC cell lines. These results were corroborated in multiple clinical datasets. Furthermore, we found elevated PGC1a and OXPHOS expression in patient tumors post-androgen deprivation therapy (ADT) (Rajan et al, 2014) and in clinical NEPC tumors compared to CRPC (Beltran, 2016). These data suggest that ADT can induce PGC1a/OXPHOS expression, which may be necessary for NEPC development. PGC1a is also highly expressed in clinical small cell lung cancer (SCLC) and OXPHOS gene deletion has the strongest killing effect in SCLC cell lines compared to NSCLC. Thus, we identify PGC1a-induced OXPHOS as a new therapeutic target for NEPC and other neuroendocrine cancers.

10:30-10:45am

POST-TRANSLATIONAL MODIFICATIONS OF SOLUBLE A-SYNUCLEIN REGULATE THE AMPLIFICATION OF PATHOLOGICAL A-SYNUCLEIN

Chao Peng, PhD
Neurology
Shujing Zhang, Ruowei Zhu, Christopher K. Williams, Shino Magaki, Harry V. Vinters, Hilal A. Lashuel, Benjamin A. Garcia, , E. James Petersson, John Q. Trojanowski, Virginia M.-Y. Lee Chao Peng

Cell-to-cell transmission and subsequent amplification of pathological proteins promotes neurodegenerative disease progression. Most research on this has focused on pathological protein seeds, but how their normal counterparts, which are converted to pathological forms during transmission, regulate transmission is less understood. Here, we show in cultured cells that phosphorylation of soluble, non-pathffological α-Syn at previously identified sites dramatically affects the amplification of pathological α-Syn, which underlies Parkinson's disease (PD) and other α-Synucleinopathies, in a conformation- and phosphorylation site-specific manner. We performed LC-MS/MS analyses on soluble α-Syn purified from PD and other α-Synucleinopathies, identifying many novel α-Syn post-translational modifications (PTMs). In addition to phosphorylation, acetylation of soluble α-Syn also modified pathological α-Syn transmission in a site- and conformation specific manner. Moreover, phosphorylation of soluble α-Syn could modulate the seeding properties of pathological α-Syn. Our study represents the first systematic analysis how of soluble α-Syn PTMs affects the spreading and amplification of pathological α-Syn, which may affect disease progression.

Wednesday, September 13
Session 2: 11:00-12:00pm

Chair: Thomas Vondriska, PhD

11:00-11:15am

GRAPH METRICS FOR THE ANALYSIS OF CELL LINEAGES

Pavak Shah, PhD
MCDB

High throughput experimental approaches are increasingly allowing for the quantitative description of cellular and organismal phenotypes. Distilling these large volumes of complex data into meaningful measures that can drive biological insight remains a central challenge. In the quantitative study of development, for instance, one can resolve phenotypic measures for single cells onto their lineage history, enabling joint consideration of heritable signals and cell fate decisions. Most attempts to analyze this type of data, however, discard much of the information content contained within lineage trees. In this work we introduce a generalized metric, which we term the branch distance, that allows us to compare any two embryos based on phenotypic measurements in individual cells. This approach aligns those phenotypic measurements to the underlying

flineage tree, providing a flexible and intuitive framework for quantitative comparisons between, for instance, Wild-Type (WT) and mutant developmental programs. We apply this novel metric to data on cell-cycle timing from over 1300 WT and RNAi-treated Caenorhabditis elegans embryos. Our new metric revealed surprising heterogeneity within this data set, including subtle batch effects in WT embryos and dramatic variability in RNAi-induced developmental phenotypes, all of which had been missed in previous analyses. Further investigation of these results suggests a novel, quantitative link between pathways that govern cell fate decisions and pathways that pattern cell cycle timing in the early embryo. Our work demonstrates that the branch distance we propose, and similar metrics like it, have the potential to revolutionize our quantitative understanding of organismal phenotype.

11:15-11:30am

DYNAMIC PROTEIN ACCUMULATION IS DIRECTLY LINKED TO GENE SILENCING DOWNSTREAM OF DNA METHYLATION

Brandon Boone
GREAT, Jacobsen lab

DNA methylation is well-studied across species, yet it is unclear how DNA methylation binding proteins facilitate gene regulation in plants. The MBD5/6 complex silences genes and transposable elements downstream of CG methylation (meCG) through genetically redundant methyl CpG-binding domain (MBD) proteins called MBD5 and MBD6, as well as three putative heat shock proteins (HSP): a HSP40 protein called SILENZIO (SLN) and two small HSPs (sHSPs) called ACD21 and ACD15. It is unclear why molecular chaperones would be associated with DNA methylation or why these proteins are essential for gene silencing downstream of DNA methylation. Using genomics, biochemistry, and microscopy, we demonstrate that all three HSPs are responsible for the proper accumulation and mobility of the MBD5/6 complex within heterochromatic compartments. We find that ACD21 and ACD15 serve two roles to both accumulate the MBD5/6 complex at high density meCG sites and recruit SLN to regulate the turnover of the MBD5/6 complex members once accumulated. We further demonstrate that accumulation of MBD6 occurs through protein-protein interaction as IDRs can enhance the heterochromatic accumulation of MBD6 but only in an ACD15 and ACD21 dependent process. Finally, using a dead Cas9 targeting technology we demonstrate that MBD5/6 complex assemblies can be utilized, in an ACD15 and ACD21 dependent process, as a tool to silence fwa, resulting in a change of flowering time. Our results demonstrate a direct link between accumulation of methylation binding proteins and gene silencing downstream of DNA methylation

11:30-11:45am

THE TRANSCRIPTION FACTOR BHLHE40 REGULATES LIPID METABOLISM IN THE LIVER AFTER FEEDING

Gabriella Rubert
GREAT, Tarling/ Vallim lab
Gabriella Rubert*,1,3, Angela Cheng1, Kelsey Jarrett2, Elizabeth Tarling1,2,3, Thomas Vallim1,2,3
Departments of Biological Chemistry1 and Medicine2, Molecular Biology Institute3

The intake of nutrients from the diet, particularly lipids, causes profound changes in many cells of the body as they rapidly adjust from low to highly elevated lipid levels. These nutrient-dependent transitions are regulated by transcriptional programs mediated by transcription factors that are not fully characterized. To explore nutrient-dependent transcriptional changes in the liver, a major site of lipid biosynthesis, our lab performed RNA-sequencing in the livers of mice that were fasted or fasted then re-fed diets containing either low or high level of lipids (Western-style diet). We identified 7,186 upregulated and downregulated genes between fasted and refed states. Interestingly, we saw unique and rapid transcriptional responses to the two diets within 4 hours. We next aimed to identify transcription factors regulated under these conditions to reveal potential mediators of these observed transcriptional changes. Our analysis identified BHLHE40 as a transcription factor whose expression is upregulated by refeeding

with the low-fat diet and further induced with the high-fat Western diet. We then generated hepatocyte-specific BHLHE40 knockouts using Bhlhe40fl/fl mice and adeno-associated virus-delivered Cre recombinase. We fasted and refed BHLHE40 L-KO mice as before and performed RNA-sequencing to determine which nutrient-dependent transitions are BHLHE40-dependent. We found that mRNA expression of cholesterol synthesis genes in the liver was no longer responsive to the low-fat diet refeed in BHLHE40 L-KO mice compared to wild-type. Furthermore, we saw a decrease in cholesterol synthesis following refeed in knockout compared to wildtype mice. Future experiments will address whether BHLHE40 induces expression of in vitro Luciferase expression via binding of promoter regions of cholesterol synthesis genes and ChIP-sequencing on mouse livers to identify genomic regions bound by BHLHE40 after fast and refeed with both low-fat and Western diet.

11:45-12:00pm

BIOINFORMATIC IDENTIFICATION OF OTX2 AND OC1 TRANSCRIPTION FACTOR BINDING SITE MUTATIONS IN THE THRBCRM1 AND THRBICR CIS-REGULATORY ELEMENTS WHICH QUANTITATIVELY AND SPATIALLY ALTER THRB TRANSCRIPTION DURING RETINAL DEVELOPMENT

Mykel Barrett
GREAT

Thrb gene products play a critical role in the establishment of unique cone cell subtypes. During retinogenesis, Thrb transcription is cis-regulated by the ThrbCRM1, ThrbCRM2, and ThrbICR, enhancer elements. Interestingly, the ThrbCRM1 and ThrbICR enhancers contain Otx2 and Oc1 motifs which differ from the nucleotide sequences the transcription factors prefer binding in vitro. For example, in vitro HT-SELEX data support Otx2 prefers binding “TAATCC,” while ThrbCRM1 is transregulated by the binding of Otx2 to an “AAATCC” sequence in vivo. Additionally, the Otx2 and Oc1 motifs diverge across species. For example, within ThrbCRM1, the genomes of rodent/lagomorph species possess a “CAATCC” Otx2 binding motif, while cats harbor a “GAATCC” motif. To explore the functional consequence of transcription factor binding site discord, plasmid vectors containing mutant cis-regulatory elements located upstream of fluorescent reporters were electroplated into developing G. gallus retinal tissue, and their activity patterns were quantitated using flow cytometry. Mutant transcription factor binding sites drive unique patterns of reporter expression. Our findings highlight the mechanistic role that mutations within the Otx2 and Oc1 transcription factor binding sites of ThrbCRM1 and ThrbICR play in quantitatively fine-tuning the transcription of the Thrb gene. The knowledge produced from this study advances understandings of enhanceropathies, and provides support for the potential of engineering enhancers that quantitatively control transcription to fall within desired windows.

Thursday September 14

Session 3: 9:00-10:30am

Chair: Anthony Covarrubias, PhD

9:00-9:15am

ENRICHMENT OF GAMETOGENIC GERM CELL POPULATION TO IMPROVE IN VITRO OOGENESIS

Isaias Roberson
CDB, Clark lab
Isaias Roberson, Alejandro Espinoza, Christian Alvarado, Sinthia Kabir Mumu, Enrique Sosa, Azra Cruz, and Amander Clark

The derivation of egg cells (oocytes) from pluripotent stem cells (PSCs) is called in vitro oogenesis (IVO), the first step of which involving the generation of primordial germ cell-like cells (PGCLC). Using the transgenic mouse pluripotent stem cell (PSC) line expressing two fluorescent reporters under the control of germline associated genes (Blimp1-mVenus/Stella-Cyan, BVSC), PGCLCs are able to be derived, identified and isolated. PGCLCs are generated in a cytokine-driven process, within a 3D aggregate and are isolated from the surrounding cells using fluorescence activated

cell sorting (FACS) based on BVSC reporter expression. BV+SC+ PGCLCS are then oocytes. On average only 0.007% of PGCLCs become oocytes for unclear reasons. To investigate possible causes of this inefficiency, PGCLC-containing aggregates were analyzed using single-cell RNA sequencing and it was revealed that the Dazl is expressed in some PGCLCs. Dazl is critical for the sexual differentiation of primordial germ cells into oocytes, and key to their survival as their gonadal niche develops. Immunofluorescence confirmed protein expression of DAZL in the PGCLC-containing aggregates. About 90% of the PGCLC population is STELLA+ only, while the remaining 10% are DAZL+STELLA+. These results indicate that most BV+SC+ PGCLCs are developmentally immature and incompetent to respond to their somatic environment at the time of combination with FOSCs, possibly explaining the low efficiency of oocyte generation in rOvaries. Given this, a new cell line has been developed to sort for DAZL-expressing cells. With this new cell line, it will be possible to evaluate whether isolating DAZL+STELLA+ PGCLCs results in a more efficient method for producing oocytes in vitro.

9:15-9:30am

TRANSPOSABLE ELEMENT REMODELING BY TRIM28 SAFEGUARDS PGC COMPETENCE FOR GAMETOGENESIS

Jonathan DiRusso
CDB, Clark lab

Primordial Germ Cells (PGCs) are embryonic precursors to the adult germline. In mammals, they are specified in the epiblast and acquire expression of core pluripotency factors, entering latent pluripotency. Once in the developing genital ridge, they undergo two key fate restriction events: 1) determination, in which the latent pluripotency network is extinguished and 2) differentiation. In particular, the molecular mechanisms driving determination are poorly understood beyond the requirement for expression of Dazl. Recently, it has become appreciated that transposable elements (TEs) of the Long Terminal Repeat (LTR) subclass are a rich source of cis-regulatory elements which are necessary for maintenance of pluripotency Tripartite Motif Containing 28 (TRIM28), an epigenetic scaffolding protein, is necessary for proper PGC determination using an in vivo mouse model. We find that expression of TEs by PGCs is dynamic during determination and differentiation by RNA-seq. We also find that three TE families associated with pluripotency: RLTR13D6, RLTR9E and RLTR9D are enriched for H3K27ac in PGCs. Following loss of TRIM28, PGCs exhibit a failure to remodel chromatin accessibility at these TEs as observed by ATAC-seq, supporting a role for silencing of cis-regulatory TEs by TRIM28 during PGC determination. Interestingly, we find that PGCs lacking TRIM28 are heterogeneous in their expression of DAZL by immunofluorescence but no longer express pluripotency factors, leading us to hypothesize that down-regulation of pluripotency factors is independent of DAZL and TRIM28. Furthermore, TRIM28 knockout PGCs fail to differentiate as confirmed by RNA-seq and immunofluorescence, demonstrating TRIM28 is necessary for differentiation. These results, collectively, support a model in which epigenetic remodeling of chromatin accessibility by TRIM28 is a separate process from determination and necessary to confer gametogenic competency prior to differentiation.

9:30-9:45am

MEF2C IS A CRITICAL REGULATOR OF HUMAN NK CELL EFFECTOR FUNCTION AND METABOLISM

Joey Li
IMMP, O'Sullivan lab

Joey H. Li, Adalia Zhou, Vignesh Senthilkumar, Siya Shah, Jeonghyun Ji, Eddie Padilla, Andréa Ball, Nedas Matulionis, Luke Riggan, Alain Greige, Fran Annese, Jessica Cooley-Coleman, Steven Skinner, Christopher Cowan, Ajit Divakaruni, Timothy E. O'Sullivan

Primary immunodeficiencies are rare but result in severe clinical sequelae for affected patients. However, identifying new gene mutations responsible for primary immunodeficiencies remains limited by the

requirement for an affected proband presenting clinically. To predict genes that may be responsible for novel natural killer (NK) cell immunodeficiencies, we performed a targeted CRISPR screen in primary human NK cells focused on transcription factors differentially expressed during NK cell development. We identified myocyte enhancer factor 2C (MEF2C) as the sole transcription factor required for NK cell proliferation, cytokine production, and cytotoxicity in vitro. Moreover, MEF2C haploinsufficiency (MCHS) patients bearing heterozygous point mutations in MEF2C displayed impaired NK cell development as well as proliferation and effector function. To examine the role of MEF2C during in vivo viral infection, we generated bone marrow chimeras from mixed wildtype and Mef2c+/- bone marrow and found that Mef2c+/- bone marrow-derived NK cells displayed impaired development and effector function in response to viral infection. RNA sequencing of MEF2C-deficient mouse and human NK cells showed decreased expression of key lipid metabolism genes controlled by SREBP1/2 pathways conserved between species. Indeed, MEF2C-deficient human NK cells had lower lipid content, uptake, and lipid transporter levels, and supplementation with the fatty acid oleate restored effector function in CRISPR-mediated or patient-derived MEF2C-deficient NK cells. Together, these findings implicate MEF2C as a previously unrecognized NK cell deficiency gene in MCHS and master regulator of NK cell function and lipid metabolism.

9:45-10:00am

EFFECTS OF HEMATOPOIETIC STEM CELL AGING ON T CELL DEVELOPMENT

Julia Gensheimer
IMMP, Crooks lab
Julia Gensheimer, Victoria Sun, Jessica Lagosh, Encarnacion Montecino-Rodriguez, Stephanie C. de Barros, Gloria Yiu, Kenneth Dorshkind, Gay M. Crooks
David Geffen School of Medicine, Department of Pathology & Laboratory Medicine, UCLA

T cell production declines with age and contributes to decreased immune function in older adults. This decline in T cell output may be due to changes in the aged progenitor cells and/or changes in the thymic microenvironment. T cells develop from hematopoietic stem cells (HSCs) that enter the thymus, an organ known to atrophy with age. Determining T cell output in vivo has relied on hematopoietic transplantation models and is confounded by the long process of engraftment and thymic recruitment. While some in vitro models have looked at T cell output from aged HSCs, these methods often start with a heterogenous population of cells and produce mixed conclusions about T cell potential. Investigation of intrinsic defects in aged HSCs is further complicated by HSC heterogeneity. During aging, the HSC pool is comprised of increasing proportions of myeloid-biased HSCs (My-HSCs) compared to lymphoid-biased HSCs (Ly-HSCs). Thus, whether reduced T cell potential is due to changes in the proportions of Ly- and My-HSCs or a reduced intrinsic capacity of each subtype is unclear. To better understand the effects of HSC aging on T cell output and development, we studied phenotypically-defined aged and young HSCs using our in vitro Artificial Thymic Organoid (ATO) system. The ATO fully recapitulates all stages of T cell development from a single HSC. This controlled system allows for detailed dissection and quantification of thymopoiesis without the influence of an aged microenvironment. Our preliminary data suggest that the lineage bias of an HSC impacts its T cell potential regardless of age, with both young and aged Ly-HSCs generating more T committed clones than aged-matched My-HSCs. Further, ATOs derived from young and aged Ly-HSCs produce comparable cell numbers, which suggests that removing aged HSCs from their inflamed microenvironment restores their T cell potential.

10:00-10:15am

SINGLE CELL ANALYSIS REVEALS DISTINCT CD8 T CELL PHENOTYPES AND FUNCTIONS IN CMV PRIMARY INFECTION AND REACTIVATION IN KIDNEY TRANSPLANT RECIPIENTS

Angela Sun
IMMP, Reed lab

Cytomegalovirus (CMV) infection is a risk factor for graft loss and patient mortality in

solid-organ transplantation. Single-cell transcriptomics of peripheral blood CD8 T (cells from kidney transplant (Tx) recipients (KTRs) pre and 1wk post-CMV viremia and 1yr post-Tx identified terminal effector populations (CD28-, TCF7-, IL7R-, KLRG1+, GZMB+) enriched in KTRs with reactivation of latent CMV infection (n=2) compared to KTRs (n=2) after primary CMV infection. Highly cytotoxic FCGRIIIA+ (CD16) clusters (GZMB+, PRF1+, GNLY+) and "NK cell-mediated cytotoxicity" and "FcγR-mediated phagocytosis" pathways (KEGG database) were enriched in KTRs at 1wk and 1yr post-reactivation. We evaluated peripheral blood CD8 T cell response of KTRs with CMV primary infection (n=4) and reactivation (n=4), with paired CMV- KTRs, pre- and ~2yrs post-Tx against CMV peptides and anti-CD16 antibody. KTRs with CMV reactivation and KTRs with poorly-controlled primary infection (peak CMV viral load >10,000 IU/mL; multiple episodes of viremia) had high CD8 T cell activation (CD69, CD137), effector cytokine secretion (IFN-γ and TNF-α) and degranulation (CD107a) to anti-CD16 stimulation, post-viremia, but primary infection resulted in poor CMV recall response 2yrs post-viremia compared to KTRs with reactivation both pre- and post-viremia. CMV-responsive CD8 T cells also lack CD16 expression. Our results suggest that CMV memory response can be difficult to establish and maintain after primary infection under immunosuppression. While latent CMV infection can be better controlled under immunosuppression, the presence of CMV-induced CD16+ CD8 T cells suggests an NK-like potential in mediating allograft injury, such as via ADCC in KTRs producing donor-specific anti-HLA antibodies.

10:15-10:30am

THE ROLE OF SENESCENT MACROPHAGES AS A DRIVER OF INFLAMMAGING AND AGING-RELATED DISEASES

Grasiela Torres
IMMP, Covarrubias lab

Aging is the number one risk factor for multiple diseases including cancer, diabetes, and neurodegeneration, creating urgency for solutions regarding health-span maintenance as lifespan increases. Cellular senescence is emerging as a main driver of age-related diseases due to the chronic low-grade inflammation that results from accumulation of senescent cells in various tissues throughout the body. Cells that undergo senescence are characterized by permanent cell cycle arrest, altered metabolism and secretion of inflammatory molecules, known as the senescent-associated secretory phenotype (SASP). Macrophages are ubiquitous innate-immune cells that serve multiple functions including protecting from pathogens, maintaining tissue homeostasis, and function to control tissue metabolism. Our lab has shown that senescent macrophages accumulate in aging tissues and can become senescent using DNA damaging stimuli such as irradiation and chemotherapeutic drugs. Our data suggest that macrophages represent a key source of senescent cells in aging tissues, however the molecular mechanisms driving senescence in macrophages and their link to age-related diseases remain unclear. Our study aims to understand role of senescent macrophages as a driver of inflammaging and aging-related diseases. To do so, we will identify genes and pathways that contribute to cellular senescence of macrophages. Our approach utilizes in vivo studies, use of a novel in vitro senescent macrophage system using primary bone marrow macrophages from WT and knockout mice, and CRISPR-Cas9 genomic editing to investigate how gene candidates regulate various features of senescence. Understanding the molecular mechanisms regulating macrophage senescence may provide insight into treating age-related diseases.

Thursday September 14
Session 4: 1:30-2:45pm

Chair: Hung Ton-That, PhD

1:30-1:45pm

NEURAL & MOLECULAR BASIS OF CHEMOSENSATION IN SKIN-PENETRATING NEMATODES

Damia Akimori
IMMP, Hallem lab

Over 610 million individuals globally are infected with the skin-penetrating intestinal parasitic nematode, Strongyloides stercoralis. S. stercoralis infect hosts exclusively as developmentally arrested infective third-stage larvae (iL3). These infectious larvae navigate through the soil searching for hosts in a poorly understood process that involves attraction to host-emitted olfactory cues. Large families of G protein-coupled receptor genes encode odorant receptors (ORs) in nematodes and have been thoroughly identified in the model nematode Caenorhabditis elegans. We have verified and manually curated putative OR genes in S. stercoralis. Through transcriptional analysis of differential gene expression between iL3s and free-living stages, we have identified five OR genes of interest that are highly expressed in iL3s. Here, we developed the first transgenic iL3s to express fluorescent reporter constructs for OR genes. These reporters will enable us to identify the neurons in which iL3-associated chemoreceptors are expressed. Additionally, we have identified specific host-associated odorants that induce skin-penetration behaviors of S. stercoralis iL3s. We aim to identify the olfactory neurons required for odor-driven host invasion and use the histamine-gated chloride channel HisCl1 to determine if chemogenetically silencing neurons eliminates responses to attractive host odorants.

1:45-2:00pm

NEURAL MECHANISMS OF OXYGEN SENSING IN A HUMAN-INFECTIVE NEMATODE

Breanna Walsh
IMMP, Hallem lab
Breanna Walsh & Elissa A. Hallem, PhD

Soil-transmitted helminths (STHs) infect at least 24% of the global population and are especially prevalent in underserved communities with poor sanitation infrastructure. Roughly 600 million individuals are infected by Strongyloides stercoralis, a skin-penetrating STH that can cause fatal disease in immunocompromised hosts. S. stercoralis infective third-stage larvae (iL3s) use external sensory cues (e.g., host body heat, host-emitted odorants) to target human hosts. In transitioning from the free-living to intra-host emitted odorants) to target human hosts. In transitioning from the free-living to intra-host environment, S. stercoralis iL3s encounter oxygen concentrations ranging from 21% (atmospheric levels) at the soil surface to ~5% in host tissues. In these contexts, we posit that oxygen serves as a key sensory cue for environmental navigation and coordinated development. However, the molecular and neural bases of oxygen sensation remain uncharacterized in S. stercoralis and all other parasitic nematodes.We found that S. stercoralis iL3s demonstrate robust changes in locomotion when exposed to acute shifts in oxygen concentration. When exposed to 7% oxygen, iL3s exhibit a gradual slowing response that is reversible when worms return to 21% oxygen. These responses are the first oxygen-evoked behaviors to be described in any parasitic nematode. We next identified four candidate oxygen sensors in S. stercoralis. The S. stercoralis candidate oxygen sensors are homologs of the soluble guanylate cyclases (sGCs) that detect oxygen in the free-living model nematode Caenorhabditis elegans. Using transcriptional reporters, we found that at least three of the S. stercoralis sGCs are expressed in neurons that bear anatomic and morphologic similarity to known C. elegans oxygen-sensing neurons. In S. stercoralis, chemogenetic silencing of putative oxygen-

sensing neurons resulted in dampened oxygen-evoked motile behaviors. We are now working to characterize the encoding properties of putative oxygen-sensing neurons in S. stercoralis iL3s via calcium imaging. Our results will illuminate how gas chemosensation shapes parasite behavior and pathogenesis.

2:00-2:15pm

DRIVERS OF LONG-TERM IMMUNE DYSFUNCTION

Ivan Salladay-Perez
CDB, Covarrubias lab

Innate immune cells retain specific epigenetic marks after activation that either augment subsequent inflammatory responses or induce a permanent state of immuno-paralysis, known as tolerance. This process is known as "trained immunity," and recent findings have leaned towards the immunological tolerance of tissue-resident macrophages (Mφ's), long-lived and self-renewing phagocytes, as the mechanisms by which pathogens avoid immune destruction. Endotoxins, such as lipopolysaccharides (LPS), are present on the cell wall of gram-negative bacteria and activate Mφ's towards a pro-inflammatory M1 state by increasing inflammatory cytokine expression and the production of intracellular antimicrobial agents such as reactive oxygen species that drive irreparable mitochondrial dysfunction. This metabolic shift makes M1's dependent on metabolic reprogramming towards glycolysis. LPS exposure is also known to induce a temporary state of tolerance in Mφ's, mediate mitochondrial programmed cell death, and recent studies have also proposed M1 Mφ's can undergo cellular senescence, a cell-cycle arrested state that results from cellular damage or stress. However, the long-term fate and function of M1 Mφ's are unclear and understudied. Therefore, it is paramount to determine whether M1 Mφ's can overcome the acute and damaging effects of LPS, including recovery of their mitochondrial function, the ability to (re)polarize to an M1 or the anti-inflammatory M2 state, and its impact on long-term functions. My early work as a doctoral student addresses this issue and provides evidence demonstrating that LPS is not a driver of cellular senescence or cell death. We have shown that M1 Mφ's regain mitochondrial function, proliferation capabilities, and escape tolerance with a prolonged recovery period. Interestingly, we observed that LPS recovered Mφ's (M1R) may foster a "trained immunity" program. We see daughter cells of M1's have an enhanced M1 response compared to tolerant Mφ's but an attenuated LPS response compared to naïve cells. We also see aberrant activity from various metabolic enzymes and pathways that maintain the metabolic status that may act as a mechanism driving impaired function. This work will provide new understandings of the biology of tissue-resident Mφ's and identify novel pharmacological targets that resurrect Mφ's from a tolerant state, which we believe is relevant for the long-term health consequences of infection and aging.

2:15-2:30pm

DEGRADATION OF THE HOST NUCLEOLAR PROTEIN CCDC137 IS AN IMPORTANT FUNCTION OF HIV-1 VPR THAT IS INDEPENDENT OF CELL CYCLE ARREST

Karly Nisson
GREAT, Fregoso lab

HIV and other primate lentiviruses encode accessory genes that antagonize cellular restriction factors to enhance viral infectivity. All but one, the conserved and essential Viral Protein R (Vpr), have been associated with a primary cellular target(s) and viral phenotype(s). Recently it was shown that Vpr targets a nucleolar protein, coiled-coiled domain containing 137 (CCDC137), for proteasomal degradation. CCDC137 degradation has been linked to multiple Vpr phenotypes, including G2/M arrest, but whether Vpr-mediated degradation of CCDC137 is an important primary function of Vpr remains unclear. Here we 1) further evaluated a causative relationship between CCDC137 degradation and G2/M arrest, 2) explored the evolution of Vpr-mediated CCDC137 degradation, and 3) investigated the requirement for Vpr, CDC137 and DCAF1 interaction in degradation. To characterize

Vpr-mediated CCDC137 degradation in the context of G2/M arrest, we delivered a diverse set of Vpr mutants and SIV orthologs to cells of their autologous hosts and assayed CCDC137 degradation alongside G2/M arrest after 28 hours. We found that infection with HIV-1 Vpr mutants that lack the ability to cause G2/M arrest resulted in potent degradation of CCDC137. Moreover, we found a lack of correlation among degradation profiles of Vpr from SIVagm isolates and their respective G2/M arrest phenotypes, together suggesting that degradation of CCDC137 is neither a cause nor consequence of G2/M arrest. Our evolutionary analysis of CCDC137, however, indicated that CCDC137 exhibits signatures of rapid evolution consistent with those observed for nearly all host targets of primate lentiviral accessory genes. Finally, through immunofluorescence and proximity ligation assays, we identified interactions between Vpr and CCDC137 in the nucleoplasm, suggesting Vpr may also alter the localization of CCDC137. Together, our data suggests that Vpr-mediated CCDC137 degradation is independent of G2/M arrest yet may be an important target of some Vpr orthologs for a still unknown function in lentiviral replication.

2:30-2:45pm

NSC95397 IS A NOVEL HIV LATENCY REVERSING AGENT

Vivian Yang
IMMP, Fregoso lab
Randilea Nichols Doyle, Vivian Yang, Yetunde Kayode, Robert Damoiseaux, Harry E Taylor, Oliver I. Fregoso

The latent viral reservoir represents a major barrier to curing HIV. One proposed method to overcome this reservoir is “kick and kill”, wherein latency reversing agents (LRAs) combined with cytotoxic agents will reactivate latent cells and target them for destruction. While many compounds that can reactivate latent T cells have been found, current LRAs do not decrease viral reservoirs in vivo, likely due to reservoir heterogeneity and the cell microenvironment. This highlights the importance of finding compounds that reactivate latent viruses through alternative pathways. Understanding how these drugs reactivate latency will also increase our knowledge of mechanisms driving latency and latency reversal. To identify novel LRAs, our lab performed an unbiased drug screen on ~4,000 compounds using a T-cell model of HIV-1 latency (J-Lat cells). We identified NSC95397, which increases HIV-associated mRNA and proteins in multiple J-Lat clones (10.6 and 5A8) with unique integration sites. To understand the mechanism(s) by which NSC95397 reactivates latently infected cells, we tested for synergy with known LRAs SAHA and prostratin, an HDAC inhibitor and PKC agonist, respectively. We found that NSC95397 has a greater than additive effect for reactivation in J-Lat 10.6 and 5A8 cells. We further assayed for changes in histone modifications H3K4me3, H3K9ac, H3K27ac, markers of open promoters and enhancers, all of which are insignificant compared to untreated cells. Together, this suggests that NSC95397 neither increases transcription via NF-κB nor changes histone modifications. We then performed transcriptomic analysis to identify cellular pathways activated by NSC95397. We found that while NSC95397 increased viral transcripts, there is no enrichment of known transcription factor sets nor tat-associated transcripts that would explain activation of the viral promoter/5’LTR. To verify that NSC95397 still reactivates in a more physiological environment, we performed the titration and combinations to the cells maintained in hypoxia and still saw reactivation. Together, our data suggest that NSC95397 reverses latency through a still unknown mechanism and provide directions to pursue for drug discovery and reactivation-based therapy.

Thursday September 14
Session 5: 3:00-4:00pm

Chair: Catherine Clarke, PhD

3:00-3:15pm

THE ANTIBACTERIAL MECHANISM OF INORGANIC SUNSCREEN FILTERS — IS IT VIABLE?

AJ Addae
CBI, Weiss lab

Sunscreen plays crucial roles in protecting the skin from the harmful effects of ultraviolet (UV) radiation, which can cause sunburn, premature aging, and increased risks of skin cancer. Commercial sunscreens often contain micron- or nano-sized particles of zinc oxide (ZnO) and titanium dioxide (TiO2) due to their superior UV protection on the skin. These metal oxides have been shown to possess antimicrobial properties, which are attributed to the production of reactive oxygen species (ROS) that disrupt mitochondrial function of skin bacteria. The role of the skin microbiome in UV-induced immune suppression has been well established. However, the interactions between ZnO and TiO2 nanoparticles and bacteria on the surface of the skin microbiome are not well understood. This talk primarily aims to demonstrate current efforts that shed light on metal oxide-induced ROS during UV light attenuation and elucidate the impact of ZnO and TiO2 nanoparticles on the vast skin microbiota in the context of sunscreen usage. Insights from this work will be helpful to attain our ultimate destination of engineering an antioxidant-doped metallic oxide nanoparticle that lessens the extent to which mitochondrial interaction disrupts the viability of skin microbiota.

3:15-3:30pm

A PRO-APOPTOTIC SMALL MOLECULE PORTIMINE: TOTAL SYNTHESIS AND MODE OF ACTION STUDIES

Alek Lotuzas
CBI, Harran lab
Lotuzas, A.; Mazariego, M., Michel, A.; Harran, P. G.*

Portimine is a unique, spirocyclic imine marine natural product containing an unprecedented 1-pyrroline motif. This small molecule has demonstrated exceptional pro-apoptotic activity against a range of cancer cell lines. Additionally, it showed drastically lower acute toxicity in mice, but increased potency against Jurkat T-Lymphoma cells than other members of its class. Even at elevated concentrations in the presence of anti-apoptotic Bcl-2 proteins, it did not cause necroptosis. The goal of this project is to finish the de-novo total synthesis of portimine and use it in conjunction with a molecular label to probe its unknown mode of action. The synthesis is designed to be modifiable to produce multiple analogs of portimine, which will further be tested for increased potency towards its targets. An efficient and diastereoselective sequence to produce a linear precursor containing all the carbons present in the natural product has already been developed. Current efforts are ongoing to close the macrocyclic structure through an exo-selective Diels-Alder reaction. Additionally, we also have constructed a novel acetyl pyrroline heterocycle which we are in the process of attaching to our linear precursor through an oxa-ene reaction. This novel molecule allows us to more easily create derivatives on the cyclic imine motif as well as the adjacent carbons, while also discovering new reactivity. Finally, once complete, Portimine's structure would allow us to attach molecular labels which we can use to probe it's unknown mode of action and derivatize to increase potency.

3:30-3:45pm

A DESIGNED IMAGING SCAFFOLD BREAKS THE BARRIER TO HIGH-RESOLUTION STRUCTURE DETERMINATION OF SMALL PROTEINS BY CRYO-EM

Roger Castells Graells, PhD
Yeates lab

Recent technical advances have made cryo-electron microscopy (cryo-EM) an attractive method for atomic structure determination, but problems of low signal-to-noise prevent routine structure determination of proteins smaller than about 50 kDa. We have

developed symmetric protein imaging scaffolds to display and solve the structure of small proteins. In earlier work (Liu Y, et al., 2019), we demonstrated the design of a novel protein cage scaffold with sufficient rigidity and modularity to reach an imaging resolution of 3.8 Å for a 26 kDa protein. In the present work, we use molecular engineering techniques to further rigidify a new cryo-EM imaging scaffold, enabling 3 Å or better resolution imaging to be achieved, even for very small proteins. We apply this system to the key cancer signaling protein KRAS (19 kDa in size), obtaining four structures of oncogenic mutational variants by cryo-EM. Importantly, a structure for the key G12C mutant bound to an inhibitor drug (AMG510) reveals significant conformational differences compared to prior data in the crystalline state. The findings highlight the promise of cryo-EM scaffolds for advancing the design of drug molecules against small therapeutic protein targets in cancer.

3:45-4:00pm

THE INCREDIBLE BULK: AI-EMPOWERED CRYOEM UNCOVERS HUMAN CYTOMEGALOVIRUS TEGUMENT ARCHITECTURES

Jonathan Jih
BBSB, Zhou lab

The compartmentalization of eukaryotic cells presents considerable challenges to the herpesvirus life cycle. The herpesvirus tegument, a bulky proteinaceous aggregate sandwiched between herpes' capsid and envelope, is uniquely evolved to address these challenges, yet these functions' structural bases remain uncharacterized. Here we use deep-learning-enhanced cyroEM to resolve tegument structures of human cytomegalovirus, revealing a portal-biased tegumentation scheme. We discover a portal-associated tegument complex, which we term PATC, and identify multiple configurations of PATC arising from layered reorganization of pUL77, pUL48 (large tegument protein), and pUL47 (large tegument binding protein) assemblies. Analyses show pUL77 seals last-packaged viral genome-end through electrostatic interactions and pUL47/48 dimers form 45-nm-long capsid-trafficking-related filaments. These results inform how herpesvirus tegument undergoes dynamic rearrangement to facilitate processes spanning viral genome packaging to delivery.

POSTER SESSION

September 13 - 4:00-6:00pm - HH Hallway and South Courtyard

1. **Jenna Giafaglione** (Goldstein lab)
2. **Melissa Emami** (Sagasti lab)
3. **Yesica Mercado-Ayon** (Butler lab)
4. **Angel Flores** (Rowat lab)
5. **Salena Gallardo** (Butler lab)
6. **Ryan Johnson** (Shah lab)
7. **Anne Roe** (Pyle lab)
8. **Tatiana Gromova** (Vondriska lab)
9. **Clover Stubbert** (Shah lab)
10. **Jocelyn Rodriguez** (Shackelford lab)
11. **Parham Peyda** (Black lab)
12. **Joshua Dolinsky** (Eisenberg lab)
13. **Hope Pan** (Eisenberg lab)
14. **Weiguang Wang, PhD** (Zhou lab)
15. **Gunalan Natesan** (Shah lab)
16. **Thalia Georgiou** (Bouchard lab)
17. **Heta Desai** (Backus lab)
18. **James Zhen** (Zhou lab)
19. **Miranda Villanueva** (Backus lab)
20. **Jordan Gonzalez** (Garg lab)
21. **Chunsheng Yan** (Tang lab)
22. **Andréa B. Ball** (Divakaruni lab)
23. **Lilith Schwartz** (Kamariza lab)
24. **Cindy Wang** (Clarke lab)
25. **Felix Fu** (Maynard lab)
26. **Kaitlin M. Hartung** (Sletten lab)
27. **Alexander Stevens** (Zhou lab)
28. **Nina Harpell** (Gelbart/Rodriguez lab)
29. **Andrew Goring** (Clubb/Loo labs)
30. **Pasquale Saggese, PhD** (Scafoglio lab)
31. **Lauren Uchiyama** (Tontonoz lab)
32. **Sari Terrazas** (Xiao lab)
33. **Daniel Velez-Ramirez, PhD** (Hill lab)
34. **Timmie Britton** (Ton-That lab)
35. **Rebecca R. Pasquarelli** (Bradley lab)
36. **Ryan Shih** (Chen lab)
37. **Carlos Galvan** (Lowry lab)
38. **Eli Biletch** (Backus lab)

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POSTER SESSION

Wednesday, September 13
4:00-6:00pm
Hershey Hall South Courtyard

1. RECIPROCAL REGULATION OF METABOLISM AND PROSTATE EPITHELIAL LINEAGE IDENTITY

Jenna Giafaglione *(CDB, Goldstein lab)*

Jenna M. Giafaglione, Preston D. Crowell, Amelie M.L. Delcourt, Takao Hashimoto, Sung Min Ha, Aishwarya Atmakuri, Nicholas M. Nunley, Mao Tian, Rachel Dang, Johnny A. Diaz, Elisavet Tika, Marie C. Payne, Deborah L. Burkhart, Dapei Li, Nora M. Navone, Eva Corey, Peter S. Nelson, Neil Y.C. Lin, Cedric Blanpain, Leigh Ellis, Paul C. Boutros, Andrew S. Goldstein

Lineage transitions are a central feature of prostate development, tumorigenesis and treatment resistance. While epigenetic changes are well-known to drive lineage transitions, it remains unclear how upstream metabolic signaling contributes to the regulation of prostate epithelial identity. To fill this gap, we developed an approach to perform metabolomics on primary prostate epithelial cells. Using this approach, we discovered that the basal and luminal cells of the prostate exhibit distinct metabolomes and nutrient utilization patterns. Furthermore, basal to luminal differentiation is accompanied by increased pyruvate oxidation. The mitochondrial pyruvate carrier (MPC) and subsequent lactate accumulation emerged as regulators of prostate luminal identity. Inhibition of the MPC or supplementation with exogenous lactate results in large-scale chromatin remodeling, influencing both lineage-specific transcription factors and response to antiandrogen treatment. These results establish reciprocal regulation of metabolism and prostate epithelial lineage identity.

2. SENSORY AXONS ALTER MIGRATORY CELL BEHAVIORS IN THE ZEBRAFISH EPIDERMIS

Melissa Emami *(CDB, Sagasti lab)*

During embryonic development, multiple cell types interact to form multicellular tissues. Contact inhibition of locomotion (CIL) is one self-organizing form of cell interactions that patterns tissues. We have found that developing sensory axons and a newly-discovered population of specialized migratory cells (MCs) in the skin undergo CIL. Live imaging and analysis of cell type-specific markers revealed that these MCs arise from EMT of basal epithelial cells, migrate through the epidermis, undergo MET into the superficial epithelial layer, and ultimately differentiate into mucus cells and ionocytes. Both MCs and touch sensing peripheral sensory axons grow in the same epidermal territory and must ultimately be distributed evenly throughout the epidermis. We hypothesize that MC migratory behavior and distribution is dependent on heterotypic CIL with sensory axons. To test this hypothesis, I am characterizing the influence of sensory axons on MCs in the presence and absence of sensory axons. Live imaging revealed that when MCs contact sensory axons, they often repolarize and migrate away, suggesting that they are undergoing heterotypic CIL. To determine how MCs are affected by sensory axons, we examined their behaviors in ngn1 morphants, which lack sensory neurons. In these morphants, MC velocities were increased and their migratory paths were less tortuous. Unexpectedly, the number of MCs in the top of the head was doubled in the absence of sensory axons. We are currently testing the hypothesis that sensory axons are barriers that inhibit ventrally-born MCs from migrating dorsally, and without that barrier more MCs reach the top of the head. We speculate that ultimately altered MC migratory paths will result in the aberrant distribution of ionocytes and mucus cells in the epidermis. To test this idea, we are using RNA-scope to map the distribution of ionocyte- and mucus

cell-specific transcripts in ngn1 morphants and controls. These experiments will reveal how sensory axon-MC interactions help to properly distribute cells in the skin, and demonstrate how a simple self-organizing system, mediated by cell repulsion, can coordinate multiple cell types during organogenesis.

3. INVESTIGATING THE CELL-TYPE SPECIFIC PRODUCTION OF NETRIN1 DURING SPINAL CORD DEVELOPMENT

Yesica Mercado-Ayon *(CDB, Butler lab)*

Neural circuits are established when axons navigate to their synaptic targets using guidance cues in the environment to direct the path of extension. Canonically axons were thought to be guided by long-range diffusible mechanisms. The textbook example of this mechanism was the ability of netrin1, produced by floor plate (FP) cells in the developing spinal cord, to guide commissural axons towards the ventral midline. However, netrin1 is also present in neural progenitor cells (NPCs), and more recent genetic studies have rather suggested that NPC-derived netrin1 is required for commissural axon extension, acting through a short-range mechanism. The extent to which FP- and NPC-derived netrin1 behave similarly or distinctly as a long-range versus short-range signal remains unresolved. We have examined this question using mouse and chicken model systems to produce tagged forms of netrin1 in either NPCs or FP cells. Our preliminary observations indicate that exogenous mouse netrin1, when electroporated into either FP or NPCs in chicken embryos, remains in very close vicinity to its source. We have further assessed whether membrane-tethered netrin1 can guide axons in a classic in vitro outgrowth assay. Our data suggests that membrane-bound netrin1 can attract axons towards source cells up to 100 microns away. Additionally, cellular fractionation studies overexpressing tagged netrin1 or membrane-tethered netrin1 in Cos7 cells, revealed netrin1 protein is found in the membrane fraction in both cases. This observation suggests netrin1 primarily associates with its source cellular membrane. Taken together, these findings indicate netrin1 acts to guide commissural neurons primarily at a short range. This insight will shed light on our ability to use netrin1 to repair damaged circuits.

4. DEFINING THE ROLE OF NUDIX5 AS A MECHANICAL REGULATOR IN OVARIAN CANCER CELLS

Angel Flores *(CDB, Rowat lab)*

Cancer metastasis requires cells to deform and migrate through confined spaces. If we could map the mechanical regulators of cancer cells—molecules that control cell deformability and migration—this would enable us to define new and complementary treatment strategies. To identify novel mechanical regulators, we developed a high throughput deformability screen testing 1280 compounds (Library of Pharmacologically Active Compounds), which revealed compounds that reduce filtration of human ovarian cancer (OVCAR5) cells through 10 μm pores. A connectivity analysis across the top drug hits that altered cell deformability revealed NUDIX5 as a predicted regulator of cellular mechanical behaviors. NUDIX5 is a member of the Nudix (nucleoside diphosphate linked moiety X) hydrolase superfamily. Analyses of publicly available patient datasets revealed NUDIX5 is highly expressed across cancer types compared to normal tissues. To test the hypothesis that NUDIX5 is a mechanical regulator through its role in producing nuclear ATP, we manipulated levels of NUDIX5 in human ovarian cancer cell lines and blocked NUDIX5 activity by treating cells with the chemical inhibitor TH5427. Our findings reveal that knockdown of NUDIX5 or blockade of NUDIX5 activity reduces whole-cell ATP levels and causes cells to be more deformable. We also find that NUDIX5 regulates cellular and nuclear shape, stiffness, and migration. Together these findings identify NUDIX5 as a mechanical regulator with implications for ovarian cancer progression and treatment.

5. INVESTIGATING HOW BMP SIGNALING IS DIFFERENTIALLY TRANSLATED BY SMAD1 AND SMAD5 DURING DORSAL SPINAL CORD DEVELOPMENT

Salena Gallardo *(CDB, Butler lab)*
Salena Gallardo, Gabriela Gajardo Del Real, Sandeep Gupta, Samantha Butler

Spinal dorsal interneurons (dIs) mediate somatosensation: our ability to sense the environment, permitting us to react to pain or touch, and move in a coordinated manner. When dIs are damaged, after injury or disease, individuals are no longer able to interpret sensory information from the periphery. Spinal dIs are generated when discrete domains of neural progenitor cells arise along the dorsal-ventral axis of the neural tube. The Bone Morphogenic Protein (BMPs) family of growth factors drive the transcriptional changes needed to direct the patterning and proliferation of dorsal neural progenitor cells towards the specification of at least six populations of dIs (dI1-dI6). BMP signaling is transduced by two R-Smads, Smad1 and Smad5, during spinal cord development. Our lab has previously demonstrated that Smad1 and Smad5 can direct different cellular functions during dI differentiation, challenging the belief that R-Smads function redundantly during embryogenesis. Continued dissection of R-Smad function has been challenging in vivo. Our recently developed 9 day directed differentiation protocol recapitulates the in vivo dorsal spinal cord BMP signaling environment, allowing us to derive bona fide dIs from mouse embryonic stem cells (mESCs). We are using this model system to investigate the hypothesis that Smad1 and Smad5 differentially regulate transcription, and thereby drive distinct cellular outcomes during dI specification. Using CRISPR-Cas9 genome editing, we have made Smad1 and Smad5 knock out (Δ) mESC lines. Using RNA-seq, we have profiled gene expression of wildtype, Smad1Δ and Smad5Δ cell lines at various in vitro defined developmental time points, spanning across our differentiation protocol. We aim to identify the developmental stages that require Smad1 and/or Smad5 for patterning and dI specification during spinal cord development. This information is critically needed to generate pure populations of dIs for drug screen platforms and/or cellular replacement therapies seeking to restore somatosensation.

6. TEMPERATURE-DEPENDENCE OF DEVELOPMENTAL TIMING IN WILD ISOLATES OF CAENORHABDITIS ELEGANS

Ryan Johnson *(Shah lab)*

Isolated populations of the same species living in different regions will acquire genetic differences over time. Some of these differences result in phenotypic variation, sometimes as an adaptation to the local environment and other times simply due to genetic drift. This phenomenon is known as natural genetic variation and to what extent this drives changes in developmental regulation has been far less extensively explored compared to morphological and behavioral diversity. Since C. elegans development is widely considered to be invariant, we can make quantitative and empirical comparisons between embryos of different genetic backgrounds using automated lineage tracing. We aim to reconstruct the embryonic cell lineage of 6 genetically diverse isolates of C. elegans collected from around the world. Although the canonical laboratory strain, N2, originally isolated from Bristol, UK, has been extensively characterized, little is known about how genetic variation affects the development of diverse representatives of the species. By characterizing the cell lineages from numerous wild isolates, this will allow us to understand the degree to which natural variation contributes to embryonic development. In particular, we find interesting differences between N2 and two strains of Pacific origin, CB4856 (Hawaii) and ECA36 (New Zealand). At ambient temperature (21-22 C), CB4856 and ECA36 exhibit increased variability in the rate of embryonic morphogenesis, with a subset of embryos exhibiting developmental delays of ~1 h relative to N2. At 18 C, ECA36 embryos require approximately 2x the median time to hatch compared to CB4856 and N2. Using

these phenotypic differences, we have initiated a screen of recombinant inbred lines generated from F1 heterozygotes between ECA36 and CB4856 with the goal of mapping genetic drivers of this phenotype. Using these resources, we aim to better understand the genetic regulation of variability in developmental timing and the thermal physiology of metazoan development.

7. 3D CULTURING OF HUMAN PLURIPOTENT STEM CELL-DERIVED SKELETAL MUSCLE ENHANCES MATURATION

Anne Roe *(CDB, Pyle lab)*

Skeletal muscle is one of the most regenerative organs in the human body, due to the muscle stem cell, called the Satellite Cell (SC). Both the SC and the skeletal muscle progenitor cells (SMPCs) are identified by PAX7. In the Pyle Lab, we use human pluripotent stem cells (hPSCs) to recapitulate muscle development. The lab has previously shown that the hPSC-SMPCs align with Week 7-12 SMPCs, only make embryonic MHC and are unable to enter the SC niche. My project is to adapt a 2D myogenic differentiation protocol to 3D, since the tissue engineering field has shown 3D culturing enhances skeletal muscle maturation. While doing this, I observed two different morphologies of the organoids. By analyzing the different morphologies separately, I noticed that morphology #2 had neural markers turn on around D30. In addition, there was higher expression of muscle genes in the organoids with neural markers present compared to the ones without. Validating that these structures exist in the organoids, transmission electron microscopy showed the presence of neurons (axons, neural tracks, neurofilament) in morphology #2. Furthermore, the muscle fibers were more organized and aligned in organoids with neurons present compared to the organoids without neurons. When performing immunofluorescence on the organoids I found that the organoids with neurons present expressed significantly more fetal and adult MHC than organoids without neurons present. PAX7 also has significantly higher expression in organoids with neurons present. All of these findings indicate a positive correlation between neurons present and more mature muscle. Next, I plan on performing single-nuclei RNA-Seq on the organoids as they develop to better understand what cell populations arise to support the skeletal muscle, as well as the stage of development the PAX7 cells are at in the organoids with more mature skeletal muscle.

8. METABOLIC-EPIGENETIC COUPLING IN CARDIOVASCULAR DISEASE

Tatiana Gromova *(CDB, Vondriska lab)*

Given rising obesity rates, understanding how metabolic changes exacerbate other cardiovascular disease (CVD) risk factors will yield novel therapeutic targets for associated comorbidities. We conducted an obesity study, showing that male mice fed a high fat diet (HFD) for 20 weeks developed metabolic syndrome as their cardiomyocytes entered a pre-diabetic state characterized by insulin resistance. To examine how obesity contributes to other CVD risk factors, we challenged the HFD mice with a second insult to the heart through phenylephrine (PE) injection. PE treatment to mice fed regular chow caused acute hypertrophy, fibrosis, and diastolic dysfunction in the heart. Surprisingly, 20-week HFD mice were ostensibly protected against these PE-induced changes, exhibiting less cardiac fibrosis, better diastolic function, and less activation of hypertrophic gene markers (ANP, bMHC) compared to controls. We hypothesize that key metabolites (FAD) and metabolically induced transcription factors (Atf3, Nr4a1) from our obesity study change the chromatin structure of cardiomyocytes to be protective against acute hypertrophy. To understand the chromatin architecture underpinning such metabolic imprinting, we will perform an assay for transposase-accessible chromatin (ATAC) sequencing and RNA-seq on isolated cardiomyocytes from male HFD or chow mice, with or without PE treatment. These experiments will identify the gene promoters undergoing chromatin reorganization in response to HFD and PE and identify their

effect on transcription and phenotype.

9. CYTOSKELETAL DYNAMICS DURING NEURONAL DEVELOPMENT IN C. ELEGANS

Clover Stubbert *(CDB, Shah lab)*

The coordination and collective polarization of neuronal bundles is a key organizer of neural networks. It was initially thought that neurons follow the classic pioneer-follower system with one neoruon that grows out first and leaves behind signals that other neurons follow, but findings in C. elegans have shown this is not always the case. The neuronal cytoskeleton is a critical player in the orchestration of process outgrowth downstream of polarity establishment. There are two major microtubule organizing centers; gamma tubulin ring complexes (ɣ-TURCS) and centrosomes within the cytoskeleton. We have been using strains labeling various cytoskeletal structures to visualize the dynamics during morphogenesis. Initial data shows that gamma tubulin structures are seen along the apical surface over length of the dendrite and in the center of neuronal rosettes. Of the major microtubule organizing centers, centrosomes play many roles during neuronal development, such as during axon assembly and spindle formation during mitosis but are not known to be associated with dendrite formation. Initial imaging of beta tubulin structure post-mitotically shows the extension of the spindle fibers, along the dendrite as well as at the base of the dendrite which we suspect to be a centrosome. Asymmetric cell divisions are critical for final cell identity and in this system occur post-miticially causing the cytoskeleton to change. We are interested in further understanding cytoskeletal dynamics and changes during neuronal polarization, morphogenesis and maturation by leveraging our labs expertise with three-dimensional live imaging and genetic perturbations.

10. HSP75 INHIBITION AS A THERAPEUTIC TARGET FOR GLYCOLYTIC LUNG SQUAMOUS CELL CARCINOMAS

Jocelyn Rodriguez *(CDB, Shackelford lab)*

In 2023, approximately 130,000 people will die of non-small cell lung cancer (NSCLC) in the United States. Lung squamous carcinoma (LUSC) is an aggressive subtype of NSCLC, which accounts for approximately 40,000 patients per year in the U.S. The 5-year survival rate for advanced NSCLC is 6%, with squamous patients having a worse prognosis than those with adenocarcinomas, underscoring the need to identify targeted therapies for the treatment of squamous tumors. Recent breakthroughs in immunotherapy have improved the treatment of this deadly disease, but unfortunately the majority of patients still do not benefit from this therapy and frequently develop resistance. LUSCs frequently carry high mutation burdens and often lack defined genetic mutations that can be successfully paired with targeted therapies such as a tyrosine kinase inhibitors (TKIs). The genetic heterogeneity intrinsic to LUSC makes it difficult to target these tumors with targeted therapies paired to defined genetic mutations. However, at a metabolic level, LUSC retain a homogeneous metabolic signature defined by high glycolytic flux. Our lab has performed in vivo analysis of metabolic signatures in combination with spatial mapping of mitochondrial networks in LUSC (Momcilovic et al., 2018; Han et al., 2023). In addition to homogeneous metabolic signatures, we identified that LUSC have homogeneous structural and functional mitochondrial phenotypes – characterized by high levels of mitochondrial stress. In this study I aim to dissect the metabolic and molecular vulnerabilities regulating mitochondrial stress responses in aggressive glycolytic tumors. I will test the hypothesis that high glucose flux in lung squamous tumors drives mitochondrial stress phenotypes and sensitivity to inhibition of heat shock proteins. The overarching goal of this proposal is to identify new therapeutic strategies to target therapy resistant LUSC while deepening our understanding of the molecular mechanisms that regulate mitochondrial stress responses in these tumors.

11. SPLICING REGULATION THROUGH COMBINATORIAL RECOGNITION OF SPLICING REGULATORY MOTIFS BY A HETEROMERIC ASSEMBLY OF RNA BINDING PROTEINS

Parham Peyda *(BBSB, Black lab)*

Alternative splicing is regulated by the combinatorial interplay between trans-acting RNA binding proteins (RBPs) and cis-regulatory elements on pre-mRNAs. The Rbfox protein family is a crucial splicing regulator, with mutations or abnormal expression of Rbfox associated with various aberrations such as familial epilepsy, pancreatic cancer metastasis, and heart conduction defects in myotonic dystrophy 1. Previous studies have primarily focused on how individual Rbfox proteins recognize cis-regulatory RNA elements and regulate splicing. However, Rbfox is part of a protein complex in the nucleus, known as the Large Assembly of Splicing Regulators (LASR), implying that its binding and activity might be influenced by other subunits within the complex. To understand this interaction, we created a transcriptome-wide footprint of this complex by sequencing nuclease-protected RNA fragments associated with LASR-bound Rbfox1. These RNA fragments contain motifs predicted to bind Rbfox and LASR subunits in tandem repeats, indicating multi-subunit recognition of these elements. We further examined sites directly bound by Rbfox using an RNA binding mutant Rbfox1(F125A). This mutant can still form a complex with LASR, but the nuclease-protected RNAs associated with LASR-bound Rbfox1(F125A) lack most sites containing the Rbfox binding motif. This finding suggests a critical role for Rbfox in recruiting LASR to these sites. Additionally, we investigated the impact of the subunit hnRNP M on LASR and Rbfox activity. Preliminary results indicate that depletion of hnRNP M partially destabilizes LASR and dysregulates certain Rbfox targets. Our study suggests that Rbfox, in conjunction with LASR, recognizes tandem RNA elements, and its regulatory activity is dependent on both target binding and the presence of an intact LASR complex.

12. STRUCTURE-BASED DESIGN OF D-PEPTIDE DRUGS AGAINST TAU AGGREGATION IN ALZHEIMER'S DISEASE

Joshua Dolinsky (*BBSB, Eisenberg lab)*

Ke Hou, Joshua Dolinsky, Carolyn Hu, Romany Abskharon, Jeffrey Zhang, Xinyi Cheng, David Eisenberg

Tau is a microtubule-binding protein that aggregates into amyloid fibrils in Alzheimer's Disease (AD). Aggregated tau is correlated with disease progression and neuronal death. Previously, our laboratory used in silico structure-based design to develop a six-residue D-peptide (D-TLKIVW, or S-DP) that inhibited tau aggregation in vitro. We found that having a seventh residue increased inhibitor efficacy, and as such, we further tested seven residue D-peptides with a variable seventh residue (D-TLKIVWX, or DP-X). These DP-X peptides were assayed on their abilities to inhibit tau aggregation, disaggregate aggregated tau, prevent seeding of AD-tau in tau biosensor cells, and protect neuronal cells from tau aggregate toxicity. D-peptides with a hydrophobic seventh residue, and to a lesser extent with a polar seventh residue, reduced tau aggregation and its effects in all tested in vitro and cellular model systems. This work uses in vitro and cellular assays to characterize in silico designed peptide drug candidates against AD-tau.

13. STRUCTURE-BASED DISCOVERY OF SMALL MOLECULES THAT DISAGGREGATE TAU AMYLOID

Hope Pan *(BBSB, Eisenberg lab)*

Alzheimer's disease (AD) is associated with the aggregation of the protein tau into amyloid fibrils. The formation of these fibrils is strongly correlated with neuron death, brain atrophy, and cognitive decline. Thus, disaggregation of tau fibrils is a potential therapeutic avenue for AD. The small molecule EGCG, abundant in green tea, has long been known to disaggregate amyloid fibrils, but EGCG has poor drug-like properties, failing to penetrate the brain. To understand how EGCG can disaggregate fibrils, we used cryo-electron microscopy to determine the atomic structure of AD brain-extracted tau bound to EGCG.

Using the EGCG binding position as a pharmacophore, we computationally screened thousands of drug-like compounds that have characteristics favoring BBB permeability. We discovered four compounds that experimentally disaggregate AD brain-derived tau fibrils in vitro and prevent seeding in a tau aggregation cell model. To experimentally verify the BBB permeability of two of the compounds, we administered each compound by tail vein injection to C57BL/6J mice. We used a liquid chromatographic–tandem mass spectrometric multiple reaction monitoring assay to detect and quantify the compounds in brain tissue. To determine whether these compounds can disaggregate tau in vivo, we assessed their ability to improve cognitive deficits and reduce tau pathology in the PS19 mouse model. PS19 mice express human tau with the P301S mutation and develop widespread neurofibrillary tangle-like inclusions4. We administered the compounds to PS19 mice by tail vein injection once a week for eight weeks. We then assessed whether the compounds improved cognitive deficits in two behavioral tests: the Barnes maze, which measures spatial memory, and the novel object recognition test, which measures recognition memory. We collected brain tissue to assess whether these compounds reduced tau pathology in the brain. Our work highlights the possibility of using structure-based methods to discover small molecule disaggregators of tau.

14. INSIGHTS INTO THE NA+-DEPENDENT TRANSPORT IN SLC4 PROTEINS FROM A CRYO-EM STRUCTURE OF THE NDCBE TRANSPORTER AND COMPUTATIONAL MODELLING

Weiguang Wang, PhD *(Zhou lab)*

Wang WW, Zhekova HR, Tsirulnikov K, Kayik G, Khan HM, Azimov R, Abuladze R, Kao L, Newman D, Noskov SY, Tieleman P, Zhou ZH, Pushkin A, Kurtz I

SLC4 transporters are involved in transport of Cl-, HCO3-, CO32-, Na+, H+, and NH3 through the membrane and are key for pH maintenance and ion homeostasis in the body. Despite their high sequence identity, the members of the family demonstrate an array of transport modes – Na+-dependent or independent transport, H+ and NH3 transport, Na+-anion symport, anion exchange and combinations thereof. However, the mechanisms governing the SLC4 modes of transport are still unclear due to lack of unambiguous evidence for the binding site locations within these proteins. We present a cryo-EM structure of the OF open SLC 4 transporter NDCBE, which exchanges Na+-carbonate for chloride. The structure resolves the Na+ and CO32- binding sites in the OF binding pocket of NDCBE. Computational modelling provides further insights into the ion dynamics in the OF permeation cavity of NDCBE and reveals separate cation and anion pathways and an additional binding site in proximity to the experimentally resolved one. Free energy calculations indicate that Na+ is required for stable binding of anions in the OF binding pocket and that the Na+-CO32- ion pair is the most likely ion load. Our results provide insights into the Na+-dependent transport of carbonate in the Na+-dependent transporters of the SLC4 family, which is controlled both by residues in the binding pocket and in the permeation cavity leading to the protein center.

15. GRAPH-BASED ANALYSIS OF CELL LINEAGES

Gunalan Natesan *(Shah lab)*

The study of cell lineages in eutelic organisms, which possess a fixed number of somatic cells, has been a powerful driving force in our understanding of fundamental developmental and biological processes. Cell lineages map intuitively to binary trees, and C. elegans' eutely property and extensive characterization make it an optimal candidate for the creation and benchmarking of distance metrics that compare tree structures. Here we adapt the tree edit distance, a metric that measures topological variations in trees, and introduce the branch distance, a generalization of the L2 norm, to the analysis of the C. elegans embryonic cell lineages. We benchmark these metrics using a published database of wild type and RNAi-perturbed C. elegans embryos, revealing previously uncharacterized heterogeneity in wild type

lineages and in the phenotypic consequences of RNAi variability on developmental timing. Extending these measurements of lineage-specific timing to the detection of patterns of similarity in anterior cell lineages of the embryo and identifies a previously unappreciated role of Notch signaling in the control of developmental timing. Finally, we apply this approach to a systematic analysis signaling in the control of developmental timing. Finally, we apply this approach to a systematic analysis of RNAi perturbations that result in cell fate transformations where we find that, while developmental timing appears to be highly sensitive to genetic perturbation, RNAi against genes in a subset of important developmental regulators generate transformations that preserve lineage-specific developmental clocks.

16. CISS-ENABLED ENANTIOSPECIFICITY BY NMR

Thalia Georgiou *(IMMP, Bouchard lab)*

Chirality plays a fundamental role in various chemical and biological processes, such as dictating particle motion and how particles and molecules recognize and interact with each other. The investigation of chirality-induced phenomena has been at the forefront of scientific exploration, with implications ranging from asymmetric synthesis to biological recognition and drug design. It is well established that the structure of many biological systems, including amino acids in proteins and nucleotides in RNA and DNA, iis influenced by chiral components, affecting specific biological functions. In bioenergetics, electron transfer (ET) reactions consistently involve spin transitions. In these reactions, the chirality-induced spin selectivity (CISS) effect is a primary factor, leading to spin polarization that influences ET reaction pathways and rates. Given that many important molecules in chemistry and biology are either chiral or contain chiral components, it is vital to understand the role of spin polarization in ET and devise strategies to harness and control it. An unexpected finding by Santos et al.1 reported enantiospecific NMR responses in solid-state cross polarization (CP) experiments, suggesting a possible novel mechanism for nuclear spin-spin coupling in chiral molecules. The connection between nuclear spin dynamics and chirality could offer new insights for molecular sensing and quantum informatics. Herein we provide the first theoretical explanation for this phenomenon, employing an effective spin-Hamiltonian derived from a DNA helix model to analyze the interplay between nuclear spins and electronic states in chiral molecules. We find that distant nuclear spins can effectively couple to conduction electrons, leading to enantioselectivity in CP through indirect nuclear spin-spin coupling and associated CP transfer rates. Such insights not only enhance our comprehension of chiral molecular systems but also pave the way for potential applications in NMR-based sensing and quantum information processing at the molecular level.

17. MULTI-OMIC STRATIFICATION OF THE MISSENSE VARIANT CYSTEINOME

Heta Desai *(BBSB, Backus lab)*

Heta Desai, Samuel Ofori, Lisa Boatner, Fengchao Yu, Miranda Villanueva, Nicholas Ung, Alexey I. Nesvizhskii, Keriann Backus

Despite the relative rarity of cysteine (2.3% of all residues in a human reference proteome), cysteine is remarkably the most commonly acquired amino acid due to missense mutations in human cancers. In fact, cysteine is frequently mutated in oncogenes. Given the unique chemistry of the cysteine thiol, including its nucleophilicity and sensitivity to oxidative stress, cysteine residues contribute to numerous biological processes, including enzymatic catalysis and redox signaling. While established proteogenomic platforms have improved our understanding of proteome variations, the widespread adoption of these methods in chemicalproteomics applications is lacking. Here, we present a chemical proteogenomic workflow to identify ligandable and putative ligandable variants. By implementing a customized FragPipe computational pipeline, we achieve accurate variant identification and MS1 quantitation for activity based protein profiling. Application of this workflow to a panel of 11 cancer cell lines, including mismatch repair deficient and

non-mismatch repair deficient lines, results in over 400 variant-containing peptides labeled with cysteine-reactive probes, including many uncharacterized variants.

18. STRUCTURE OF CANCER-CAUSING HUMAN GAMMAHERPESVIRUSES AND COMPARISON WITH ALPHA- AND BETAHERPESVIRUS

James Zhen (*BBSB, Zhou lab)*

Discovered in 1964, Epstein-Barr virus (EBV) is the first identified virus to cause human cancer and recent evidence suggests that it is also the causative agent of multiple sclerosis. EBV is classified into the gammaherpesvirus subfamily of Herpesviridae, which also includes Kaposi's sarcoma associated herpesvirus (KSHV), the latest member of human cancer-causing viruses. The gammaherpesvirus subfamily stands out from its alpha- and betaherpesvirus relatives due to the tumorigenicity of its members. Although previous studies of human alpha- and betaherpesvirus structure by cryo-electron tomography (cryo-ET) have been reported, 3D reconstructions of intact human gammaherpesvirus remain elusive. Here, we structurally characterize extracellular virions of EBV and KSHV in their near-native state by cryo-ET and report comparisons of their morphology with prototypic alpha- and betaherpesvirus, herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV). Each human herpesvirus species has an eccentric capsid position and polarized distribution of tegument. Envelope glycoproteins are clustered to localized areas on the viral membrane with greater volumes of underlying outer tegument. The envelopes of EBV and KSHV are less densely populated with glycoproteins than those of HSV-1 and HCMV. Additionally, fusion protein gB trimers occupy triplet arrangements, which suggests a directed organization of the herpesvirus envelope. Human herpesviruses share conserved assembly and maturation pathways, but nuanced differences in the tegument and envelope architectures possibly reflect differences in cell tropism and infection between the different types of herpesvirus.

19. CHEMOPROTEOMIC APPROACHES TO DECIPHER NOVEL MECHANISMS OF NON-VESICULAR CHOLESTEROL TRANSPORT

Miranda Villanueva *(BBSB, Backus lab)*

Cholesterol plays important roles in membrane fluidity, lipid raft formation and in immunity. Its concentration and location must be tightly regulated to avoid dysregulation and toxicity. The details of cholesterol transport within cells require further investigation to fully understand its transport and distribution throughout cellular components. Previous chemoproteomic studies utilizing photoaffinity cholesterol probes have proven powerful as they successfully identified cholesterol interactors and binding sites. However, their workflows fail at identifying site of labeling or require specific instrumentation and complicated manual data deconvolution. Importantly, none of these studies have focused on evaluating trafficking in hepatocytes with HDL-delivered probe. We are developing a novel chemoproteomics workflow and plan to apply it in parallel with established photoaffinity protein level enrichment for the identification and mapping of novel cholesterol interactions involved in HDL and LDL mediated transport. First, a novel photoaffinity cholesterol probe with a clickable handle, NBII-165, has been successfully validated to enrich cholesterol interacting proteins. Additionally, we are developing a novel photolabeling footprinting method that selectively labels two residues, tyrosine and histidine, and enables enrichment of the modifications through biorthogonal chemistry for interaction site mapping of cholesterol interacting partners. Uniquely, we accomplished delivery into HepG2 cells through loading of HDL or LDL particles. Preliminary results identified 42 proteins enriched in HDL-conjugated NBII-165 treated cells with 34% overlap compared to conventional sterol delivery control. Next, our footprinting method has been established to work with purified proteins as well as whole cell lysates with about 30% labeling efficiency. Lastly, isotopically labeled capture reagents have been validated and will enable MS1

quantitation of cholesterol treated and control samples for downstream binding site mapping. Our novel chemoproteomic footprinting method will circumvent the challenges of working with sterol-crosslinked proteins while still mapping interactions sites.

20. TOWARDS THE TOTAL SYNTHESIS OF (–)-KERAMAPHIDIN B

Jordan Gonzalez *(CBI, Garg lab)*
Jordan A. M. Gonzalez, Milauni M. Mehta, Logan Bachman, Matthew S. McVeigh, and Neil K. Garg

The chemical synthesis of natural products is an important area of research that provides a platform for the development of therapeutics and the strategic application of new reactions. This presentation will detail ongoing efforts toward the total synthesis of (–)-keramaphidin B, a manzamine alkaloid with anti-cancer activity. Our synthetic strategy leverages a highly reactive strained cyclic allene to build the core of the natural product. Furthermore, plans for late-stage synthetic manipulations will also be discussed.

21. GENOME MINING FROM AGRICULTURALLY IMPORTANT FUNGI LED TO A D-GLUCOSE ESTERIFIED POLYKETIDE WITH A TERPENE-LIKE CORE STRUCTURE

Chunsheng Yan *(CBI, Tang lab)*
(Chunsheng Yan, Wenyu Han, Qingyang Zhou, Yalong Zhang, Kanji Niwa, Zuodong Sun, Zhongshou Wu, Steven E. Jacobsen, K. N. Houk, Yi Tang *)

A complete inventory of secondary metabolites (SMs) is helpful to investigate the interaction between agricultural-associated fungi and plant. Genome mining helps discover the the biosynthetic gene clusters (BGC) and the associate natural products. Here, we mined a gene cluster that can produce 5 new compounds and one known compound. The final product was a polyketide product featured terpene like structure. Also, the pathway suggested the 2nd example of (4+3) cycloaddition in biosynthesis and a new strategy of glycosylation.

22. PRO-INFLAMMATORY MACROPHAGES MAY NOT REQUIRE COLLAPSE OF MITOCHONDRIAL RESPIRATION

Andréa B. Ball *(CBI, Divakaruni lab)*
Andréa B. Ball, Anthony E. Jones, Wei-Yuan Hsieh, Kaitlyn B. Nguyễn, Steven J. Bensinger & Ajit S. Divakaruni

As part of the innate immune system, pro-inflammatory macrophages play key roles in the response to invading pathogens. Historically, studies investigating how pro-inflammatory macrophages metabolically adapt in response to stimuli have suggested that these macrophages shift away from mitochondrial respiration and towards glycolysis for ATP production. Despite being less efficient, this switch is meant to allow for the repurposing of mitochondria as signaling organelles by accumulating signaling metabolites such as succinate and itaconate. However, we have observed that during early activation pro-inflammatory macrophages retain robust rates of mitochondrial respiration despite significant increases in pro-inflammatory gene expression and accumulation of succinate and itaconate. Therefore, it remains unclear to what extent mitochondrial respiration is involved in the pro-inflammatory macrophage phenotype. Respirometry studies of a panel of pro-inflammatory stimuli have revealed that not all stimuli decrease respiration. Metabolomics have revealed that the accumulation of pro-inflammatory metabolites occurs independently of mitochondrial respiration even at later timepoints. Finally, with both loss-of-function and gain-of-function approaches, we determined that pro-inflammatory gene expression is independent of mitochondrial respiration. Our loss-of-function approach took advantage of the knowledge that nitric oxide drives the loss in mitochondrial respiration. Macrophages from mice lacking nitric oxide production showed no alteration in gene expression in comparison to wild-type mice. The gain-of-function approach used several well-known mitochondrial effector compounds to determine if inducing mitochondrial defects could impact pro-inflammatory gene

expression. Again, we observed no change in gene expression when defects in mitochondrial respiration were induced. Overall, it appears that pro-inflammatory macrophages can both accumulate mitochondrial signals and increase pro-inflammatory gene expression with or without intact mitochondrial respiration.

23. PROGRESS TOWARD DRUG SUSCEPTIBILITY TESTING (DST) FOR MULTIDRUG RESISTANT (MDR) TUBERCULOSIS USING SOLVATOCHROMIC PROBES

Lilith Schwartz *(CBI, Kamariza lab)*

Annually, tuberculosis (TB) kills more people than any other infectious disease caused by a single agent, except for COVID-19. A significant barrier to combating TB spread is a lack of inexpensive and rapid diagnostic tests for Mycobacterium tuberculosis (Mtb). In particular, there's an urgent need for point-of-care detection methods for drug-resistant TB. Previously, environment-sensitive probes, 4-N,N-dimethylamino-1,8-naphthalimide (DMN) and 3-hydroxychromone (3HC) trehalose conjugates, demonstrated efficient fluorescence labeling of Mtb in minutes. Importantly, these probes only label live Mtb cells, and fluorescence diminishes as cells die. We are developing a drug susceptibility testing (DST) platform using these probes to improve efficient decision-making during the course of TB treatment. Using optical density measurements, and flow cytometry fluorescence analysis, we demonstrate the effects of the four frontline TB drugs, namely rifampicin, isoniazid, pyrazinamide, and ethambutol, on probe-labeling kinetics of Mycobacterium smegmatis (Msmeg), a BSL1 model organism for Mtb. We also assess whether the plate reader assay provides labeling readouts that are consistent and reliable for detection of Msmeg labeling. Plate readers are much more commonly found in low-income clinical laboratories compared to flow cytometers, allowing for more financially accessible DST in countries with high TB burden. These experiments are carried out in both liquid broth cultures and clinically relevant solvents such as artificial serum and sputum. Overall, a plate reader DST assay using these trehalose-conjugated fluorescent probes may improve point-of-care diagnostics for MDR TB.

24. CAN WE PREDICT COENZYME Q DEFICIENCY IN PATIENTS? THE CASE OF COQ5, A C-METHYLTRANSFERASE IN COENZYME Q BIOSYNTHESIS

Cindy Wang *(CBI, S. Clarke/C. Clarke labs)*

Coenzyme Q (CoQ) is an essential lipid that functions as an electron carrier in the mitochondrial electron transport chain. In its reduced form (CoQH2), it can also act as a chain-terminating antioxidant providing protection against lipid peroxidation. A defective CoQ biosynthetic pathway in humans can cause a wide array of illnesses, including cardiovascular, kidney, and neurodegenerative disorders, through a condition known as primary CoQ deficiency. We focus on COQ5, a gene encoding an S-adenosylmethionine (AdoMet)-dependent C-methyltransferase in the CoQ biosynthetic pathway. COQ5 missense single nucleotide variants (SNVs) are found in the population in primarily heterozygotes, but little is known about their clinical presentation. Here, we identify potentially deleterious COQ5 missense SNVs based on structural, functional, and sequence information. Select SNVs will be characterized in the yeast Saccharomyces cerevisiae, whose CoQ biosynthetic pathway is highly homologous to that of humans. As a proof of principle, we show impaired respiratory growth, reduced whole cell CoQ6 content, and altered flux through the pathway in previously identified yeast Coq5 point mutants. From these results we infer the state of the CoQ synthome, a large complex whose Coq5-dependent assembly is required for CoQ biosynthesis. We are also developing an LC-MS/MS-based activity assay for yeast Coq5 in Escherichia coli to assess the impact of each SNV on catalytic activity in particular. Our results will shed light on the structure-and-function relationship of COQ5 and present a strategy for facilitating the screening and diagnosis of primary CoQ deficiency.

25. LONG-ACTING BASAL INSULIN USING SELF-IMMOLATIVE PROTEIN-POLYMER CONJUGATES

Felix Fu *(CBI, Maynard lab)*

Basal insulin is a long-acting insulin that diabetes patients receive to mimic the constant insulin production by the pancreas and control glucose level outside of eating. There are three different basal insulins currently available in the market: glargine, detemir, and degludec. However, the longest duration of those basal insulins is around 40 hours, meaning the patients need to receive daily injections, as well as having no liver specificity, causing nocturnal hypoglycemia. Eli Lily developed a PEG-insulin conjugate, peglispro, it ultimately failed due to signs of liver injury during clinical trials. We propose a novel polymer-insulin conjugate that utilizes the self-immolative linker our group developed. We will first explore accelerating the rate of release by leveraging the reduced aromaticity of some polycyclic self-immolative linker cores. Then, we will examine the release profile of the linker with different polymer lengths and types. Once we select the linker to use based on the desired half-life, we will investigate the site-specific conjugation of the linker on GlyA1 by screening various conjugation conditions. By selectively conjugate to the A-chain N-terminus of the insulin, we can ensure the conjugate to have significantly reduced activity, while released insulin regains full activity. Next, we plan to test the bioactivity of the conjugate using blood glucose depression tests in mouse. Finally, we will study the in vitro and in vivo release profile, bioavailability, safety, and pharmacokinetics of the conjugate. If successful, the proposed work will provide a new class of long-acting basal insulin that will have tunable half-life by using different self-immolative linker as well as polymer size and chemistry. This basal insulin will also have liver specificity, mimicking the endogenous insulin production, while also avoiding the peripheral capillary permeability issue that caused liver damage with peglispro

26. REACTIONS AND REARRANGEMENTS OF THE OLYMPICENYL KETONE, 6H-BENZO[CD]PYREN-6-ONE

Kaitlin M. Hartung *(CBI, Sletten lab)*
Kaitlin M. Hartung and Ellen M. Sletten

Polycyclic aromatic hydrocarbons (PAHs) are featured interests in areas ranging from astrochemistry to responsive materials. One such scaffold is 6H-benzo[cd]pyrene, known and studied for its propensity to form cationic, anionic, or radical aromatic species and the potential applications arising from each. While attempting to utilize the oxidized from of this PAH, 6H-benzo[cd]pyren-6-one, as a synthetic intermediate, we encountered challenges working with its addition products; namely, a new reactive intermediate appeared to form under relatively mild conditions, hindering purification but inviting further investigation. Initial observations of a second species by mass spectrometry corresponding to a loss of water – and presumably, formation of an aromatic cation – led us to probe the characteristics of the 6-position, which led to a series of exploratory reactions that both elaborate on previous observations and add new entries to the scattered literature on this scaffold. Summarized here are our findings on the reactivity and rearrangements of addition products to 6H-benzo[cd]pyren-6-one as an entry to the full understanding of 6H-benzo[cd]pyrene derivatives and their applications in organic synthesis.

27. ASYMMETRIC RECONSTRUCTION OF THE AQUAREOVIRUS CORE AT NEAR-ATOMIC RESOLUTION AND MECHANISM OF TRANSCRIPTION INITIATION

Alexander Stevens *(BMSB, Zhou labs)*

The Reoviridae family of dsRNA viruses is characterized by its members' capacity for endogenous transcription of their multipartite genomes within proteinaceous capsids of 1 to 3 layers. These viruses share inner core particles (ICPs) that conform to icosahedral, T=2*, symmetry, but differ in two major respects: first, the presence or absence of RNA-capping turrets at each icosahedral vertex; second, the number of additional host-specific capsid layers that are often lost upon cell entry. While the role of these additional

layers in host infection is generally understood, the absence of asymmetric ICP structures from turreted, multilayered reoviruses has obfuscated our understanding of how successive removal of these external layers impact the structural organization of the ICP and transcription initiation. Here, we present the 3.3 Å resolution structure of the aquareovirus (ARV) ICP, and atomic models of the capsid proteins VP3 and VP6, transcriptional enzymatic complex (TEC) subunits VP2 and VP4, and RNA-capping turret protein VP1. These structures reveal significant differences when compared to those of the coated ARV, as well as their counterparts in single-layered cytoplasmic polyhedrosis virus (CPV). Compared to the double-layered ARV virion and infectious subvirion particle structures, the ARV ICP undergoes significant capsid expansion and widening of the nucleotide processing channels in its TEC and turret. Thus, the loss of outer capsid layers may regulate transcription initiation in ARV, unlike CPV which relies solely on allosteric regulation by binding transcriptional cofactors. These results shed new light on the mechanism of transcription initiation amongst turreted, multilayered members of Reoviridae.

28. STRUCTUAL INSIGHTS INTO TOBACCO MOSAIC VIRUS ENDS

Nina Harpell *(BMSB, Gelbart/Rodriguez labs)*

Tobacco Mosaic Virus (TMV) has a remarkable history as the first virus ever isolated and has since been extensively studied through X-ray crystallography and cryoEM. At present, there are several high-resolution structures of TMV deposited in the PDB that have identified key structural motifs and RNA-binding sites; however, due to current helical averaging techniques, variations across the capsid are averaged out in favor of a higher resolution overall structure, as asymmetric averaging and refinement is not currently possible due to the large size of TMV. This method eliminates the ability to identify any structural differences at refinement is not currently possible due to the large size of TMV. This method eliminates the ability to identify any structural differences at the ends of the capsid in comparison to the center. The ends of TMV play a significant role in its viral life cycle including initiation of disassembly. Furthermore, RNA content at either end differs significantly from that at the other end and from that at the center of the capsid. The 5’ end completely lacks any guanine bases, which have the strongest interaction with capsid protein (CP) while the 3’ end consists of 40% guanine bases. To investigate the role of RNA sequence composition on TMVs disassembly mechanism, we have introduced a virus-like particle (VLP) that packages only the 5’, packaging signal, and 3’ regions of the RNA for cryoEM imaging and reconstruction. This “short” TMV VLP (mTMV) can be imaged at high magnification suitable for high-resolution single-particle analysis. Additionally, a structure of this mTMV VLP will provide insight into the effects of in-vitro reconstitution on capsid structure and assembly.

29. DISSECTING THE ACTINOBACTERIAL SURFACE PROTEOME

Andrew Goring *(BMSB, Clubb/Loo labs)*

My research is focused on gaining a fundamental understanding on the molecular basis of heme acquisition by pathogenic Actinobacteria using novel chemical probes combined with quantitative mass spectrometry. The Actinobacteria phylum is home to some of the most clinically important gram+ pathogens, including Mycobacterium tuberculosis which is the second leading cause of death from a single infectious agent after COVID-19. They possess a distinct cell envelope architecture marked by a thick, hydrophobic and highly impermeable outer mycolic acid membrane (MM) surrounding their peptidoglycan cell wall (PG). The MM attenuates efficacy of widely used antibiotics and the permeability of β-lactams through the mycobacterial cell wall is 100-fold slower than the Escherichia coli. Understanding the molecular mechanisms employed by pathogenic actinobacteria to import molecules across the MM could lead to the identification of surface-exposed or secreted virulence factors, serving as novel drug targets amenable to cell-impenetrable therapeutics

such as antibodies. My work is focused on defining the heme acquisition system of Corynebacterium diphtheriae, a model actinobacterium. I am using a novel chemical crosslinking probe combined with quantitative proteomics to gain high-resolution spatial information of protein organization across the cell envelope. The organization of protein machinery across the cell envelope gives insight into the mechanism of heme-acquisition and on how the bacterium employs a broad range of virulence factors to mount an infection.

30. GLUCOSE DEPRIVATION PROMOTES PSEUDO-HYPOXIA AND DE-DIFFERENTIATION IN LUNG ADENOCARCINOMA, DRIVING AN AGGRESSIVE PHENOTYPE.

Pasquale Saggese, PhD *(Scafoglio lab)*
Pasquale Saggese, Aparamita Pandey, Abbie Hall, Eileen Fung, Jane Yanagawa, Erika F. Rodriguez, Tristan R. Grogan, Giorgio Giurato, Giovanni Nassa, Annamaria Salvati, Alessandro Weisz, Steven M. Dubinett, Claudio Scafoglio.

Increased utilization of glucose, beyond that necessary for ATP synthesis, is a hallmark of cancer. Several studies are investigating the efficacy of glucose restriction on solid cancers, by glucose transporter blocking or glycolysis inhibition. However, the adaptations of cancer cells to glucose restriction, leading to treatment resistance, are not known. Here, we report the discovery that glucose restriction in lung adenocarcinoma (LUAD) cells induces cancer cell de-differentiation, leading to a more aggressive phenotype. Glucose deprivation causes a reduction in alpha-ketoglutarate (αKG), leading to attenuated activity of αKG-dependent histone demethylases and consequent histone hypermethylation. We further show that this de-differentiated and aggressive phenotype depends on unbalanced EZH2 activity, causing inhibition of Prolyl-4-hydroxylase domain 3 (PHD3) and increased expression of hypoxia-inducible factor 1α (HIF1α).

HIF1α activation by low glucose leads to activation of Slug and triggers epithelial to mesenchymal transition (EMT). Finally, we identified a HIF1α-dependent transcriptional signature with prognostic significance in human LUAD. Our studies further current knowledge of the relationship between glucose metabolism and cell differentiation in cancer, characterizing the epigenetic adaptation of cancer cells to glucose deprivation and identifying novel targets to prevent the development of resistance to metabolic therapies targeting glucose uptake and glycolysis.

31. ENDOPLASMIC RETICULUM-LIPID DROPLET CONTACT SITE PROTEIN CALSYNTENIN-3B IN LIVER PHYSIOLOGY

Lauren Uchiyama *(GREAT, Tontonoz lab)*
Lauren Uchiyama, Alexander Nguyen, Kevin Qian, Xu Xiao, Liujuan Cui, and Peter Tontonoz

The ketogenic diet has become a popular dietary intervention for rapid weight loss, and promotes fat utilization in the absence of carbohydrates. This diet has favorable effects in the dietary intervention for metabolic disease, neurodegeneration, polycystic ovary disease, and cancer. Aside from the direct enzymatic pathway, the molecular and intracellular mechanisms required for ketogenesis in this context have been poorly characterized. Excess neutral lipids are stored in lipid droplets (LDs)–dynamic organelles that quickly expand and shrink depending on the metabolic needs of the cell. Recent studies have led to the discovery of Calsyntenin-3b (Clstn3b), an endoplasmic reticulum-lipid droplet protein that increases lipid utilization. However, the physiologic or pathologic contexts in which the liver requires Clstn3b remain to be determined. Here, we show that Clstn3b is a PPARa target gene whose expression is highly induced by ketogenic diet. Under these dietary conditions, liver-specific KO of Clstn3b impairs ketogenesis, with no differences in fatty acid oxidation or triglyceride storage. Furthermore, absence of Clstn3b decreases mitochondrial respiration. These findings define the physiologic relevance of an ER/LD organelle contact site protein that modulates ketogenesis, which may reveal novel opportunities for therapeutics in under ketogenic diet.

32. FUNCTIONAL IMPACT OF ADAR1 ON THE DIFFERENTIAL STABILITY OF DSRNA-CONTAINING ALTERNATIVE 3' UTRS

Sari Terrazas *(GREAT, Xiao lab)*
Sari Terrazas, Ting Fu, and Xinshu Grace Xiao

The 3' untranslated regions (3' UTRs) of mRNAs are critical hubs of gene regulation, affecting RNA stability, localization, and translation. The functional roles of 3' UTRs are enabled by intricate interactions between RNAbinding proteins (RBPs) and cis-acting elements in the 3' UTRs. Dysregulation of these regulatory mechanisms can lead to substantial changes in gene expression, as shown for various diseases. In addition, many 3' UTRs harbor double-stranded RNA (dsRNA) structures, a source of endogenous dsRNAs. Presence of cytosolic dsRNA is expected to initiate interferon (IFN) response through anti-viral signaling pathways involving cytosolic dsRNA sensors such as MDA5 or RIG-I. Other RBPs, such as ADAR1, may counteract the immunogenic potential of dsRNAs to avoid abnormal immune activation. As a primary RNA editing enzyme, ADAR1 achieves this function by editing dsRNAs, which is expected to alter dsRNA recognition by MDA5. However, it remains unclear whether other RBPs, RNA processing mechanisms, or cis-elements may be involved in regulating dsRNAome in human cells. In our previous study, we observed abundant binding of ADAR1 to 3' UTRs, contributing to 3' UTR-mediated gene regulation. Recently, our lab carried out RNA-seq for the nuclear and cytoplasmic fractions of control and ADAR1 knockdown (KD) HeLa cells. Upon ADAR1 KD, we observed significant induction of IFN-stimulated genes (ISGs). Moreover, for genes with alternative 3' UTRs due to alternative polyadenylation (APA), we observed a significant enrichment of longer 3' UTRs upon ADAR1 KD in the cytoplasmic, but not the nuclear RNA. Interestingly, the extension regions of these 3' UTRs are enriched with Alu repeats, potentially forming dsRNAs and serving as ADAR substrates. Thus, we hypothesize that dysregulation of ADAR1 leads to an enrichment of dsRNA in long 3' UTRs which stimulates antiviral signaling and ISG expression.

33. ENVIRONMENTAL SENSING IN UNICELLULAR EUKARYOTES

Daniel Velez-Ramirez, PhD *(Hill lab)*
Daniel Vélez-Ramírez, Adelaide Leung, Michelle Shimogawa, Jihui Sha, James Wohlschlegel, Kent Hill

Trypanosoma brucei is a flagellated protozoan, and causative agent of sleeping sickness, a fatal vector borne disease in Sub-Saharan Africa. Despite the recent advances in developing safe and effective treatments, the eradication of it is still out of sight. A critical aspect of T. brucei biology is its ability to survive in two diametrically different environments: the tsetse fly (T. brucei's vector), and the human body. Therefore, T. brucei requires constantly sensing its environment and responding to it, and there is cumulative evidence that shows its flagellum is a cAMP signaling platform. However, the molecular mechanisms are almost completely unknown and such are potential drug targets. In our working model, receptor-type adenylate cyclases (ACs), localized at the tip of the flagellum, produce cAMP upon activation by an unknown environmental signal. The proper functioning of this cAMP pathway, allows T. brucei cells to establish infection within the tsetse fly, making possible T. brucei transmission to humans. T. brucei ACs are single-pass transmembrane proteins with an intracellular cyclase domain, a single-transmembrane alpha helix, and two extracellular ligand-binding domains that resemble bacterial Periplasmic Binding Proteins (PBPs). In bacteria, PBPs bind small molecules, and are involved in sensing, transport, and chemotaxis. We theorize that the PBP-like domains present in T. brucei ACs, function as environmental sensors, transducing extracellular signals to a chemical one: cAMP. In order to determine the nature of those extracellular signals, we are planning to use the 3D structure of the PBP-like domains. Our first AC for structural determination and extracellular signal

prediction is AC1, a flagellum tip-specific AC. So far, we have endogenously tagged AC1 with a HA tag, performed anti-HA pulldowns, and confirmed the presence of AC1-HA on the eluates. Currently, we are scaling up the purification of AC1-HA to obtain enough material for structural determination.

34. CURRENT PROJECTS OF THE TON-THAT LABORATORY

Timmie Britton *(IMMP, Ton-That lab)*

The Ton-That lab studies mechanisms of pilus tip assembly and oxidative protein folding in the Gram-positive pathogens, Actinomyces oris, and Corynebacterium diphtheriae, respectively. We are also focused on identifying novel virulence mechanisms of the Gram-negative opportunistic pathogen, Fusobacterium nucleatum, an oral pathobiont associated with several extra-oral diseases.

35. IMC43 PLAYS AN ESSENTIAL ROLE IN FORMATION OF THE TOXOPLASMA GONDII DAUGHTER IMC

Rebecca R. Pasquarelli *(IMMP, Bradley lab)*
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 survival is dependent on a specialized organelle named the inner membrane complex (IMC) which is used to invade host cells and replicate via an unusual process of internal budding called endodyogeny. During endodyogeny, the IMC acts as a scaffold for developing daughter buds. While many daughter IMC proteins have been identified, most are recruited after budding has been initiated and are not essential for parasite fitness. We recently discovered IMC43, a novel daughter IMC protein that is recruited at the earliest stages of daughter bud initiation. Using the auxin-inducible degron system we showed that depletion of IMC43 results in aberrant morphology, dysregulation of endodyogeny, and an extreme defect in replication. Deletion analyses revealed a C-terminal domain that is essential for the protein's function. We then performed both TurboID proximity labelling and a yeast two-hybrid screen using IMC43 as bait, which identified two binding partners: the essential daughter protein IMC32 and a novel daughter IMC protein which we named IMC44. We found that IMC43 is responsible for regulating the localization of both IMC32 and IMC44 at specific stages of endodyogeny. This regulation was found to be dependent on the essential C-terminal domain of IMC43. Using pairwise yeast two-hybrid assays, we determined that this region is also sufficient for binding to both IMC32 and IMC44. Current studies aim to identify additional components of this essential complex and explore how these proteins collaborate to launch the daughter cell scaffold. As these proteins are parasite-specific, they represent potential targets for therapeutic intervention of T. gondii and related parasites.

36. TANDEM CAR-T CELLS PROMOTE FAVORABLE TUMOR MICROENVIRONMENT REMODELING AND INTERCELLULAR COMMUNICATION IN GBM

Ryan Shih *(IMMP, Chen lab)*

Glioblastoma (GBM) is an aggressive primary brain malignancy with numerous resistance mechanisms and poor response to multimodal treatment. Chimeric antigen receptor (CAR)-T cell therapy, which uses a patient's own T cells engineered to express a synthetic tumor-targeting receptor, has emerged as a promising approach. However, GBM tumors are characterized by (1) a profoundly immunosuppressive tumor microenvironment (TME) that actively renders immune cells dysfunctional, and (2) antigen heterogeneity that provides an escape mechanism from therapies targeting single antigens. To combat both of these features, we previously designed a tandem CAR (tanCAR) with specificity to the soluble immunosuppressive molecule TGF-β and membrane-bound tumor antigen IL-13Rα2. These tanCAR-T cells activate in response to TGF-β (instead of becoming suppressed) and mediate tumor cell killing through engagement with IL-13Rα2, facilitating improved tumor control in vivo hypothesized to stem from recruited

endogenous immune cells reactive against diverse tumor antigens. Single-cell RNA sequencing reveals tanCAR-T cells favorably alter the composition of both myeloid and lymphoid compartments in the TME.

37. DEFINING METABOLIC FLEXIBILITY IN HAIR FOLLICLE STEM CELL INDUCED SQUAMOUS CELL CARCINOMA

Carlos Galvan *(CDB, Lowry lab)*
Carlos Galvan, Aimee Flores, Victoria Cerillos, Itzetl Avila, William Lowry

Among the numerous changes associated with the transformation to cancer, cellular metabolism is one of the first discovered and most prominent. However, despite the knowledge that nearly every cancer is associated with the strong upregulation of various metabolic pathways, there has yet to be any clinical progress on the treatment of cancer by targeting metabolism directly. We previously showed that inhibition of the Warburg Effect in cancer cells of origin had essentially no effect on the initiation or progression of cutaneous squamous cell carcinoma, suggesting that these cancers are potentially metabolically flexible enough to produce the necessary metabolites required for sustained growth in the absence of glycolysis. Here we focused on Glutaminolysis, another metabolic pathway frequently implicated as important for tumorigenesis in correlative studies. We genetically block Glutaminolysis in cancer cells of origin, and found that this has little effect on tumorigenesis, similar to what we previously showed for the Warburg effect. Tumors with genetic blockade of glutaminolysis instead upregulated their lactate uptake and utilization to power the TCA cycle, providing further evidence of metabolic flexibility. To define the limits of metabolic flexibility in cancer initiating hair follicle stem cells, we genetically blocked both glucose and glutamine metabolism and found that frank carcinoma was not compatible with abrogation of both of these carbon utilization athways. These data point towards metabolic flexibility mediated by regulation metabolite uptake and suggest that treatment of cancer through metabolic manipulation will require multiple interventions on distinct pathways.

38. DRUG TARGET IDENTIFICATION BY PHOTOSENSITIZER-DEPENDENT OXIDATION AND CAPTURE BY AMINE

Eli Biletch *(CBI, Backus lab)*

For every 24 therapeutic candidates in the development pipeline, only one on average will reach FDA approval. The most common cause of this attrition is a lack of efficacy or safety, indicative of poor target binding and/or deleterious off-target interactions. Accordingly, innovation in target identification and validation techniques is key to improving clinical success. State-of-the-art target identification strategies such as photoaffinity labeling and microenvironment mapping often struggle to elucidate transient interactors such as those involved in target complexes and molecular glue effects. Here, we present the early development of a photosensitizer-dependent target identification strategy based on the oxidation and capture of target proteins by clickable amines. Preliminary data suggest that the efficiency and radius of labeling may be suitable for the characterization of transient interactors and target complexes, a hypothesis we are working to validate by pulling down well-established targets of the kinase inhibitor dasatinib.