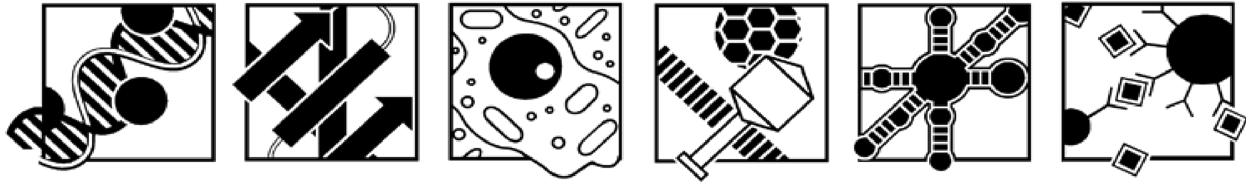


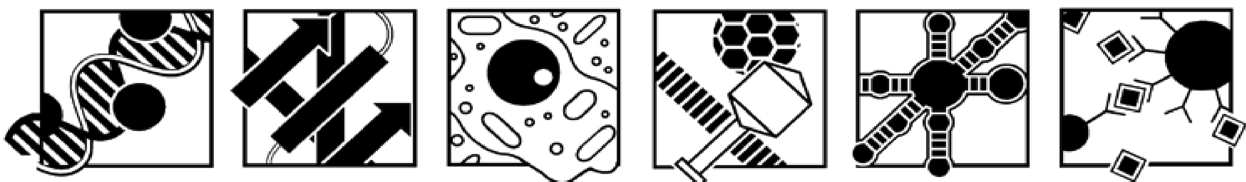
GRADUATE PROGRAM IN CELLULAR AND MOLECULAR BIOLOGY

40TH ANNUAL SYMPOSIUM AND POSTER SESSION

50TH ANNIVERSARY CELEBRATION

PROGRAM AND ABSTRACTS

FRIDAY, MAY 20, 2022



MEDICAL SCHOOL
CELLULAR & MOLECULAR BIOLOGY
UNIVERSITY OF MICHIGAN

40th ANNUAL SYMPOSIUM

Friday, May 20, 2022

Myron Levine Lecture

Dr. Erika Holzbaur

Professor, Department of Physiology
University of Pennsylvania

"Actin dynamics regulate spatial mixing of mitochondria in dividing cells and mitochondrial homeostasis in interphase cell"

CMB Introductions (12:00 p.m. – 12:10 p.m.)
12:00 p.m. - 1:00 p.m.
The Kensington Hotel Auditorium

CMB STUDENT/FACULTY POSTER SESSION
1:00 p.m. – 4:00 p.m.
Grande Ballroom, The Kensington Hotel
Appetizers and refreshments to be served

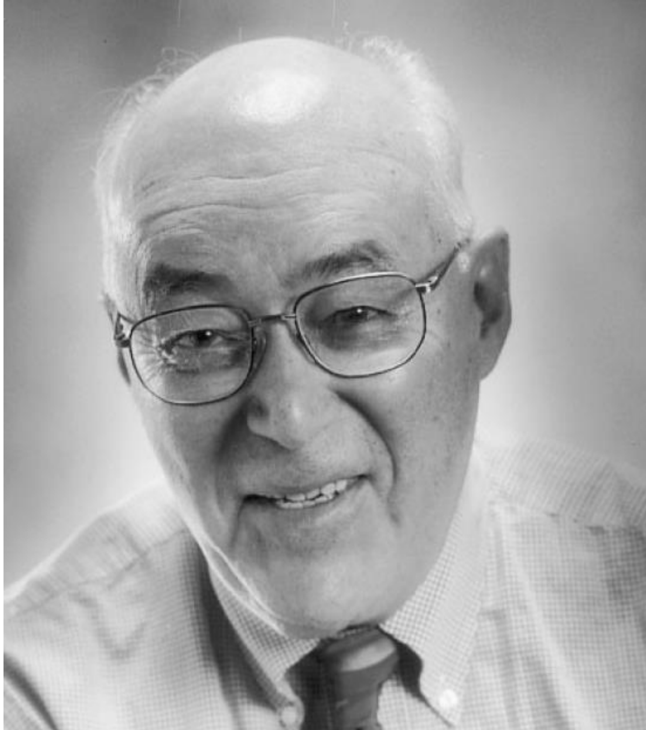
Questions: Contact Carolyn Walsh in the CMB Office: cmbgrad@umich.edu or (734) 615-1660

Biography – Erika Holzbuar, Ph.D.

Erika Holzbaur's laboratory is focused on the microtubule-based motor cytoplasmic dynein and its activator dynactin. Dynein and dynactin are required for vesicular trafficking, mitotic spindle assembly, and development of polarity. We are interested in mechanisms of force production and motor function, mechanisms of cargo coupling and regulation, effects of dynein and dynactin on dynamics of the cytoskeleton, and the analysis of neurodegenerative diseases resulting from impairments in dynein/dynactin function. Disruptions in dynein/dynactin function cause motor neuron degeneration and muscle atrophy, leading to motor neuron diseases similar to ALS. Approaches in the lab include in vitro motility assays, cellular transfection assays, live cell microscopy, and development and characterization of transgenic mouse models for motor neuron disease.

The Twenty-First Myron Levine Lecture
May 20, 2022

Myron Levine, Ph. D.
July 28, 1926 - December 12, 2012



"His was the voice of reason — clear, insightful, dispassionate and humane, a model of the academic virtues."

Myron Levine was a founder of the CMB Program and Director from 1974 to 1990. The program was initiated to provide a meeting ground for faculty scattering among different departments across the campus who had overlapping research interests in the rapidly developing fields of cellular and molecular biology. The continuing success of the program over nearly four decades is a confirmation of wisdom of its founders. During his long tenure as Director, Mike was particularly sensitive to the needs of CMB students. While always attentive to the goals of the program, his flexibility facilitated positive problem-solving, and his wise counsel helped students overcome personal and academic difficulties to succeed in the program. Mike was very proud of the continued success of CMB under the Robert Bender, Dave Engelke and Jessica Schwartz, and he considered the program to be one of his most important achievements.

Mike carried out his undergraduate work at Brooklyn College in New York City. A summer research program at Cornell University in Ithaca was influential in his commitment to biological research. A faculty advisor encouraged him to apply to graduate school in the Biology Department at the University of Indiana in Bloomington, which was then at the forefront of genetics research. His teachers there included Herman Muller and Tracy Sonneborn. As a postdoctoral fellow with Salvador Luria, who was later to win the Nobel Prize, Mike attended the famed Phage Group meetings at Cold Spring Harbor Laboratory in the 1950s and 60s. The accommodations at the Laboratory were primitive at that time but the intellectual atmosphere was rarefied, and the Levine family spent several memorable summers there.

Mike joined the Department of Human Genetics at the University of Michigan in 1961. The early work in his laboratory focused on the genetic regulation of lysogeny in bacteriophage P22, which

has a genome of 44 kb encoding 64 genes. With postdoctoral fellow Hamilton Smith, who later went on to win a Nobel Prize, he used pulse–labeling to identify the sequential activation and repression of phage genes during the establishment of lysogeny, work that was published in *Science* and considered by Mike as some of his best. Smith's recollection of his years in Mike's lab can be heard in an interview at the Cold Spring Harbor Laboratory oral history web site. As a Ph. D. student with Mike, David Botstein used temperature-sensitive phage mutants to dissect the intermediates in phage replication. Botstein went on to chair the genetics departments at Stanford and Princeton. The Levine lab also carried out cell-free assembly of pre-formed tail-less heads and head-less tails into active phage. He reviewed the work of this period in an article on Phage Morphogenesis in the Annual Review of Genetics, Vol. 3, 1969, that still reads well today.

In the 1970s and 80s, Mike applied similar logic to dissect the process of latent neuronal infection by the Herpes virus, which has a 150 kb genome encoding 100 genes. With students and postdoctoral fellows Fred Homa, Roz Sandri-Goldin and Al Goldin, the laboratory studied viral replication and the establishment of latency. In an interdisciplinary collaboration with neurologist David Fink and virologist Joe Glorioso, this virus was developed as a carrier for gene therapy, taking advantage of the natural tropism of the virus for neuronal ganglia. This work was continued in the Fink and Glorioso laboratories, leading to delivery of the human proenkephalin gene for treatment of chronic pain by administration of herpes virus through the skin (Ann. Neurol. 2011). More than 25 years after initiation of the work, clinical trials are now in progress.

In addition to his contributions to education and research, with publication of more than 120 articles, Mike served the scientific community on editorial and review panels, as Editor of the Journal of Virology, and member and chair of the NIGMS committee on the Genetic Basis of Disease. He was Acting Chair of the Department of Human Genetics for 3 terms and was honored with the UM Distinguished Faculty Achievement Award, the Distinguished Biomedical Lecture, and the Annual CMB Myron Levine Lecture. He served as a member of the Wallenberg Executive Committee for more than 20 years.

Mike was greatly valued by his faculty colleagues. At faculty meetings in the Department of Human Genetics, his was a voice of reason – clear, insightful, dispassionate and humane, a model of the academic virtues. People leaned forward to hear his quiet, measured contributions. One of my first evenings in Ann Arbor was spent at a CMB student/faculty get-together in the Levines' home on Hillsdale. The room was full, with students sitting and standing everywhere. The atmosphere was warm and collegial, imbued with the graceful hospitality that Mike and Bobbie so generously shared with the University community. After his retirement in 1996, Mike remained active as an Emeritus Professor, continuing to participate in many CMB and Human Genetics functions. For more than 40 years, Mike Levine made unique contributions to the quality of academic life at the University of Michigan. He will be long remembered and deeply missed.

Miriam Meisler
Myron Levine Professor of Human Genetics

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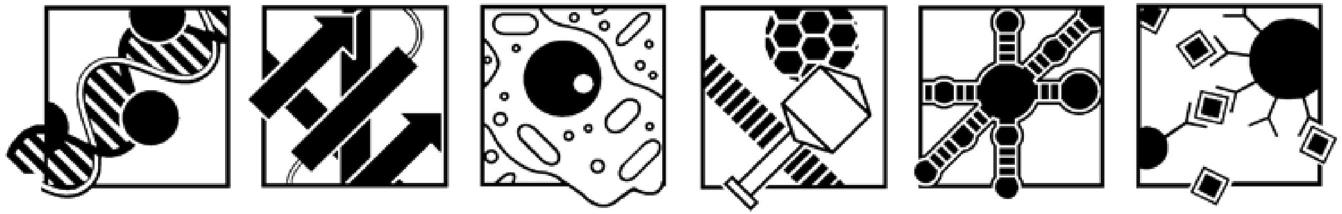
Acknowledgments

We wish to thank everyone who has helped to make the symposium both a successful event and a strong and lasting tradition for CMB, this year and in previous years. Our thanks also go out to the student volunteers who have helped in setting up the facilities.

This year we celebrate 50 years as a CMB program! The program has a long-standing history of excellence in research. We could not make significant medical strides without your support. Thank you for being part of this wonderful program.

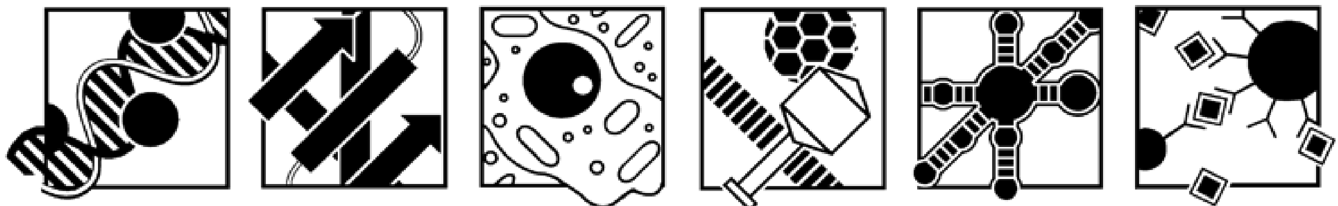
Thanks for your tremendous participation!

40th Annual Symposium Committee



CMB Thanks YOU!

CMB Faculty and Students
2021-2022



CMB thanks the many students and faculty who have helped with CMB activities in 2021-2022:

Student Committees

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Margarita Brovkina
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Kaitlyn Speckhart*
Hari Milaganur Mohan*

Rackham Student Government:

Ashley Melnick*

*Students on committee more than one year

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Prelim Workshop	Manoj Puthenveedu Ben Allen
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2021/2022 CMB Recruiting	ABRCMS**:** Ben Allen (Rackham Diversity Ally)		
Rackham Diversity Allies	Ben Allen		
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Newsletter Advisor	Karl Desch		
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PIBS 504 (R&R)	Scott Barolo		

	Chad Brenner	Sunny Wong
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		Dan Goldman

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Wu, Josephine – Barmada (Mentor)
Andy Lieberman (Chair), Stephanie Moon, Peng Li, Hayley McLoughlin

ABSTRACTS
BY CATEGORY

Cell Biology, Cellular Biochemistry, Cellular Physiology, Structural Biology

CMB Affiliate	Poster #	Abstract Title & Authors
Truong, Hanh	1	**The Ciliary Transition Zone Protein, Tectonic1, Forms a Membrane Diffusional Barrier in Mouse Rod Outer Segments** Hanh Truong*, Jason Willer, Jorge Martinez-Marquez, Amanda Travis, Jillian Pearring
Machlin, Jordan	2	**Female Reproductive Biology and Tissue Engineering** Jordan Machlin*, Ariella Shikanov
Royzenblat, Sonya	3	**Escherichia coli chromosome conformation mapping** Sonya Royzenblat*, Peter Freddolino
Mohan, Harihar Milaganur	4	**Understanding the role of RTL8 proteins in Ubiquilin-2 biology** Harihar Milaganur Mohan*, Hanna Trzeciakiewicz, Emily Crowley, nathaniel Safren, Henry Paulson, Lisa Sharkey
Chen, Brandon	5	**Endoplasmic reticulum-mitochondria contact sites (ERMCS) signal cellular epigenetic state to lipid reprogramming** Brandon Chen*, Tito Cali, Jonathan Sexton, Costas Lyssiotis, Yatrick Shah
Eberhardt, Emily	6	**N-terminal GTPase-mediated regulation of the outer-mitochondrial membrane protein Miro1** Emily Eberhardt*
Pletan, Madison	7	**Motor-dependent transport of misassembled Nups to the endoplasmic reticulum** Madison Pletan*, Billy Tsai

Cell Biology, Cellular Biochemistry, Cellular Physiology, Structural Biology
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CMB Affiliate	Poster #	Abstract Title & Authors
Connolly, Sarah	8	**Structural analysis of Helicobacter pylori VacA's channel in membrane** Sarah M. Connolly*, Amanda Erwin, Megan Sabb, Georgia Caso, Timothy L. Cover, Melanie D. Ohi
Melnick, Ashley	9	**Polymerase-associated factor complex (PAFc) protects Notch-induced leukemia cells from DNA damage and mitochondrial stress** Ashley Melnick*, Anna McCarter, Shannon Liang, Nicole Dean, Elizabeth Choe, Nicholas Kunnath, Brain Magnuson, Qing Wang, Mats Ljungman, Mark Chiang
Knupp, Jeffrey	10	**Revisiting the role of sperm protamine proteins in organismal development, fertility, and evolution** Jeffrey Knupp*, Yu-Jie Chen, Peter Arvan, Billy Tsai
Orosco, Amanda	11	**Understanding the role of Keratin 16 in maintaining tissue homeostasis in response to stress** Amanda Orosco*, Pierre Coulombe
Collie, Sam	12	**FLAP mediates Signal Relay in Neutrophils during Chemotaxis** Sam Collie*
Vangos, Nicholas	13	**Deciphering the tubulin code: mechanisms of tubulin recognition by EML2** Nicholas Vangos*, Michael Cianfrocco
Pineda, Christopher	14	**Specification mechanisms for Keratin 17 during the DNA Damage Response** Christopher Pineda*, Raji Nair, Pierre A. Coulombe

Cell Biology, Cellular Biochemistry, Cellular Physiology, Structural Biology
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Vizurraga, Alexander	15	**Structure-Aided Discovery of First-in-Class Small Molecule Adhesion GPCR Modulators** Alexander Vizurraga*, Gregory G. Tall
Huang, Wesley	16	**Defining the role of ferroptosis in inflammatory bowel disease (IBD)** Wesley Huang*, Nupur Das, Yatrik Shah
Yuan, Ye	17	**Towards a Cell Biology Model of the Circadian Clock in Drosophila** Ye Yuan*, Yangbo Xiao, Dunham Clark, Swathi Yadlapalli
Forson, Jacqueline	18	**Traversing the secretory pathway in search of degradation signals** Jacqueline Forson*, Rachel Plumb, and Ryan Baldridge
Wu, Josephine	19	**Neuronal activity regulates MATR3 in a calcium and calmodulin dependent manner** Josephine Wu*, Ahmed Malik, Quinn Doctrove, Christie Gillies, Sami Barmada
Williams, Jonathan	20	**The Structural Basis of Meiotic Prophase Chromosome Movements by the Linker of Nucleoskeleton and Cytoskeleton (LINC) Complex** Jonathan D. Williams*, Ritvija Agrawal, Kirsten Brenner and Jayakrishnan Nandakumar

Developmental Biology, Organogenesis, Neurobiology, Aging

CMB Affiliate	Poster #	Abstract Title & Authors
Conchola, Ansley	21	**Stable iPSC-derived NKX2-1+ Lung Bud Tip Progenitor Organoids Give Rise to Airway and Alveolar Cell Types** Ansley S. Conchola*, Renee F.C. Hein, Alexis S. Fine, Zhiwei Xiao, Tristan Frum, Charlie J. Childs, Yu-Hwai Tsai, Emily M. Holloway, Sha Huang, John Mahoney, Jason R. Spence
Wang, Zhong	22	**14-3-3 binding motif phosphorylation disrupts Hdac4 organized inhibitory condensates to stimulate cardiac reprogramming** Liu Liu, Ziad Sabry, Ienglam Lei, Shuo Tian, Wenbin Gao, Yijing Guo, Zhaokai Li, Y. Eugene Chen, and *Zhong Wang*
Dean, Kendall	23	**Determining the role of Ankyrin-G in GABAergic deficits related to bipolar disorder using patient-derived neurons** Kendall Dean*, Paul Jenkins
Gordian, Desiree	24	**Identifying the Role of GPR10 and CALCR-Expressing Neurons in the Area Postrema in the Aversive and Non-Aversive Control of Food Intake** Desiree Gordian*, Martin G. Myers, Jr.
Elkahlah, Najia	25	**FruM interacts with neuronal lineage to sexually differentiate neurons** Najia Elkahlah*, Josie Clowney
Hergenreder, Ty	26	**Dscam gene triplication causes neocortical overinhibition in Down syndrome** Hergenreder, Ty, Liu H, Caballero-Florán RN, Yang T, Hull JM, Pan G, Li R, Veling MW, Isom LL, Kwan KY, Huang ZJ, Fuerst PG, Jenkins PM, and *Y,e Bing*
Tuckowski, Angela	27	**The role of fmo-4 in longevity and stress resistance** Angela Tuckowski*, Marshall Howington*, Hillary Miller, Scott F. Leiser
Wallace, Gunseli	28	**Auditory lipidomics, an approach to identify unique molecular effects of noise trauma** Gunseli Wallace*, Lingchao Ji, MBBS, PhD, Costas Lyssiotis, PhD, Gabriel Corfas, PhD

Developmental Biology, Organogenesis, Neurobiology, Aging
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CMB Affiliate	Poster #	Abstract Title & Authors
Manske, Gabriel	29	**Centromere Identity during Germ Cell and Embryonic Development Defies the Somatic Cell One-to-One Maintenance Mechanism** Gabriel Manske*, Kelsey Jorgensen, Saikat Chakraborty, Binbin Ma, Mansour Abo-Elenin, Samantha Schon, Ben Black, Karen Schindler, Xin Chen, Saher Sue Hammoud
Henry, Evelynn	30	**Identifying and characterizing a longevity control center in the Drosophila brain** Evelynn Henry*, Tuhin Chakraborty, Scott Pletcher
Kim, Sumin	31	**TorsinA regulates nuclear pore complex assembly and localization during neuronal maturation** Sumin Kim*, Daniela Boassa, Sébastien Phan, Thomas R. Shaw, Samuel S. Pappas, Mark H., Ellisman, Sarah L. Veatch , Sami J. Barmada, William T. Dauer
Munneke, Allyson	32	**The serotonin receptor 5-HT2A mediates nutrient-specific longevity in Drosophila** Allyson S. Munneke*, Saige S. Porter, and Scott D. Pletcher

Genetics and Genomics, Gene Regulation

CMB Affiliate	Poster #	Abstract Title & Authors
Serrano-Zayas, Candilianne	33	**Investigating the transcriptional regulation of the LDL Receptor** Candilianne Serrano-Zayas*, Taslima G. Khan, David Ginsburg, Brian T. Emmer
McShane, Ariel	34	**Exploring the relationship between transcription regulation and chromatin organization** Ariel McShane*, Michelle Paulsen, Ruchisree Garuda, Ishwarya Venkata Narayanan, Karan Bedi,Brian Magnuson, Tom Wilson, Mats Ljungman
Ryan, Charles	35	**A RING1 contribution to neurogenesis and schizophrenia** Charles Ryan*, Yao Tsan, Samamtha Regan, and Stephanie Bi
Brovkina, Margarita	36	**Fruitless decommissions regulatory elements to implement cell-type-specific neuronal masculinization** Margarita V. Brovkina*, Rachel Duffié, Abbigayl E. C. Burtis, and E. Josephine Clowney

Molecular Mechanisms of Disease, Cancer Biology, Genetics of Disease, Non-Human Models of Disease

CMB Affiliate	Poster #	Abstract Title & Authors
McLoughlin, Hayley S.	37	**Gene silencing in SCA3 disease mice rescues spatiotemporal oligodendrocyte impairments** Kristen H Schuster, Annie J Zalon, Danielle DiFranco, Alexa Putka, Sabrina Jarrah, Nicolas Stec, Arsal Naeem, Zaid Haque, Hongjiu Zhang, Yuanfang Guan, *Hayley S. McLoughlin*
Michmerhuizen, Anna	38	**Multiomics analysis to uncover the mechanism of radiosensitization of androgen receptor-positive triple negative breast cancers with AR inhibition** Anna R. Michmerhuizen*, Andrea M. Pesch, Benjamin C. Chandler, Lynn M. Lerner Connor Ward, Leah Moubadder, Stephanie The, Breanna McBean, Caleb Cheng, Lori J.Pierce, Corey W. Speers
Wang, Zhong	39	**Acetyl-CoA production by specific metabolites promotes cardiac repair after myocardial infarction via histone acetylation** Lenglam Lei, Shuo Tian, Wenbin Gao, Liu Liu, Yijing Guo, Paul C Tang, Y. Eugene Chen, *Zhong Wang*
Azaria, Ruth	40	**Leveraging Proteostasis for Niemann-Pick C Gene Therapy** Ruth Azaria*, Mark Schultz, Andrew Lieberman
Rios-Doria, Jonathan	41	**INCB090244 is a selective and orally bioavailable small molecule that potently inhibits PD-L1 and induces antitumor immunity** **Jonathan Rios-Doria, Alla Volgina, Prafulla C. Gokhale, Hao Liu, Christina Stevens, Nina Zolotarjova, Darlise DiMatteo, Kanishk Kapilashrami, Elham Behshad, Pramod Thekkat, Gengjie Yang, Leslie Hall, Chrysi Kanellopoulou, Mark Rupar, Christopher Maddage, April Horsey, Krista Burke, Yan-ou Yang, Maryanne Covington, Steve Wang, Phillip Liu, Richard Wynn, David A. Reardon, Holly Koblish **CMB Alum

Molecular Mechanisms of Disease, Cancer Biology, Genetics of Disease, Non-Human Models of Disease

(continued)

CMB Affiliate	Poster #	Abstract Title & Authors
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Balbin-Cuesta, Ginette	43	**Reactivation of Gamma Globin Expression in β-Hemoglobinopathies** Ginette Balbin-Cuesta*, Rami Khoriaty
Bidlack, Christopher	44	**Deep Mutagenesis Scan of SERPINC1 to inform variants affecting thrombosis risk** Christopher Bidlack*, Krista Golden, Mary Underwood, and Karl Desch
Elvira, Carina	45	**Ankyrin-G in Developmental and Epileptic Encephalopathy** Carina Elvira*, Paul Jenkins
Correia, Adele	46	**Combatting Cholesterol Accumulation in Niemann-Pick Type C Disease** Adele Correia*, Andrew Lieberman
Nakatsuka, Erika	47	**Targeting DNA Double Strand Break Repair in Homologous Recombination Proficient High Grade Serous Ovarian Cancers** ***Erika Nakatsuka, MD, PhD, Lijun Tan, Caroline Foster, **Karen McLean, MD, PhD *** Postdoctoral Fellow **CMB Alum
Gensterblum-Miller, Elizabeth	48	**CRTC1-MAML2 fusion translocation and transcription regulation in mucoepidermoid carcinoma** Gensterblum-Miller E*, Heft Neal M.E., Bhangale A.D., Brenner J.C.
Tseng, Yi-Ju (Lulu)	49	**Ribosomal quality control in repeat-associated non-AUG translation of GC rich repeats** Yi-Ju Tseng*, Peter K. Todd

Molecular Mechanisms of Disease, Cancer Biology, Genetics of Disease, Non-Human Models of Disease
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CMB Affiliate	Poster #	Abstract Title & Authors
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Bell, Hannah	51	**Microbiota metabolic exchange is critical for colorectal cancer redox homeostasis and growth** Hannah Bell*, Joshua Goyert, Yatrik Shah
Van Pelt, Kate	52	**FIC-1/FICD-mediated AMPylation of HSP70 family chaperones modulates polyglutamine toxicity** Kate M. Van Pelt* , Matthias C. Truttmann
Pun, Matthew	53	**Evaluating shared molecular features and potential vulnerabilities of low-H3K27me3 pediatric brain tumors** Matthew Pun*, Sriram Venneti MD, PhD
McMillan, Michael	54	**RNA methylation influences TDP43 binding and disease pathogenesis in models of amyotrophic lateral sclerosis and frontotemporal dementia** McMillan M*, Gomez N*, Bekier M, Li X, Miguez R, Tank EM, Barmada SJ
Gomez, Nicholas	55	**Exogenous UPF1 corrects TDP-43-directed NMD-sensitization of the transcriptome in neuronal models of ALS** Nicolas Gomez*, Elizabeth Tank PhD, Sami Barmada MD PhD
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#01

The Ciliary Transition Zone Protein, Tectonic1, Forms a Membrane Diffusional Barrier in Mouse Rod Outer Segments

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Primary cilia are thin microtubule-based organelles that relays extracellular signals to the cell through receptors enriched in the ciliary membrane. The distinct protein composition of the cilium is maintained by a membrane diffusional barrier, formed by the transition zone. In primary cilium, the transition zone generally retains receptors in the cilia; however, in the specialized light-sensing photoreceptor cilium (aka the outer segment) the transition zone acts to prevent non-resident proteins from accumulating in the cilia. While previous work has showed that membrane proteins utilize ciliary transport carriers (such as TULP, IFT and BBSome) to enter and exit the cilium, the molecular components of the diffusion barrier remain unknown. We now show that Tctn1, a component of the transition zone, forms a physical diffusional barrier for membrane proteins in mouse rod outer segments. Specific loss of Tctn1 in rods results in normal outer segment formation and transition zone structure; however, overtime non-resident membrane proteins accumulate into the outer segment and rods degenerate. We find that ciliary transport carriers are not affected by loss of Tctn1 and instead the rate of membrane protein diffusion within the transition zone is increased suggesting that Tctn1 acts as a physical gate. We also identified a *Tctn1* nonsense mutation in a family with retinitis pigmentosa that results in mislocalization of Tctn1 from the transition zone in photoreceptors. Together our work shows that the tectonic complex acts as a physical barrier within the membrane slowing diffusion through the transition zone and allowing for proper removal of non-resident membrane proteins from the photoreceptors outer segment.

#02

Female Reproductive Biology and Tissue Engineering

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Research in the Shikanov lab aims to develop means to restore ovarian reproductive and endocrine function in young women and girls with premature ovarian insufficiency (POI). POI is a common complication of anticancer treatments and it causes sterility and complications related to absent ovarian endocrine function such as premature osteopenia, muscle wasting, impaired cognitive development, and accelerated cardiovascular disease. The various projects in our laboratory address the critical need for safe fertility preservation options and contribute to our ability to extend fertility preservation to a population of young girls currently devoid of this option without the risk of cancer recurrence. We engineered a biomimetic environment for ovarian tissue with controlled physical and biological properties that promotes the survival and development of ovarian follicles. The biomimetic environment for in vivo applications, such as ovarian tissue implantation, promotes graft remodeling, revascularization and restoration of fertility and endocrine function. In vitro culture of the ovarian follicles in the biomimetic matrices mimics the physiological environment and leads to maturation of healthy eggs. Additionally, we designed a novel immuno-isolation device that protects the implanted tissue from rejection, allows diffusion of nutrients, oxygen and hormones, and accommodates structural and functional changes of growing follicles. Furthermore, we have created a mouse model to mimic T therapy for the female-to-male (FTM) gender transition. These mice manifest defects in ovarian and uterine architecture. Creation of a FTM mouse model provides a powerful tool to clarify the effects of T therapy on fertility, in a manner not possible in humans. The goal of these projects is to use FTM mouse model to investigate the effects of cross-sex T treatment on reproductive phenotype and function, and determine the reversibility of these effects following cessation of T. Finally, we are utilizing single cell RNA sequencing (scRNAseq) to create a single cell atlas of the human ovary. Our goal is to create a comprehensive transcriptional map of the cell types in the human ovary using scRNAseq, allowing us to decipher the transcriptional differences between follicular cells at different stages of development and identify stromal cells performing supportive functions to follicle development.

#03

Escherichia coli chromosome conformation mapping

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Like all cells, the *Escherichia coli* genome has a large ratio of DNA to cell size and thus, there is a need for the chromosome to be compacted into a manageable size into the nucleoid. Unlike in eukaryotes, which have histones around which the DNA winds, prokaryotes use nucleoid-associated proteins to modulate their DNA compaction. The organization of the chromosome in three-dimensional space is still a topic under scrutiny. Given that chromosomal position has been shown to have a strong effect on transcription, and that specific highly expressed genes have been observed to cluster into transcriptional factories, information on chromosomal conformation is critical in further studying the regulation of bacterial gene transcription. Currently, the field is divided between two theories, the first being that the chromosome of *E. coli* folds into macrodomains, and the second being that it folds into a well-mixed boundary-less conformation with each part equally interacting.

More recently, alternative methods have been developed that avoid conditions that could alter the chromosome conformation, such as the use of Mu transposable phage that integrates its DNA into *E. coli* at a high frequency with virtually no location bias and transposes via replicative transposition. I plan to expand upon this knowledge in the field by (1) characterizing a 3D DNA-DNA contact map using a high-resolution variant of the Mu transposition-based method and (2) determining how long-range contacts correlate with gene regulation. The overall impact of this work will allow the field to finally move forward in determining relationships between DNA structure and function, by creating a 3D *E. coli* DNA:DNA contact map. Furthermore, the proposed Mu method can be used as a tool to characterize genome structure in a more definitive manner that can inform future work in DNA interaction mapping and the study of gene regulation in other organisms.

#04

Understanding the role of RTL8 proteins in Ubiquilin-2 biology

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The brain expressed ubiquilins (UBQLNs) 1, 2 and 4 are a family of ubiquitin adaptor proteins that participate broadly in protein quality control (PQC) pathways, including the ubiquitin proteasome system (UPS). One family member, UBQLN2, plays a role in numerous neurodegenerative diseases including ALS/FTD and Huntington's disease. UBQLN2 typically resides in the cytoplasm but can translocate to the nucleus under proteotoxic stress and in disease to facilitate nuclear PQC. How UBQLN2 translocates to the nucleus and clears aberrant nuclear proteins, however, is not well understood. In a mass spectrometry screen to discover UBQLN2 interactors, we identified a family of small (13 kDa), highly homologous uncharacterized family of proteins, retrotransposon Gag-like 8 (RTL8). We then confirmed the interaction between UBQLN2 and a representative RTL8 family member, RTL8A, both *in vitro* using recombinant proteins and *in vivo* using mouse brain tissue. We further show that RTL8A promotes nuclear translocation of UBQLN2. The robust effect of RTL8A on nuclear translocation of UBQLN2 does not extend to the other brain-expressed ubiquilins, UBQLN1 and UBQLN4. Depletion of all RTL8 proteins in human cell lines perturbs nuclear translocation of UBQLN2 following heat shock. Finally, compared to UBQLN1 and UBQLN4, UBQLN2 preferentially stabilizes endogenous RTL8 levels in cells and mouse brain, supporting functional heterogeneity among UBQLNs. As novel UBQLN2 interactors that recruit UBQLN2 to specific subnuclear compartments, RTL8 proteins may regulate the role of UBQLN2 in nuclear PQC.

#05

Endoplasmic reticulum-mitochondria contact sites (ERMCS) signal cellular epigenetic state to lipid reprogramming

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Organelles provide unique environment for complex metabolic reactions to occur. Healthy organellar functions and robust inter-organellar communication are thoroughly controlled to sustain tissue homeostasis and cellular physiology. Organellar dysregulations have been associated with tumorigenesis, yet the roles of inter-organellar communications in cancers have been elusive. Physical inter-organellar contacts allow signal transduction via direct molecule exchange, protein-protein interaction and post-translational modification. Mitochondria, the cellular metabolic and bioenergetic hubs, form one of the most robust contact sites with the endoplasmic reticulum (ER). **ER**-**m**itochondria **c**ontact **s**ites (**ERMCS**) have critical roles in lipid metabolism, calcium homeostasis, and reactive oxygen species (ROS) handling. Dynamic ERMCS remodeling is critical to sustain mitochondrial respiration, ER stress adaptation and metabolic rewiring. ERMCS dysregulations have been observed in metabolic disorders such as diabetes, obesity and cancer. However, we have very limited understanding of how ERMCS is associated with tumor metabolic adaption and rewiring.

Here, I utilized a novel split-GFP ER-mitochondria proximity reporter to identify critical regulators of ERMCS with an image-based phenotypic FDA-approved drug repurposing screen platform. I identified Fedratinib, a novel JAK2/BRD4 inhibitor, and other BRD4/BET inhibitors as potent inducers of ERMCS formation across a panel of 10 cancer cell lines of various tissue origins. Interestingly, the role of BRD4 in ERMCS remodeling is independent of the canonical pTEFb (CDK9 and cyclin T1)-BRD4 transcription elongation complex, as inhibition CDK9 does not phenocopy BRD4 inhibitors. Utilizing lattice light sheet microscopy, we discovered the induction of ERMCS occurs as early as 2-4 hours and maximizes around 10-12 hours. Then, we conducted bromouridine-tagging RNA sequencing, or Bru-Seq, to identify nascent RNA transcript modulation within 30 minutes and 2-hour post Fedratinib treatment. We identified downregulation of gene set associated with hypoxia and various lipid metabolic pathways, and a drastic upregulation of integrated stress response (ISR) and ER stress genes. Interestingly, exposure to long term 2% oxygen (hypoxic) decreased basal ERMCS and reverted Fedratinib's activity to induce ERMCS formation. Surprisingly, hypoxic mimetic via PHD inhibitors did not reverse the phenotype, indicating uncoupling of HIF and oxygen signaling. We then focused on the connection between lipid metabolism and oxygen sensing as one the rate-limiting enzyme in coordinating fatty acid desaturation, Stearoyl-CoA desaturase (SCD), is an oxygen dependent fatty acid desaturase. Pharmacological inhibition of SCD1 similarly reverted the phenotype of ERMCS induction, suggesting hypoxia is modulating ERMCS via manipulating fatty acid saturation and lipid reprogramming. We hypothesize ERMCS is a central hub in integrating cellular fatty acid distribution and signaling hub for stress response. In conclusion, understanding metabolic implications of **ER**-**m**itochondria **c**ontact **s**ites (**ERMCS**) and cellular responses upon ERMCS remodeling will give us novel insights into targeting metabolic vulnerabilities in cancers.

#06

N-terminal GTPase-mediated regulation of the outer-mitochondrial membrane protein Miro1

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Microtubule-based trafficking of mitochondria is critical to fulfill the energy needs of the cell. Failure to regulate this process has detrimental effects on neuronal health and can result in neurological disorders such as Parkinson's disease. Of particular importance to mitochondrial trafficking is Miro1, an outer-mitochondrial, membrane protein. Despite almost 20 years of intensive study, many aspects of Miro1-directed trafficking remain unknown. Cell-based approaches have been unable to arrive at mechanistic descriptions for the orchestration of mitochondrial trafficking, although one hypothesized mechanism for regulation includes the GTPase domains of Miro1. Mutations locking the GTPase domain in specific nucleotide states have been well-characterized in another family of G proteins and have been applied to Miro1 to stabilize the protein in the GTP or GDP-bound state. Of particular interest is the Miro1 P13V mutant (GTP-locked nGTPase). Not only does Miro1 P13V appear to be associated with an apoptotic pathway, but it also appears to cause severe phenotypic effects on mitochondrial dynamics and trafficking. Using the baculovirus expression system in *Spodoptera frugiperda* cells, I have expressed and purified human, Miro1 proteins and investigated their biochemical and structural properties. Using a combinatorial approach including size-exclusion chromatography and transmission electron microscopy, I have determined that P13V Miro1 appears distinct from WT Miro1. I have also begun work to further characterize the differences between these proteins through 1) comparison of the melting temperature of both proteins using a thermal shift assay and 2) interaction with the downstream, binding partner TRAK1. I hypothesize that not only are Miro1's regulatory domains modulated in response to GTP state, but that this regulation is critical for mitochondrial trafficking.

#07

Motor-dependent transport of misassembled Nups to the endoplasmic reticulum

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Dysfunctional nuclear pore complexes (NPCs) leading to mislocalization of the NPC components nucleoporins (Nups) are frequently observed in age-related neurodegenerative diseases, as well as certain cancers and viral infections. Here we clarify one mechanism of Nup mislocalization. By impairing NPC integrity via depletion of the phenylalanine-glycine (FG)-rich Nup component Nup98, we find that the resulting misassembled FG Nups - including Nup62 – are targeted to a discrete punctum in the endoplasmic reticulum (ER) called the ER-focus. Strikingly, the Nup-localized ER-focus co-localizes with a previously-established ER site that functions as a repository for polyomavirus undergoing ER-to-cytosol escape, a critical infection step. The ER membrane protein kinectin, in concert with its cytosolic kinesin-1 motor, binds to and promotes proper transport of the misassembled Nups to the ER-focus. Surprisingly, the mislocalized Nups are not subject to turnover by proteasomal or lysosomal degradation. Further work is being conducted to determine whether the mislocalized Nups have the capacity to recycle back into functional nuclear pore complexes; this would suggest a model where the ER-foci serve as critical storage sites for displaced Nups. Together, our results describe a motor-dependent transport mechanism that targets misassembled Nups to a discrete ER site for storage and possible recycling back to the nuclear membrane.

#08

Structural analysis of *Helicobacter pylori* VacA's channel in membrane

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Helicobacter pylori is a Gram-negative bacterium that infects the human stomach and causes gastric inflammation. *H. pylori* colonizes the stomachs of over half of the world's population and can result in the development of chronic gastritis, peptic ulcer disease (PUD) and gastric cancer. Vacuolating cytotoxin A (VacA) is a key virulence factor expressed by *H. pylori* that increases the risk of developing PUD and gastric cancer. Following secretion by *H. pylori*, VacA binds to the membranes of gastric epithelial cells, oligomerizes, and forms anion-selective channels on various cell membranes. Several of VacA's subsequent intercellular effects including vacuolation and membrane permeabilization depend on the formation of VacA's active transmembrane channel. VacA is secreted by *H. pylori* as an 88kDa monomer (p88) consisting of an N-terminal p33 region and a C-terminal p55 region. The p33 region contains a hydrophobic stretch that is required for channel formation. Deletion of this region results in mutant VacA that is unable to vacuolate mammalian cells and exhibits ineffective channel activity in lipid bilayers. Despite the determination of VacA's soluble structure using single-particle cryo-electron microscopy (cryo-EM), the structure of VacA's channel region remains unknown due to a lack of defined density for the hydrophobic channel region in the soluble VacA structure. Given the importance of VacA's transmembrane channel activity for its cellular effects, determining the channel structure and mechanism of channel insertion is critical to further functional understanding of VacA's role in *H. pylori* infection. To address the structural basis of VacA's channel activity in pathogenesis, we analyzed VacA in the membrane context of liposomes using single-particle cryo-EM and cryo-electron tomography (cryo-ET). 2D classification of VacA bound to small unilamellar vesicles (SUVs) revealed that VacA oligomers interact with the SUV membrane but fail to insert fully into the lipid bilayer. This analysis suggests that VacA exhibits a hemipore state where it partially inserts into membrane but the full transmembrane channel is not formed. Using cryo-ET, we investigated the formation of VacA channels in liposomes. Through segmentation of tomograms, we observed both VacA that was partially inserted and VacA that fully formed its channel through the liposome membrane. These data suggest that, upon binding membrane, VacA undergoes an initial transition to a partially inserted channel state before full channel insertion. This study offers significant insights into the structural basis of VacA's transmembrane channel and the mechanism of channel insertion into membrane. Given the importance of VacA's channel in intercellular effects, this study provides a structural understanding of channel insertion that will be used to inform studies on VacA's function in the context of pathogenesis.

#09

Polymerase-associated factor complex (PAFc) protects Notch-induced leukemia cells from DNA damage and mitochondrial stress

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About 60% of T-cell acute lymphoblastic leukemia (T-ALL) patients have activating *NOTCH1* mutations, making *NOTCH1* the most prevalent oncogene in this cancer. In early clinical trials, pan-Notch inhibitors like gamma-secretase inhibitors (GSI) caused excessive toxicity because Notch has essential functions in tissue homeostasis. To circumvent this toxicity, work by my lab and others supports an alternative strategy of targeting context-dependent transcriptional cofactors that are required for Notch to activate transcription. In theory, inhibiting specific Notch cofactors might have less toxicity than inhibiting all Notch functions. Cell division cycle 73 (Cdc73) is a scaffold component of the polymerase-associated factor complex (PAF1c). Like flies with mutant Notch, flies with mutant PAF1c display notched wings and impaired expression of Notch target genes, suggesting that PAF1c is linked to the Notch pathway. The canonical function of PAF1c is to activate mRNA synthesis by recruiting histone modifier enzymes and transcriptional machinery to the most highly expressed genes. PAF1c also has non-canonical functions in regulating enhancers through eRNA synthesis. My project examines the role of Cdc73 in promoting proliferation of Notch-induced T-ALL. I hypothesized that Cdc73 facilitates the activation of response elements that drive oncogenic gene expression programs in T-ALL. In support of this hypothesis, we showed that Cdc73 knockdown impaired proliferation of murine and human Notch-activated T-ALL cells and prolonged survival of leukemic mice. Further, *Cdc73* deletion abrogated development of Notch-dependent pre-T cells, which are the normal cells of origin for T-ALL. Mechanistically, Bru-seq analysis showed that Cdc73 and Notch shared similar pathways, particularly in DNA repair and oxidative-phosphorylation. Within DNA damage repair, Atr, a gene essential for DNA damage repair, appeared as one of the strongest Cdc73 induced DNA repair genes. Consistently, *Cdc73* deletion induced gH2AX signals and AnnexinV-positive apoptosis, and decreased cell membrane potential. Inhibition of Atr decreased cell proliferation in several human and mouse T-ALL cell lines. BruUV-seq analysis showed that Cdc73 knockdown did not affect eRNA signals near Cdc73 target genes but did affect signals at the promoters of DNA repair and oxidative phosphorylation genes. Additionally, we found that Cdc73 binds the promoters of DNA repair and oxidative phosphorylation genes such as Lig4 and Ndufb4 respectively, suggesting that DNA repair and oxidative phosphorylation genes are regulated by Cdc73 via promoters rather than enhancers. Since Notch1 occupancy was associated with the promoters of Cdc73-regulated genes, we considered the possibility that Cdc73 physically interacts with Notch1. However, endogenous co-IP assays did not detect a strong interaction. These data suggest Cdc73 promotes gene expression programs essential for Notch-induced T-cell development though canonical functions at promoters, which are then hijacked to promote Notch-induced leukemogenesis. Cdc73 might possess essential functions in the DNA damage repair response and oxidative phosphorylation for which there is no literature, making Cdc73 a novel gene to pursue.

#10

Degradation of misfolded prohormones through two distinct routes of ER-coupled autophagy

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Secretory proteins produced in the endoplasmic reticulum (ER) facilitate countless biological processes within the body. Proper secretory protein folding precedes secretion, and misfolded secretory protein buildup within the ER can cause disease. In the Type I Diabetic condition known as MIDY (Mutant-INS-gene-Induced Diabetes of Youth), dominant-negative genetic mutations within the proinsulin coding sequence produce misfolded proinsulin products that are incompetent for secretion, leading to blood glucose dysregulation. MIDY proinsulin accumulates within the ER and forms detergent-insoluble complexes that entrap wildtype proinsulin (in heterozygous patients) that are degraded through a selective ER-coupled autophagy pathway (ER-phagy). How these toxic complexes are recruited into the ER-phagy pathway is poorly understood. Using an IP-mass spectrometry approach, we have identified multiple factors that are required for MIDY proinsulin degradation through ER-phagy, including the ER membrane proteins PGRMC1 and SigmaR1. These proteins interact with misfolded proinsulin and represent separate routes of entry into lysosomal degradation of misfolded ER secretory proteins. Modulation of these proteins may provide therapeutic relief for MIDY patients, as pharmacological inhibition of PGRMC1 promotes the secretion of wildtype proinsulin.

#11

Understanding the role of Keratin 16 in maintaining tissue homeostasis in response to stress

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Tissue homeostasis is the delicate balance of proliferation and cellular loss in complex epithelia such as the skin. Effective homeostasis is critically important in skin epithelia, a high turnover tissue that must cope with a broad array of environmental stressors. The most superficial compartment of the skin, the epidermis, is comprised of various cell types with keratinocytes making up more than 80% of the cells present. Differentiating keratinocytes arise from stem cells that actively proliferate in the basal layer of the epidermis. As these cells differentiate into keratinocytes, they increase their expression of various keratin intermediate filament (IF) proteins. A major role of the keratin filaments is to endow epithelial cells and tissues with the ability to withstand the various forms of stress the skin experiences daily. Keratin filaments are obligatory heteropolymers comprised of two types of intermediate filament (IF) proteins, known as type I and II. The type I keratin 16 (gene: *Krt16*; protein: K16) and its partner type II keratins 6a and 6b (K6a, K6b) are constitutively expressed in the palms and soles of the feet and in all ectoderm-derived epithelial appendages (hair, nail, glands, tooth, thymus). K16, its type II partners, keratin 6 (K6a, K6b), and another type I keratin 17, are not expressed in interfollicular epidermis unless it is subjected to stress (e.g., UV, injury, infection, disease). Genetic mutations that affect the coding sequence of K16 protein are causative for pachyonychia congenita (PC), a rare autosomal dominant disease characterized by aberrant differentiation and faulty homeostasis. PC features symptoms affecting ectoderm-derived appendages, giving rise to oral leukokeratosis, nail dystrophies, sebaceous cysts, natal teeth, and palmoplantar keratoderma (PPK). PPK—the symptom that PC patients seek treatment for owing to incredible pain and discomfort associated with the lesions—consists of extreme thickening of the skin of the palms and especially soles, which are exposed to high levels of mechanical/pressure stress. Treatments available for PC patients are palliative in nature.

The skin of mice homozygous for a *Krt16* null allele shows a markedly abnormal response to stress. Moreover, PPK lesions that morphologically resemble PC arise spontaneously at sites of mechanical pressure in *Krt16* null mouse footpad skin. The study of these mice has so far revealed multiple facets of K16's involvement in the skin's response to stress, the regulation of the innate immune response and of skin barrier function and, possibly, of keratinocyte differentiation. The *Krt16* null mouse does not provide, however, a genetically relevant model for PC, nor does it offer the possibility of testing new therapies for this disorder. In collaboration with the University of Michigan Transgenic Core, CRISPR-Cas9 gene editing was used to introduce an arginine to cysteine mutation at amino acid position 123 in the mouse *Krt16* gene. This allele accounts for 33% of all cases of PC due to mutations at the *KRT16* locus. This model is currently being vetted and may provide us the opportunity to understand how missense mutations alter the function of K16 in response to stress and maintenance of tissue homeostasis in palm and sole skin, and to test for therapies for PC patients. Of particular interest to us, at this time, is testing the immunosuppressive drug, rapamycin, to determine whether this is a suitable form of treatment for PC. This drug is currently in a clinical trial but it has yet to be tested in an *in vivo* preclinical setting. Rapamycin binds to mTOR, inhibiting the formation of the mRNA-binding translation ititiation complex, thus, suppressing translation of 5'TOP motif-containing mRNAs, which reportedly include *KRT6A* and *KRT16*. Both the *Krt16* null and *Krt16* R123C mutant mice provide an unparalleled opportunity to test the effectiveness of rapamycin in reducing levels of K6, and possibly K16 in an *in vivo* model of PC, while ultimately trying to understand the molecular and biochemical nature of K16's involvement in the stress response.

#12

FLAP mediates Signal Relay in Neutrophils during Chemotaxis

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As the most abundant cells of the innate immune system, neutrophils are first responders to sites of injury and infection and play many essential roles in inflammation. They are involved in the pathology of numerous diseases including pathogenic infections, autoimmune disorders, and cancer. Neutrophils reach injured and infected sites by recognizing gradients of chemical cues such as the peptide N-Formylmethionyl-leucyl-phenylalanine (fMLF) and migrating directionally towards them. fMLF activation also results in the secretion of a secondary chemoattractant Leukotriene B_4 (LTB_4), and its release amplifies fMLF-mediated responses by forming a secondary gradient that recruits distant cells in a process called signal relay.
LTB_4 is synthesized on the nuclear envelope by the enzymes 5-Lipoxygenase (5-LO), 5-LO Activating Protein (FLAP), and Leukotriene A_4 Hydrolase (LTA_4H). Stimulation of neutrophils with fMLF causes the development of nuclear envelope buds containing these enzymes. These buds eventually form multivesicular bodies (MVBs) with intraluminal vesicles. The MVBs go on to fuse with the plasma membrane and release their intraluminal vesicles as exosomes which release LTB_4 to the extracellular environment.

The mechanism that leads to the formation of nuclear envelope buds containing LTB_4 is poorly understood. While we know that the generation of ceramides by neutral sphyngomylenase1 (nSmase1) is essential for this process, any role of the LTB_4-synthesizing enzymes is currently unknown. The transmembrane protein FLAP forms a trimer with an anisotropic structure in response to chemoattractant stimulation. The FLAP trimer might help promote the membrane curvature that leads to bud formation at the nuclear envelope.

To test this, I will perform a CRISPR knockout of FLAP in a neutrophil-like cell line. Then I will express either wild-type FLAP or a mutant form that is incapable of trimerizing and assess the cells' ability to form buds and engage in signal relay. Additionally, to test whether FLAP is acting as a scaffold to recruit other proteins to the sites of nuclear envelope budding, I will use immunoprecipitation to pull down a pure sample of FLAP and FLAP interacting proteins. I can then use mass spectrometry to analyze the contents of this sample and determine potential proteins involved in the formation of nuclear buds and secretion of LTB_4 such as ESCRT complex members.

Aberrant production of LTB_4 can have deleterious consequences for various respiratory and cardiovascular diseases. Although previous drugs targeting LTB_4 synthesis initially proved very promising for the treatment of asthma, atherosclerosis, and chronic kidney disease in early clinical trials, these compounds were not pursued further often due to off target effects. Gaining a better understanding of the function of FLAP in LTB_4 synthesis and secretion will allow for the development of more specific and effective therapeutics.

#13

Deciphering the tubulin code: mechanisms of tubulin recognition by EML2

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Microtubules play varied and orchestrated roles in cells, such as mitosis, cargo transport, and cellular motility. Microtubule diversity is central to these functions and manifests in genetic isotypes and post-translational modifications, both of which can modulate microtubule dynamics and recruit specific microtubule-associated proteins (MAPs). This combinatorial diversity supports the “tubulin code” hypothesis in which tubulin modifications are maintained by “reader” and “writer” proteins in a similar way to the histone code. Writer proteins modify tubulin in ways only specific reader proteins can interpret. The reader proteins modulate microtubule dynamics and recruit other effector proteins, creating a regulatory network along microtubules.

Tubulin tyrosination is a tightly regulated tubulin post-translational modification with long-ranging effects from individual protein function to organism level survival. Proper maintenance of tubulin tyrosination is necessary for mitosis, retrograde axonal transport, cardiomyocyte contraction, and brain development. Tyrosinated (Y-) tubulin function is mediated by several Y-tubulin reader proteins. However, many Y-tubulin reader proteins remain unknown, creating a gap in our literacy of the tubulin code. Given the regulatory importance of known Y-tubulin reader proteins, the identification and characterization of new readers could elucidate unknown cellular functions of tubulin tyrosination homeostasis.

Our collaborators have identified the echinoderm-MAP-like protein 2 (EML2) as a putative Y-tubulin reader with additional microtubule tip-tracking behavior. I plan to characterize EML2’s association with Y-tubulin by assessing its *in vitro* co-sedimentation with recombinant human microtubules as well as its *in vitro* effects on microtubule dynamics. I will also solve its microtubule-bound structure to characterize the binding interface. Given two proximal surface patches separately implicated in binding, I hypothesize that EML2 engages both the tubulin globular domain and the modified C-terminal tubulin tail along its binding interface. In defining its mode of tubulin association, I hope to add EML2 to the growing lexicon of MAPs within the tubulin code.

#14

Specification mechanisms for Keratin 17 during the DNA Damage Response

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Keratin 17 (K17) is a stress-responsive intermediate filament protein that is upregulated in chronic skin diseases and many different types of carcinomas. For many cancers, high levels of K17 expression correlate with a poor prognosis for the patient. We recently reported that the nuclear pool of K17 impacts the cellular response to DNA damage in skin keratinocytes. K17 is induced following DNA damage and promotes keratinocyte survival. Through interactions with many key effectors of DNA damage response (DDR) including but not limited to gamma-H2AX, 53BP1 and DNA-PKcs, K17 is required for the timely recruitment of the DDR machinery. Genetic loss of K17 results in an attenuation of DDR signaling and increased apoptotic cell death. Re-expression of GFP-tagged K17 in *KRT17* null A431 tumor keratinocytes rescues many K17-dependent traits including abnormal DDR signaling. Going forward, we aim to further decipher the mechanisms involved in K17-dependent DDR processes in the nucleus. We are doing so using a multi-prong approach to identify: i) the relevant cis-acting determinants in K17 protein, which features three well-delineated domains (N-term head, central rod, C-term tail), via domain mapping studies; ii) the nature and role of site-specific post-translational modifications specific to K17 protein engaged in DDR; and ii) additional DDR effectors capable of binding K17 at sites of DNA damage. These efforts will help further define the processes and mechanisms involving K17 during DDR and their relevance to cancer.

#15

Structure-Aided Discovery of First-in-Class Small Molecule Adhesion GPCR Modulators

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GPR56 (ADGRG1) is a member of a subset of G protein coupled receptors called adhesion GPCRs (AGPCRs) which are included in the B class of GPCRs. These GPCRs possess a highly conserved GPCR autoproteolysis-inducing (GAIN) extracellular domain that cleaves the receptor into two fragments prior to activation, deemed the extracellular N-terminal fragment (NTF) and the membrane-embedded C-terminal fragment (CTF). Cleavage occurs at a location proximal to the start of the seven transmembrane spanning (7TM) domain such that a small stalk sequence of the 7TM remains embedded within the GAIN. Once the NTF and CTF dissociate from one another, this stalk region acts a tethered agonist (TA) by binding to an orthosteric site to maximally activate G protein signaling. Despite being clinically relevant to several biological processes, most AGPCRs are classified as orphans with no identified extracellular ligands. This has significantly impeded the study of AGPCR activation in endogenous tissue systems. Here, we conducted a large-scale high throughput screen to find novel small molecule agonists of GPR56. Over 200,000 small molecules across three libraries were screened at UM's Center for Chemical Genomics (CCG) using a cell-based serum response element (SRE) luciferase gene reporter assay in HEK293T cells. Primary screening yielded 74 candidate agonists with robust activity in the SRE-Luc assay comparable to that of the synthetic peptide agonist positive control. Follow-up biochemical GPCR reconstitution assays narrowed this pool of compounds to 14 unique structures. For two of highest-activity structures, we are pursuing structure-activity relationship (SAR) studies via commercially available analogs. Using the recently solved cryo-EM structure of activated GPR56, we designed alanine point mutants at critical residues in either the TA, or TA-binding residues in the 7TM. We were able to see agonist-induced activation of TA mutants, but not of TA-binding site mutants, supporting orthosteric binding. With several other AGPCR structures being solved recently, knowledge of these receptors is growing immensely. These compounds may serve as leads for future GPR56 therapeutic drugs with additional derivatization.

#16

Defining the role of ferroptosis in inflammatory bowel disease (IBD)

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Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gut that impacts over a million people in the U.S. yearly. There are two major forms, Crohn's disease (CD) and ulcerative colitis (UC). Chronic inflammation and unregulated mucosal immune activation lead to epithelial damage in a self-perpetuated cycle that is difficult to treat. The enhanced production of reactive oxygen species (ROS) is a hallmark for both UC and CD. The increase in ROS is thought to be a major mechanism of epithelial cell death in IBD. Mouse models of colitis are highly protected from tissue injury following antioxidant treatment. However, inhibiting ROS in IBD has only led to modest benefits in disease activity in patients. It is known that ROS can induce cell death via regulated and unregulated cell death pathways and better characterization of these are needed in IBD. We mined a large IBD RNA-seq database and identified an iron and lipid signature. Iron-induced lipid peroxidation is a hallmark of a non-apoptotic form of cell death termed ferroptosis. Consistent with the gene expression signature of ferroptosis, we show that lipid peroxidation is induced in chronic models of intestinal injury and in IBD patient samples, but not acute colitis models. A major antioxidant pathway for basal protection from ferroptosis is mediated by System Xc- and glutathione peroxidase (GPX)4. System Xc- transports cystine which is converted to cysteine for production of the antioxidant glutathione. GPX4 utilizes glutathione to reduce lipid peroxides. Intestinal epithelial deletion of System Xc- or GPX4 does not alter basal intestinal homeostasis or increase injury in acute models of colitis. However, disruption of System Xc- or GPX4 increases intestinal injury and cell death in chronic intestinal injury models. Mechanistically, we show novel connection between lipid dysregulation and cell death in chronic models of intestinal injury. We screened almost 100 lipids and identified several modulators of ferroptosis. Recent work demonstrates that ferroptosis is highly integrated to cellular lipid metabolism and the long-chain-fatty-acid-CoA ligase (ACSL)4 is a central executioner of ferroptosis. In depth analyses of transcriptomic data demonstrated that ACSL4 is highly induced in RNA-seq data from several IBD studies. Consistent with this data, ACSL4 was highly induced in chronic models of intestinal injury and in IBD patient samples. To understand if ACSL4 is a critical trigger for cell death in chronic models, a in vivo inhibitor of ACSL4 was assessed. Inhibition of ACSL4 significantly improved tissue injury in chronic modls of IBD in both wild-type and GPX4 knockout mice. Currently the first mouse models of ACSL4 are being generated to determine the role of lipid metabolism in IBD. Our work clearly provides a more targeted approach to oxidative cell death in IBD.

#17

Towards a Cell Biology Model of the Circadian Clock in *Drosophila*

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Most organisms synchronize their physiology to the diurnally changing environment for optimized resource utilization. Decades of research, mostly biochemical and genetic assays, unveiled the cell-autonomous molecular clock, a network of interlocked transcription-translation feedback loops. However, the molecular clock oscillator could not be satisfactorily modeled *in silico* assuming well-mixed cell compartments.

With wider adoption of high-resolution microscopy and scarless gene editing techniques, it is now feasible to characterize subcellular spatiotemporal distribution of core clock components *in vivo*. Here, we present our current work in which subcellular organization of core clock mRNAs and proteins is interrogated in *Drosophila* clock neurons. Mounting evidence shows that the cell is not a homogeneous mix, and our work demonstrates that the molecular clock assumes intricate subcellular organization at all levels of DNA, RNA, and protein, and throughout repression and activation phases of the circadian clock.

#18

Traversing the secretory pathway in search of degradation signals

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Protein quality control pathways are critical regulators for protein quantity and quality in cells. These pathways function in conjunction with protein degradation machinery to mitigate protein misfolding and prevent the toxic accumulation of misfolded or aberrant proteins. Accumulation of misfolded proteins has been implicated to cause a variety of human diseases such as familial protein folding disorders, diabetes, and neurodegenerative diseases. Degradative protein quality control at the ER and post-ER exists in multiple forms: ER-associated degradation (proteasomal), autophagy (lysosomal) and Golgi associated degradation (proteasomal and lysosomal). ER-associated degradation (ERAD) is a highly conserved pathway that has been well characterized in *Saccharomyces cerevisiae* (budding yeast). During ERAD, protein substrates are recognized, retrotranslocated across the membrane to the cytosol, where it becomes polyubiquitinated, extracted from the membrane and degraded via the proteasome. While the later steps have become increasingly clear, the principles of substrate recognition remain unclear. Furthermore, even less is known about substrate recognition during post-ER protein quality control pathways. This work describes a recently identified *degron*, a critical element within a protein needed for substrate selection and degradation. Through a combination of cell sorting and DNA sequencing techniques of a generated and screened N-terminal library, we have identified a degron that is dependent on the proteasome and Hrd1 for degradation. Continuing with this approach, we are in the process of screening additional libraries to identify features that are identified by ERAD and post-ER protein quality control systems. *We hypothesize that protein quality control in the secretory pathway recognize non-overlapping set of features that function as degrons.* Identifying degrons in the secretory pathway will be a major advancement in the fields of protein quality control and target protein degradation. This work will further our mechanistic understanding of substrate recognition in degradative protein quality control pathways.

#19

Neuronal activity regulates MATR3 in a calcium and calmodulin dependent manner

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RNA binding protein (RBP) dysfunction and abnormal neuronal excitability are hallmarks of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD). Even so, it remains unclear if and how activity dependent processes regulates RBPS. In a subset of familial ALS cases, the DNA-RNA binding protein matrin-3 (MATR3) causes both ALS and FTD. MATR3 pathology, including cytoplasmic mislocalization, and increased nuclear staining also occurs in sporadic cases of ALS, suggesting an important role of MATR3 in disease pathogenesis. In a rat primary neuron model, both knockdown and overexpression of MATR3 results in neurotoxicity, indicating proper MATR3 homeostasis is essential for neuronal survival. Moreover, we have observed that MATR3 RNA binding is crucial to maintaining MATR3 solubility and nuclear distribution within neurons. Thus, physiological regulators of MATR3 abundance and RNA binding are important modifiers of toxicity in ALS and FTD. We and others found that stimulation of neuronal activity with the glutamate receptor agonist NMDA leads to the rapid degradation of the disease-associated RNA binding protein matrin-3 (MATR3). Both calcium and calpains—a family of calcium-sensitive cysteine proteases—are required for MATR3 degradation in response to NMDA. Furthermore, treatment with W-7, an inhibitor of the calcium signal transduction protein calmodulin (CaM), blocks MATR3 turnover. Previous studies hinted at a direct interaction between CaM and the RNA binding domain of MATR3, and we found that NMDA treatment rapidly inhibits MATR3's ability to bind its RNA targets. These observations suggest a model in which NMDA receptor activation results in calcium influx, CaM binding to MATR3 and release of its RNA substrates, and subsequent MATR3 degradation via calpains. This model may also help explain the toxicity of a newly-identified *MATR3* mutation affecting a conserved residue (F488L) adjacent to MATR3's RNA binding domains. In addition, bioinformatic algorithms predict the presence of CaM binding regions buried with RNA recognition motifs of other ALS/FTD-associated RNA binding proteins including TDP43, FUS, hnRNPA1, and TIA1. Thus, our data link two conserved phenomena in ALS and FTD—neuronal hyperactivity and RNA binding protein pathology—and may have direct implications for the function of these proteins as well as disease pathogenesis.

#20

The Structural Basis of Meiotic Prophase Chromosome Movements by the Linker of Nucleoskeleton and Cytoskeleton (LINC) Complex

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Mammalian meiosis requires the linker of nucleoskeleton and cytoskeleton (LINC) protein complex at the nuclear envelope (NE). In meiotic prophase I (prior to NE breakdown), telomeres of chromosomes travel along the NE, temporarily assembling into a bouquet confirmation in a localized region. Prophase telomere movements enable homolog synapsis and recombination. Recombination results in crossing over that ensures homolog segregation and contributes to genetic variation. The LINC complex, consisting of SUN1 and KASH5, enables this movement. SUN1, an inner-nuclear-membrane (INM) protein, attaches telomeres to the NE. KASH5, an outer-nuclear-membrane (ONM) protein, links SUN1 to dynein, which travels along cytoplasmic microtubules. Yet it is unknown how the LINC complex transmits the forces required for these complex chromosome movements.

Previously, our lab showed that KASH5 protein homodimerizes and collaborated with Dr. Morgan DeSantis to identify KASH5 as a novel dynein activating adaptor. A crystal structure of the C-terminal SUN1 SUN domain bound to C-terminal KASH5 peptides gave rise to a model in which SUN1 and KASH5 assemble in a 6:6 ratio. Importantly, however, this structure excluded the predicted coiled-coil (CC) regions of SUN1 and KASH5, which mediate oligomerization, and the SUN1 autoinhibitory domain (AID) proposed to regulate SUN domain trimerization. Thus, the relevance of this model for meiotic chromosome movement remains unknown.

We hypothesize that the LINC complex assembles into an oligomeric structure of at least 2 SUN1 trimers and 3 KASH5 dimers that transmits forces from multiple dynein motors to move chromosomes. Furthermore, we predict that the CC and AID regions of SUN1 interact functionally to determine this structure.

To determine the oligomeric state of the meiotic LINC complex as it connects dynein to chromosomes, we will employ size-exclusion chromatography with multi-angle light scattering (SEC-MALS) and electron microscopy (EM) of SUN1-KASH5 complexes assembled in a membrane-free system. In addition, we will collaborate with Dr. Sue Hammoud to perform quantitative immunoblotting of endogenous SUN1 and KASH5 proteins to determine LINC complex stoichiometry in mouse spermatocytes.

To determine how the SUN1-KASH5 interaction modulates prophase chromosome movement, we will perform maltose-binding protein (MBP) pull-down assays of dynein-binding to the SUN1-KASH5 complex. Furthermore, we will collaborate with the DeSantis lab to perform single-molecule total internal reflection fluorescent microscopy (smTIRF) and peroxisome trafficking assays of dynein motility while it is bound to SUN1-KASH5. Finally, we will collaborate with Dr. Hiroki Shibuya to assay chromosome movement in SUN1- or KASH5-deficient mouse spermatocytes expressing wildtype or mutant SUN1 or KASH5 proteins.

These discoveries would elucidate how the LINC complex functions with dynein to move meiotic chromosomes.

DEVELOPMENTAL BIOLOGY, ORGANOGENESIS, NEUROBIOLOGY, AGING

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#21

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Stable iPSC-derived NKX2-1$^+$ Lung Bud Tip Progenitor Organoids Give Rise to Airway and Alveolar Cell Types

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Bud tip progenitors (BTPs) in the developing lung give rise to all epithelial cell types found in the airways and alveoli. The current work aimed to develop an iPSC organoid model enriched with stable NKX2-1$^+$ BTP-like cells. Building on prior work, we optimized a directed differentiation paradigm to generate spheroids with robust NKX2-1 expression. Spheroids were expanded into organoids that possessed NKX2-1$^+$/CPM$^+$ BTP-like cells, which increased in number over time. Single cell RNA-sequencing analysis revealed a high degree of transcriptional similarity between induced BTPs (iBTPs) and *in vivo* BTPs. Using FACS, iBTPs can be purified and expanded as induced bud tip organoids (iBTO), which maintain an enriched population of bud tip progenitors. When iBTOs are directed to differentiate into airway or alveolar cell types using well-established methods, they give rise to organoids composed of organized airway or alveolar epithelium, respectively. Collectively, iBTOs are transcriptionally and functionally similar to *in vivo* BTPs, providing an important model to study human lung development and differentiation.

#22

14-3-3 binding motif phosphorylation disrupts Hdac4 organized inhibitory condensates to stimulate cardiac reprogramming

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Limited understanding of the molecular mechanisms of induced cardiomyocyte (iCM) reprogramming is a key obstacle preventing its effective clinical applications. We report here the identification of a phosphorylation code in 14-3-3 binding motifs (PC14-3-3) that greatly stimulates iCM formation. PC14-3-3 is identified in pivotal functional proteins for iCM reprogramming, including transcription factors and epigenetic factors. Akt1 kinase and PP2A phosphatase are a key writer and eraser of the PC14-3-3 code, respectively and PC14-3-3 activation induces iCM formation with only Tbx5 but without Mef2c and Gata4. In contrast, PC14-3-3 inhibition by mutagenesis or degradation-mediated code removal abolishes reprogramming. We further discover that key PC14-3-3 embedded factors, such as Mef2c, Nrip1, and Foxo1, form inhibitory nuclear condensates with Hdac4 under hypo-phosphorylation state and PC14-3-3 activation disrupts these condensates to promote cardiac gene expression. This study provides a framework in decoding post-translational modifications for cell reprogramming and organ regeneration.

Highlights

1. A PC14-3-3 (phosphorylation code in 14-3-3 binding motifs) is identified in pivotal functional proteins, such as Mef2c and HDAC4, that stimulates iCM formation.
2. Akt1 kinase and PP2A phosphatase are a key writer and a key eraser of the PC14-3-3 code, respectively.
3. PC14-3-3 code activation can replace Mef2c and Gata4 and induce iCM formation with the presence of only Tbx5.
4. PC14-3-3 activation disrupts nuclear inhibitory condensates comprised of Hdac4 and other PC14-3-3 embedded factors to promote cardiac gene expression.

#23

Determining the role of Ankyrin-G in GABAergic deficits related to bipolar disorder using patient-derived neurons

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Bipolar disorder (BD) is a chronic, debilitating, psychiatric disorder which affects over 1% of the global population. Despite its prevalence, the underlying causes of BD remain unknown. BD is a highly heritable disorder, suggesting that genetics play a critical role in BD etiology. Through genome wide association studies, *ANK3* has been identified as one of the most significant risk loci for BD. Our long-term goal is to determine how *ANK3* variants contribute to BD etiology. *ANK3* encodes ankyrin-G, which is essential to the formation of plasma membrane subdomains critical for neuronal function. Recently, we discovered a novel role for ankyrin-G in the development of inhibitory (GABAergic) neuronal synapses. GABAergic synapses mediate the synchronization and function of neuronal networks in the brain, and deficits in GABAergic signaling have been linked to BD. We hypothesize that a subset of BD-associated *ANK3* variants disrupt ankyrin-G function at inhibitory synapses and generate functional deficits in GABAergic signaling. We propose human induced pluripotent stem cell (hiPSC)-derived neurons as a model to test this hypothesis. This approach allows for the generation of relatively pure cortical-like neuron cultures that retain the genetic background of the patient and form mature GABAergic synapses. Additionally, the structure and functionality of these GABAergic synapses can be measured using immunocytochemistry and calcium imaging, respectively. For the first time, this will allow us to study the mechanisms linking BD-associated *ANK3* variants to GABAergic deficits in an exact BD patient genetic background. Understanding these mechanisms will ultimately allow for the development of novel BD therapeutics targeting this pathway.

#24

Identifying the role of *Gpr10* and *Calcr*-expressing Neurons in the Area Postrema in the Aversive and Non-Aversive Control of Food Intake

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Gut signals promote meal termination via the area postrema (AP) in the brain. In addition to containing neurons that suppress food intake and mediate non-aversive responses to feeding-related signals from the gut, some circuits mediate aversive responses to gut malaise (e.g., nausea and vomiting). The mechanisms that mediate these distinct valence responses remain incompletely understood. In collaboration with the Tune Pers' lab, we generated snRNA-seq atlases of AP neurons, identifying one group of GABAergic (GABA) and two populations of glutamatergic neurons (GLU10 and GLU4). GLU10 neurons express the calcitonin receptor (*Calcr*) and the receptor activity modifying protein-3 (*Ramp3*; complexes of *Calcr* and *Ramp3* represent the amylin receptor, AmyR). AmyR agonism in the AP does not promote aversive responses, suggesting the non-aversive nature of GLU10 cells. The GLU4 group expresses GDNF family receptor alpha-like (*Gfral)*, *Glp1r*, and prolactin-releasing peptide receptor (*Gpr10). Gfral* and *Glp1r*-expressing cells project to the lateral parabrachial nucleus (LPBN) and overlap with calcitonin gene-related peptide (CGRP)-expressing cells. CGRP-expressing cells induce aversion. Single-cell sequencing by the Liberles group suggested that *Gfral* and *Gpr10*-expressing cells may represent distinct subsets of AP neurons. This notion was by the Pers group. We generated a $\textit{Calcr}^{\textit{Cre}}$ and $\textit{Gpr10}^{\textit{Cre}}$ rat to study these AP cell populations. I will examine whether physiological or pathophysiological signals activate $\text{Calcr}^{\text{Cre}}$ and $\text{Gpr10}^{\text{Cre}}$- expressing cells in the AP, identify their downstream projection sites and distinguish whether they overlap with CGRP cells. Finally, I will determine whether $\text{Calcr}^{\text{Cre}}$ and $\text{Gpr10}^{\text{Cre}}$ -expressing cells induce aversion or non-aversive food suppression.

#25

FruM interacts with neuronal lineage to sexually differentiate neurons

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Most animal species reproduce sexually by fusing disparate gametes. Developmental sex differentiation of the nervous systems programs mating and social behaviors. In mammalian brains, nuclear hormone receptors fill this role; in insects, brain sex is defined by the male-specific BTB-POZ transcription factor Fruitless (FruM). While transcription factors thought to specify circuit masculinization have been known for decades, the developmental logic by which the genome builds different circuits remains elusive. Using Drosophila melanogaster as a model, we are investigating how FruM alters gene expression during development to program social behaviors.

fruitless neurons arise from dozens of different neuroblasts (neural stem cells) and are anatomically diverse. Many types of *fruitless* neurons are sexually dimorphic in arborization patterns or cell number. For example, mAL neurons are more numerous in the male due to developmental apoptosis in the female. mAL neurons arise from a single neuroblast, and preliminary data from our lab suggests that sister neurons from the same stem cell do not express *fruitless*. While the importance of FruM for behavior is established, less is known about how the genetic identity of *fruitless* neurons is specified and how only a subset of neurons per stem cell express *fruitless.* We have successfully applied a genetic technique to label entire neuroblast lineages including several that produce *fruitless* neurons in both sexes. We isolated those lineages and subjected them to scRNA-seq. Using this data, we are asking, (1) How are *fruitless* neurons different from non-*fruitless* expressing neurons from the same hemi-lineage? (2) Does FruM act on the same or different targets in neurons that descend from different hemi-lineages?

Ultimately, we would like to ask how some neurons activate transcriptional programs that are sensitive to cellular sex, and how those factors cause the same neurons in males and females to wire differently and produce distinct behaviors. By studying these neurons in the context of their lineage we will define their developmental identity and how the sex-specific production of FruM interacts with cell identity during development in males.

#26

Dscam gene triplication causes neocortical overinhibition in Down syndrome

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A growing number of molecules have been identified as regulators of inhibitory synapse development, but whether dysregulated expression of these molecules contribute to brain disorders is poorly understood. Here we show that Down syndrome cell adhesion molecule (Dscam) regulates the inhibition of neocortical pyramidal neurons (PyNs) in a level-dependent fashion. Loss of Dscam impairs inhibitory neuron development and function. In the Ts65Dn mouse model for Down syndrome, where Dscam is overexpressed, GABAergic innervation of cortical PyNs by chandelier and basket cells is increased. Genetic normalization of Dscam expression rescues the excessive GABAergic innervation and the increased inhibition of PyNs. These findings demonstrate excessive GABAergic innervation and inhibition in the neocortex of Down syndrome mouse model and identify Dscam overexpression as the cause. They also implicate dysregulated Dscam levels as a potential pathogenic driver in related neurological disorders.

#27

The role of *fmo-4* in longevity and stress resistance

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Aging is the leading risk factor for the most common chronic diseases, including cardiovascular disease, dementia, and cancer. Hence, a deeper understanding of the pathways that affect aging and how they may lead to disease could result in treatments that slow or prevent the development of multiple diseases simultaneously. In this way, scientists have employed the use of a wide range of model organisms, from yeast to mammals, to define various genetic and environmental pathways that influence aging. Our lab has identified *flavin containing monooxygenase- 2* (*fmo-2*) as a key regulator of longevity in *C. elegans.* Tissue-specific upregulation of *fmo-2* in the intestine increases stress resistance, healthspan, and longevity in worms, while loss of *fmo-2* leads to abrogation of these benefits. Further, *fmo-2* overexpressing (OE) worms exhibit an extended lifespan compared to wild-type (WT) worms, while *fmo-2* knockout (KO) worms have similar lifespan to WT. We also find that *fmo-2* is necessary and sufficient to promote longevity downstream of multiple processes, such as the hypoxic response and dietary restriction (DR).

My project focuses on the role of other members of the FMO family of enzymes, particularly the *C. elegans* FMO-4. FMO-4 is structurally similar to FMO-2 and is also required for health benefits in multiple longevity pathways, including DR. Interestingly, we find that *fmo-4* is required for *fmo-2*-mediated longevity and is both required and sufficient to improve stress resistance downstream of *fmo-2.* However, while *fmo-2* is required for the hypoxic response and DR-mediated longevity, *fmo-4* is only required for some forms of DR-mediated longevity. Because *fmo-2* is expressed primarily in the intestine while *fmo-4* is primarily expressed in the hypodermis, it is possible each gene plays a similar role in different tissues. Taken together, these data lead me to hypothesize that FMO-2 enzymatic activity in the intestine leads to induction of *fmo-4* in the hypodermis. I predict that FMO-4 will have tissue-specific metabolic functions both overlapping and distinct from *fmo-2* that regulate longevity and stress resistance in worms. Going forward, my project will focus on *fmo-4* and its interactions with *fmo-2,* the distinct functions of *fmo-4,* and the downstream processes being altered by *fmo-4* to regulate longevity.

#28

Auditory lipidomics, an approach to identify unique molecular effects of noise trauma

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Noise induced hearing loss is a problem of epidemic proportions, but we do not yet fully understand how noise damages the ear. It is well-established that synapses between inner hair cells (IHCs) and auditory neurons are the most labile part of the cochlea, and that their loss in response to mild noise exposure leads to a pathology called Hidden Hearing Loss. This synaptopathy is apparent immediately after a 2-hour exposure, suggesting it is caused by changes in metabolism rather than gene expression. To understand how noise damages the cochlea, our group previously compared the inner ear metabolome of exposed and control mice by measuring aqueous metabolites. To expand upon the metabolic changes induced by noise, we are now exploring the lipidome and the implications of its dysregulation.

Hearing and deaf mice were noise-exposed (2 hr, 98-100 dB @8-16 kHz) to distinguish between metabolic changes induced by IHC mechano-transduction or just mechanical stimulation. Immediately following exposure, the otic capsules were removed and untargeted lipidomics was performed on the tissues. Four hundred ninety-one unique species were detected, which were organized into 51 lipid classes. Unsaturated (monounsaturated and polyunsaturated) free fatty acids (FFA) were the only lipid type significantly altered ($p<0.05$) by noise, and this occurred only in hearing animals. Furthermore, primarily long-chain FFAs were altered with noise.

Long-chain FFA are metabolized for energy primarily through beta-oxidation in the mitochondria, where they are brought in by the rate limiting enzyme CPT1A. To investigate if the inner ear depends on long-chain FFA metabolism for energy, we inhibited CPT1A globally by treating 8-week-old mice with 35 mg/kg Etomoxir daily. Etomoxir treatment did not alter hearing as measured by Acoustic brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) on days 1, 3, 7 and 14 of treatment. On day 16 of treatment, mice were noise exposed (2 hr, 100 dB @8-16 kHz) and Etomoxir treatment was ceased. There was no difference in the recovery from noise between Etomoxir and saline treated mice as measured by ABR and DPOAE several weeks post-noise.

Though preliminary, to our knowledge this is the first lipidomic study following noise exposure. Furthermore, our results indicate that function of the inner ear does not seem to be dependent on long chain FFA beta-oxidation in the mitochondria. In future experiments we will validate the decrease in the lipids reported with a larger cohort, as well as examine the dependence of metabolite levels on noise intensity. Furthermore, as starvation causes increases in FFAs in the blood, we will investigate the role of nutrient deprivation on normal hearing and the noise response.

#29

Centromere Identity during Germ Cell and Embryonic Development Defies the Somatic Cell One-to-One Maintenance Mechanism

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The centromere-specific histone variant CENP-A is an excellent model to study epigenetic memory in mitotic cells. CENP-A is a monogenic histone H3 variant that epigenetically defines the location of centromeres. The current model for centromeric inheritance relies on DNA-bound CENP-A nucleosome serving as a scaffold to direct the deposition of a newly generated CENP-A nucleosome at the end of telophase and the beginning of G1, tightly regulating the quantitative propagation and maintenance of the genomic location of centromeric chromatin. However, germ cells pose a unique challenge to the regulation of centromeres. In the female germline, oocytes initiate meiosis in utero and can remain arrested at prophase I of meiosis throughout the reproductive lifespan of the organism. In the male germline, post-meiotic haploid spermatids undergo a genome-wide reorganization of their chromatin: replacing up to 99% of their histones with small basic proteins called protamines. These events raise a fundamental question: how is centromere identity maintained and propagated across generations? To address this question, we developed a mouse model to genetically tag the centromeric histone variant CENP-A and followed centromeric chromatin dynamics throughout gametogenesis and into the totipotent embryo. Our data shows that the undifferentiated spermatogonia and oogonia have expanded CENP-A levels at their centromeres which are significantly parred down during differentiation and only slightly reduced thereafter. This centromeric expansion and reduction is conserved in *Drosophila* gametogenesis, and notably proceeds the predicted reduction occurring during the histone-to-protamine exchange. The extent of CENP-A reduction is a sex specific process consequently leading to the generation of terminally differentiated gametes with unequal centromeres. The CENP-A nucleosomes retained in oocytes and sperm are inherited and maintained in early embryos. Importantly, centromere equalization in zygotes occurs at the 1-cell stage and relies on a redistribution (recycling) of chromatin-bound maternal CENP-A onto paternal centromeres, revealing a novel mechanism for centromere equalization/expansion. Finally, the absolute levels of CENP-A continue to increase in early zygotic cell cycles and can be modulated by the available pool of CENP-A and HJURP protein levels. Therefore, unlike adult mitotic cells, the germline and early embryo centromeres are remarkably flexible – undergoing programmed expansions and contractions within a single generation within a species.

#30

Identifying and characterizing a longevity control center in the *Drosophila* brain

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Aging is the leading risk factor for diseases like cancer, neurodegeneration, and heart disease, and even in the absence of these diseases, aging profoundly impairs quality of life. The aging process is not as inexorable as it once seemed, however—decades of research have demonstrated that lifespan and healthspan can be positively influenced by environmental and pharmaceutical interventions. Given that all human beings are subject to the complications of aging, it represents a high-impact target for the investment of time and resources towards mitigating human suffering. Biogerontology researchers are tackling many facets of this complex phenomenon in order to understand its causes and identify targets for intervention. One area of emerging importance is the contribution of the nervous system to physiological aging. While it has been demonstrated that some aspects of lifespan modulation are neurally mediated, we know little about the scope of the brain's involvement in organismal aging or the mechanisms underlying these contributions. Understanding how the brain influences aging has important implications for human health, as neural regulation of aging represents a theoretical upstream point of intervention in multiple aging processes. A neural structure shown to control aging via multiple pathways would offer a promising proof of this concept. Our lab has identified one such structure in the ellipsoid body (EB), a neuropil in the *Drosophila* brain that regulates aging in different environmental contexts. The EB, whose diverse roles include behavioral and physiological regulation, sensory integration, and memory formation, is a component of the central complex (CX), a region functionally and structurally analogous to the mammalian basal ganglia (BG). We expect that a deeper understanding of the role of the EB and the broader CX in modulating lifespan will provide a framework for understanding how aging is controlled by mammalian brains. The mapping of the *Drosophila* hemibrain connectome and recent advances in functional imaging offer powerful new tools for dissecting the circuits through which the brain coordinates aging processes. Our lab has extensive experience in investigating how the brain regulates aging, and we are well poised to use these new technologies to study how environmental cues are transduced by the brain into aging outcomes. To address gaps in our understanding of how the brain controls aging, we will (i) probe the scope of the EB's involvement in known lifespan phenotypes; (ii) determine how these neurons are recruited by environmental stimuli; and (iii) use functional imaging to trace their downstream effects. Completion of this work will provide a picture of what different brain-mediated lifespan phenotypes have in common, presenting precise upstream targets for intervention in diverse aging processes. Given the functional homology between the CX and mammalian BG, we expect these findings to lay the groundwork for identifying similar points of intervention in mammalian—and ultimately, human—brains.

#31

TorsinA regulates nuclear pore complex assembly and localization during neuronal maturation

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Nuclear pore complexes (NPCs) are large protein complexes composed of hundreds of nucleoporins that mediate nucleocytoplasmic transport of protein and RNA. NPC abnormalities are implicated in DYT1 dystonia, a neurodevelopmental movement disorder caused by a loss-of-function mutation in the gene encoding torsinA, a AAA+ protein localized to the endoplasmic reticular (ER)/nuclear envelope (NE) endomembrane space. Despite increasing evidence implicating NPC dysfunction in DYT1 and other neurological diseases, NPC biogenesis in neurons remains poorly understood. Furthermore, the biological function of torsinA and the molecular defects underlying DYT1 dystonia remain largely unknown. In mouse primary neurons, we find a steady upregulation in NPC biogenesis during neuronal maturation. While NPCs are uniformly distributed in wild-type neurons, torsinA-null neurons develop mislocalized clusters of NPCs. To elucidate the dynamics of NPC formation and localization, we developed a novel HaloTag-Nup107 mouse line and conducted pulse-chase studies of existing and newly-formed NPCs. We observe that in torsinA-null neurons, new pores form adjacent to existing pores, thereby resulting in increasingly severe abnormal clusters of NPCs as neurons mature. In contrast to the drastic difference in localization between wild-type and torsinA-null neurons, NPC density is normal in torsinA-null neurons, suggesting that torsinA regulates NPC localization, but not number.

Similar to prior findings *in vivo*, primary cultures of torsinA-null neurons develop evaginations of the inner nuclear membrane (NE buds). The emergence of NE buds in primary neurons coincides with the formation of mislocalized NPC clusters, thereby implicating a temporal association between these events. To understand the spatial relationship between abnormal NPC clusters and NE buds, we performed multiple-tilt electron tomography. We observe that NE buds form in clusters and that the base of NE buds form NPC-like pore structures at the inner nuclear membrane. These data are consistent with a model in which loss of torsinA causes stalled NPC assembly, resulting in formation of aberrant NE buds at sites of new NPC formation. Yet, contrary to current models, torsinA does not seem to be required for completion of NPC assembly: as torsinA-null neurons further mature, abnormal NE buds are resolved and NPC assembly resumes, incorporating a late-recruited cytoplasmic component to the stalled pores. Taken together, our findings suggest a critical function of torsinA in spatially and temporally regulating NPC assembly during a key period of neuronal development and implicate aberrant NPC biogenesis in the pathogenesis of DYT1 dystonia.

#32

The serotonin receptor *5-HT2A* mediates nutrient-specific longevity in *Drosophila*

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Perception of environmental cues has long been known to influence longevity, and this is often mediated by conserved neuromodulators, such as serotonin (5-hydroxytryptamine, or 5-HT). However, the direct role of serotonin in modulating lifespan remains elusive. The model system *Drosophila* provides the ideal system by which to dissect this, with their relatively short lifespan and conservation of basic neural circuitry with higher organisms. Because serotonin signaling is essential for mediating a wide range of behaviors and physiological processes, broad manipulations of serotonin levels are difficult to interpret, and mechanistic insight is limited. By focusing the investigation at the level of the receptor, we can more easily untangle the specific mechanisms by which serotonin signaling modulates lifespan. *Drosophila* possess five receptors for serotonin homologous to those found in humans (*5-HT1A*, *5-HT1B*, *5-HT2A*, *5-HT2B*, and *5-HT7*) and receptor mutants display a remarkable variability in their effects on longevity. Most interestingly, *5-HT2A* mutants display a sexually dimorphic lifespan extension. Further characterization of these long-lived mutants revealed that they do not display changes in many behaviors indicative of general health, including sleep and activity; however, *5-HT2A* mutants, specifically, display altered feeding behaviors. *5-HT2A* mutants alter total food consumption based on the availability of dietary protein, and the lifespan extension is dependent upon specific nutrient ratios. Additionally, optogenetic activation of *5-HT2A*$^{+}$ neurons promotes interaction with a protein-containing solution. Together, these data indicate *5-HT2A* mediates a protein valuation neuron state and this nutrient perception state modulates lifespan. Given the high conservation of serotonin signaling across taxa, this work will provide important insight into the biological mechanisms governing longevity.

GENETICS AND GENOMICS, GENE REGULATION

ABSTRACTS 33- 36

#33

Investigating the Transcriptional Regulation of the LDL Receptor

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High levels of cholesterol in the bloodstream increase an individual's risk of cardiovascular disease, the leading cause of death in the United States. Cholesterol homeostasis is highly regulated through receptor-mediated endocytosis of low-density lipoproteins (LDL) by the LDL receptor (LDLR). Our lab previously applied a genome-wide CRISPR-Cas9 screen to identify genes that disrupt LDL uptake in HuH7 and HepG2 cells. Two novel regulators were identified, CHD7 and PROX1, decreasing LDL uptake via reduction in LDL receptor transcription in HuH7 cells. This work led us to expand our efforts in understanding LDLR transcriptional regulation, by focusing on the functional role of candidate cis-regulatory elements (cCREs) near the *LDLR* gene. We designed a novel CRISPR/Cas9 screen with 12,375 gRNA sequences spanning ~200kb of noncoding genomic space associated with predicted regulatory activity near the *LDLR* locus, including control gRNAs targeting coding regions of *LDLR* and *MYLIP*. The functional dissection of these noncoding regions will not only functionally map regulatory sequences relevant to physiological functions, advance the interpretation of human genetic variation and potentially represent a new therapeutic venue to further lower the hypercholesterolemia risk of certain patients.

#34

Exploring the relationship between transcription regulation and chromatin organization

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The genome is organized into a series of compartments, domains, and loops that work together to position chromatin and allow proper execution of various essential cell processes, including transcription of RNA. From the model of phase separated transcription hubs to the role of chromatin loops in facilitating enhancer-promoter interactions, there is a well demonstrated link between 3D chromatin organization and transcription, although all the intricacies of how chromatin structure does or does not impact transcription have not been fully elucidated. We are interested in exploring the link between genome structure and RNA production using nascent RNA-sequencing to connect different mechanisms and features of active transcription—including enhancer-promoter looping interactions, enhancer RNA (eRNA) transcription, read-through transcription, and promoter divergent transcription (PROMPTs)—with the 3D structure of the genome. Specifically, we are using Bru-seq and BruUV-seq data from 16 common human cell lines, generated for the ENCODE project, to explore active transcription throughout the genome to identify these features of transcription genome-wide. We then plan to use matched ENCODE data from various chromatin assays, such as Hi-C, ChIA-PET, ATAC-seq, etc., in addition to other curated datasets to explore these transcription features in the context of the chromatin landscape. In parallel with this analysis exploring transcription across different cell lines, we are also using modified HCT116 cell lines containing auxin inducible degrons targeting various proteins known to be integral to transcription and chromatin organization to explore how these perturbations impact gene expression. From these analyses, we hope to provide catalogs of transcription features and insight on how they may be impacted by the chromatin architecture of the cell.

#35

A *RING1* contribution to neurogenesis and schizophrenia

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During corticogenesis, neural stem cells (NSCs) undergo rapid proliferation to produce carefully titrated populations of neurons. Expediated NSC divisions rely on efficient maintenance of genome integrity. Polycomb repressive complex 1 (PRC1) plays an increasingly recognized role in DNA damage repair. *RING1* is an E3-ubiquitin ligase within PRC1 that catalyzes monoubiquitination of histone 2A (H2AUb1). A de novo pathogenic *RING1* missense variant was detected in an individual with early-onset schizophrenia and developmental delay. NSCs differentiated from *RING1*$^{G284A/G284A}$ human embryonic stem cells exhibit reduced genome wide H2AUb1, increased baseline DNA damage, and delayed DNA repair following chemotoxic stress. Additionally, an accumulation of NSCs in the G2/M phase were observed in forebrain organoids characterized by single-cell RNA sequencing. Cell cycle defects translated into altered timing of neural differentiation and increased apoptosis. These results indicate that PRC1-mediated H2AUb1 is necessary for efficient DNA damage repair and cell cycle progression in developing human NSCs.

#36

Fruitless decommissions regulatory elements to implement cell-type-specific neuronal masculinization

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Sex differentiation is a profound developmental program, often inducing differences in growth, morphology, physiology, and even behavior to support sexual reproduction. Innate and sex-specific social behaviors, such as courtship, aggression, and care of offspring, are found across vertebrates and invertebrates alike, even though the precise social roles of female and male animals are diverse. Studies of model organisms, such as worms, fruit flies, and mice, have shown that sex-varying social behaviors are hard-wired in the brain by differentiated neurons and neural circuits. Untangling how sex differentiation interacts with the specification, maturation, and circuit connectivity of neurons to pattern subtly but critically different behaviors remains a major experimental and conceptual hurdle. In the insect nervous system, the male-specific transcription factor Fruitless regulates the development and activation of innate, female-directed courtship behavior. While much is known about how Fruitless-dependent neuronal differences generate sex-specific behaviors, little is known about the transcriptional and chromatin events executed by Fruitless to pattern courtship behavior during development of the circuit. Bridging this gap, we isolated Fruitless neurons and profiled chromatin accessibility states using ATAC-seq. We find that Fruitless directs numerous changes in chromatin accessibility across neurons of the courtship circuit in a highly cell-type specific manner, and further, validate regulatory elements as Fruitless-specific enhancers *in-vivo*. Fruitless regulatory targets are preferentially near genes involved in axon guidance and synaptic connectivity, and, further, regions closed in courtship circuit neurons are likely to be direct targets of Fruitless. We are currently investigating 1) where Fruitless binds chromatin and how it influences gene expression and 2) how these events unfold over developmental time. We hypothesize that Fruitless alters neurons of the courtship circuit early in development by decommissioning enhancers whose regulated genes will come into play later on. This work will provide a model case for how sex differentiation pathways intersect with other aspects of neuronal identity and development to generate subtly but critically different circuits.

MOLECULAR MECHANISMS OF DISEASE, CANCER BIOLOGY, GENETICS OF DISEASE, NON-HUMAN MODELS OF DISEASE

ABSTRACTS 37-57

#37

Gene silencing in SCA3 disease mice rescues spatiotemporal oligodendrocyte impairments

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Spinocerebellar Ataxia type 3 (SCA3) is the most common dominantly inherited ataxia. Currently, no effective preventative or disease-modifying treatments exist for this progressive neurodegenerative disorder. The disease is caused by a CAG repeat expansion in the mutant gene, *ATXN3*, which produces an abnormally enlarged poly-glutamine tract in the mutant ATXN3 protein (mutATXN3). Studies evaluating the pathogenic mechanisms of mutATXN3 primarily focus on neuronal implications. Consequently, therapeutic interventions have been generated to target these deficiencies, often overlooking non-neuronal contributions to disease. Our lab has shown that oligodendrocytes in SCA3 display some of the earliest and most progressive dysfunction in disease (Schuster et al. 2022, *JNeurosci*). Here, we extend that study to show that oligodendrocyte dysfunction can be rescued with antisense oligonucleotide (ASO) treatment in a symptomatic mouse model of SCA3. By transcriptionally profiling the most vulnerable SCA3 brain regions in disease mice injected with anti-ATXN3 ASO treatment and comparing to vehicle-injected disease and wildtype mice, we found anti-ATXN3 ASOs rescued differentially expressed genes important for oligodendrocyte maturation. We confirmed temporal rescue of oligodendrocyte impairments across vulnerable SCA3 brain regions by biochemical and histological assessments. We further validated ASO rescue of myelination by TEM analysis of the corticospinal tract. In summary, our data suggest a severe, but modifiable, deficit in OL maturation caused by the gain-of-toxic function of mutATXN3 early in SCA3 disease progression. We are actively exploring the role oligodendrocyte dysfunctions plays in SCA3 pathogenesis and the possible implications of our studies in other neurodegenerative diseases.

#38

Multiomics analysis to uncover the mechanism of radiosensitization of androgen receptor-positive triple negative breast cancers with AR inhibition

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Expression of the androgen receptor (AR) has been identified as a driver of tumor growth in triple negative breast cancers (TNBC), and previous work has nominated AR inhibition as a strategy for radiosensitization in AR+ TNBC. Despite its role in radioresistance in AR+ TNBC, the mechanistic role of AR and specifically its role in mediating DNA damage repair in response to radiation therapy (RT) remains unknown. To understand the role of AR in response to RT *in vitro,* we assessed the cellular localization of AR in AR+ TNBC cell lines (ACC-422, MDA-MB-453) in the presence of hormones with treatment of second-generation anti-androgens, including enzalutamide (ENZA), apalutamide (APA), or darolutamide (DARO). Cells were alternatively cultured in media containing charcoal stripped serum (CSS) without hormones with R1881 stimulation. RNA-sequencing was performed to compare AR+ TNBC cells treated with CSS or R1881 stimulation alone or in combination with ionizing radiation. Reverse phase protein arrays were performed in cells treated with ENZA, RT, or combination treatment. While stimulation with R1881 was sufficient to induce nuclear translocation of AR in MDA-MB-453 cells, AR inhibition with ENZA, APA, or DARO blocked AR nuclear translocation under CSS or FBS growth conditions. When cells were treated with R1881+RT, AR nuclear translocation was induced at similar or greater levels compared to R1881 alone in MDA-MB-453 and ACC-422 cells. Combination treatment of RT with ENZA in the presence of hormones reduced AR nuclear localization (39% reduction in MDA-MB-453 cells and 32% reduction in ACC-422 cells) compared to RT alone. These results suggest that decreased promoter region binding, and gene expression upregulation may be a mechanism of radiosensitization with AR inhibition. In addition, transcriptomic analyses demonstrated at least 979 genes differentially expressed in multiple models. Pathway analyses in these models showed common affected pathways included ECM-receptor interaction, PPAR-gamma activation, PI3K-Akt signaling pathway, and the MAPK/ERK signaling pathway. Proteomic analysis in the same cell lines identified apoptosis, DNA damage, and cell cycle pathway changes after RT when AR-signaling was blocked. Common affected pathways in combined analyses identified PI3K-Akt and MAPK/ERK signaling pathway changes that may be responsible for this radiosensitizing phenotype. Our data suggest that AR inhibition in AR+ TNBC is sufficient to inhibit AR nuclear translocation suggesting that AR may play a nuclear role in response to RT to promote DNA repair and radioresistance. We identify potential pathways, including ECM-receptor interaction, PI3K-Akt signaling pathway, and the MAPK/ERK signaling pathway that may be regulated by AR in response to RT and therefore may be responsible for radioresistance.

#39

Acetyl-CoA production by specific metabolites promotes cardiac repair after myocardial infarction via histone acetylation

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Myocardial infarction (MI) is accompanied by severe energy deprivation and extensive epigenetic changes. However, how energy metabolism and chromatin modifications are interlinked during MI and heart repair has been poorly explored. Here, we examined the effect of different carbon sources that are involved in the major metabolic pathways of acetyl-CoA synthesis on myocardial infarction and found that elevation of acetyl-CoA by sodium octanoate (8C) significantly improved heart function in ischemia reperfusion (I/R) rats. Mechanistically, 8C reduced I/R injury by promoting histone acetylation which in turn activated the expression of antioxidant genes and inhibited cardiomyocyte (CM) apoptosis. Furthermore, we elucidated that 8C-promoted histone acetylation and heart repair were carried out by metabolic enzyme medium-chain acyl-CoA dehydrogenase (MCAD) and histone acetyltransferase Kat2a, suggesting that 8C dramatically improves cardiac function mainly through metabolic acetyl-CoA-mediated histone acetylation. Therefore, our study uncovers an interlinked metabolic/epigenetic network comprising 8C, acetyl-CoA, MCAD, and Kat2a to combat heart injury.

#40

Leveraging Proteostasis for Niemann-Pick C Gene Therapy

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Niemann-Pick disease type C is a childhood-onset, autosomal recessive, lysosomal and lipid storage disease. Clinical phenotypes are heterogeneous, but typically include progressive neurodegeneration, hepatomegaly, and early death. Niemann-Pick C is commonly (~95% of cases) caused by loss-of-function mutations in the *NPC1* gene, which encodes a transmembrane glycoprotein required for exporting cholesterol from late endosomes and lysosomes. Loss of function in this protein causes a disease-characterizing build-up of cholesterol in late endosomes and lysosomes. Addition of exogenous NPC1 restores cholesterol storage defects; thus, gene therapy has been explored to treat Niemann-Pick C disease. Work in this area has previously focused on transduction of cells with human wild type *NPC1* to correct cholesterol exporting defects. However, there is little information about the folding and function of the newly expressed human WT-NPC1 protein. Surprisingly, we demonstrate that mouse WT-NPC1 traffics to the lysosome more efficiently than human WT-NPC1. Additionally, we find that mouse WT-NPC1 more efficiently effluxes accumulated cholesterol. Thus, use of mouse WT-NPC1 may increase the effectiveness of Niemann-Pick C gene therapy through improved proteostasis. To test this, we will perform intracerebroventricular delivery of an improved, neuronal-targeting AAV containing human or mouse WT-NPC1 in NPC1-null mice. We will evaluate these mice to determine the extent to which treatment with each species' gene improves Niemann-Pick C disease pathology.

#41

INCB090244 is a selective and orally bioavailable small molecule that potently inhibits PD-L1 and induces antitumor immunity

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Background: Blocking the PD-L1 immune checkpoint axis with therapeutic antibodies against either the ligand or PD-1 has proven to be an effective treatment modality for multiple cancer histologies. Small molecules targeting the PD-L1/PD-1 axis represent an alternate modality of blocking this pathway. INCB090244 is a small molecule that binds PD-L1, blocks the PD-L1/PD-1interaction and restores T cell function similar to the clinical stage PD-L1 inhibitor INCB086550.

Methods: MDA-MB-231 or CHO cells overexpressing PD-L1 were used to investigate effects of INCB090244 on PD-L1 dimerization, and intracellular trafficking. In vivo, CD34+ humanized mice harboring MDA-MB-231 tumors or C57Bl/6 mice bearing GL261 subcutaneous or orthotopic tumors were used to investigate the efficacy, biodistribution, and pharmacodynamic effects of INCB090244. Human specific gene expression changes in tumors from MDA-MB-231 bearing humanized mice were analyzed by RNA sequencing.

Results: In vitro, INCB090244 potently disrupted the PD-L1:PD-1 interaction, induced PD-L1 dimerization, and inhibited PD-1-mediated negative signaling, resulting in enhanced IFN gamma and IL-2 production in primary human immune cells. Following dimerization, INCB090244 induced internalization of PD-L1 resulting in co-localization with the Golgi apparatus and partial localization in the nucleus. After cell treatment and washing, full restoration of PD-L1 at the cell surface was observed after 5 days of culture in vitro. In vivo, INCB090244 reduced tumor growth in CD34+ humanized mice bearing MDA-MB-231 tumors, to similar levels as atezolizumab. Antitumor activity was completely abrogated in immunodeficient mice, confirming the pharmacologic dependency on a competent immune system. RNA sequencing analysis on tumors from these mice demonstrated similar T cell activation gene signatures as clinical checkpoint blockade antibodies. Biodistribution studies in mice bearing both subcutaneous and orthotopically implanted GL261 glioma tumors demonstrated higher accumulation of INCB090244 in tumor tissue compared to PD-L1 antibodies.

Conclusions: INCB090244 effectively disrupted the PD-L1/PD-1 interaction, induced dimerization and internalization of PD-L1, restored immunity in *in vitro* and *in vivo* tumor models, and is a suitable surrogate for the clinical candidate INCB086550. RNA sequencing demonstrated T cell activation signatures similar to those observed in patients receiving checkpoint blockade antibodies. Biodistribution studies demonstrated higher subcutaneous and brain tumor penetration by INCB090244 compared to PD-L1 antibodies, suggesting a potential advantage of small molecule PD-L1 inhibitors in accessing intratumoral regions. These data further support the clinical evaluation of small molecule PD-L1 inhibitors for cancer immunotherapy

#42

Overcoming Adaptive Resistance to MEK inhibition by activation of Protein Phosphatase 2A in KRAS-mutant cancers.

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A quarter of all human cancers harbor KRAS mutations, yet to date there are no clinically approved therapies specifically targeting this genetic aberration, as KRAS is considered an undruggable target[1]. Deregulation and aberrant activation of the mitogen-activated protein kinase (MAPK) pathway is a hallmark of KRAS-mutant tumors[2]. To date, treatment approaches have centered on the development of KRAS inhibitors and strategies to target KRAS effector kinases RAF, MEK, and ERK[3]. Although some patients initially respond well to these treatments, efficacy is limited by the development of resistance[4]. Thus, understanding the mechanisms regulating treatment response dynamics upon MAPK inhibition is necessary to identify novel opportunities to deepen treatment response, attenuate resistance mechanisms and improve overall treatment outcomes.

Under physiologic conditions, MAPK activity is balanced by negative feedback mechanisms driven by its own activity. KRAS-mutant tumor cells actively dysregulate negative feedback mechanisms to maintain augmented MAPK activity in response to MAPKi[5]. Protein Phosphatase 2A (PP2A) describes a family of highly conserved serine/threonine phosphatases that have diverse substrates and functions – including the negative regulation of MAPK activity. The Narla lab, and others, have shown inactivation of PP2A influences MAPKi sensitivity based on the following observations: 1) A high-throughput drug screen identified PP2A as a determinant of MEK inhibitor response in KRAS-mutant lung cancer cell lines[6]; and 2) A recurrent PP2A mutation in a KRAS-mutant colorectal cell line increased resistance to MEK inhibition and potentiated MAPK signaling[7]. Collectively, these findings suggest an important role for PP2A in the development of resistance to MAPKi in KRAS-mutant cancers. However, the precise mechanism by which PP2A is inactivated in response to MAPKi is unknown. My research will focus on illuminating the mechanism by which PP2A inactivation drives MAPKi resistance. We will use cellular and in vivo models of KRAS-mutant lung cancer to assess the effects of MEK and ERK inhibition on PP2A function and whether reactivation of PP2A using pharmaceutically tractable small molecular modulators of the phosphatase will drive more durable responses and delay the development of treatment resistance.

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#43

Reactivation of Gamma Globin Expression in β-Hemoglobinopathies

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Oxygen delivery to the body's tissues and organs is mediated by hemoglobin, a tetrameric protein made up of two α- and two β-like subunits. β-hemoglobinopathies are the most common monogenic disorders worldwide, and are defined based on whether patients have quantitative (β-thalassemia) or qualitative (Sickle Cell Disease (SCD)) defects in β-globin synthesis. Unfortunately, there are very few treatment options for β-hemoglobinopathies. Increased expression of γ-globin, an alternative β-like subunit, can compensate for the β-globin defect in SCD and β-thalassemia. Therefore, strategies that increase γ-globin expression may form novel therapies for β-hemoglobinopathies. To date, few potential activators of γ-globin expression have been discovered. I have thus performed a pooled genome-wide CRISPR activation screen that identified multiple novel candidate genes that activate γ-globin expression. In preliminary results, I have validated several of these candidate γ-globin activators, and plan to perform additional studies to determine the mechanisms by which these candidate genes increase γ-globin expression. Additionally, in parallel, I have performed a pooled genome-wide CRISPR activation screen to identify genes that impact erythroid differentiation. From this work, I expect to identify novel genes that promote γ-globin expression without impairing erythroid differentiation. This work is expected to further our understanding of the regulation of γ-globin expression and may result in the development of novel therapeutics for β-hemoglobinopathies.

#44

Deep Mutagenesis Scan of *SERPINC1* to inform variants affecting thrombosis risk.

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Background

Venous thromboembolism (VTE) includes both formation of pathogenic thrombi in the deep veins (deep vein thrombosis) and embolization of thrombi to the arterial circulation of the lungs (pulmonary embolism). VTE is a complex genetic disorder with an estimated heritability of 40-60% from family, sibling, and twin studies. Our lab recently conducted an exome sequencing study in ~400 VTE cases and found an excess of rare likely loss of function variants in three anticoagulant genes, *PROC, PROS1 and SERPINC1*. Antithrombin (AT), encoded by the *SERPINC1* gene, is a critical anticoagulant protein that inactivates thrombin and factor Xa (FXa). Homozygosity for *SERPINC1* deficiency is lethal, while partial absence leads to an increased risk of VTE. Damaging *SERPINC1* variants can be categorized into two major types of antithrombin deficiencies: Type I deficiency due to decreased antithrombin concentrations in the bloodstream (quantitative deficiency), or Type II deficiency due to altered antithrombin function without necessarily affecting quantity (qualitative defects).

Objectives

Since AT deficiency is a strong risk factor for VTE, a comprehensive functional database of *SERPINC1* variants could guide the interpretation of sequencing results in VTE patients and lead to a better understanding of AT structure and function.

Design/Methods

To perform a deep mutational scan, we will transfect the variant *SERPINC1* cDNA library into cells bearing the Bxb1-based recombinase "landing pad" system described by Matreyek et al. To identify variants causing AT retention, we will tag our segmented *SERPINC1* libraries with *eGFP* reporter genes (AT-eGFP), and subject our cells to fluorescence-activated cell sorting (FACS) followed by next gen sequencing to identify variants associated with poor AT secretion. To identify variants affecting antithrombin function we will add a transmembrane domain of glycophorin B (*GYPB*) instead of the c terminal eGFP tag (AT-GYPB). This will allow us to perform antithrombin functional screens using flow cytometry. Specifically, we will treat library cells with thrombin or factor Xa, two canonical ligands of antithrombin, to determine which variants disrupt AT function. Thrombin-Antithrombin (TAT) complex ELISA has confirmed the functionality of our SERPINC1-eGFP construct, and Sanger sequencing has confirmed successful mutagenesis of eight known *SERPINC1* variants.

Results and Conclusions

We have currently developed AT-eGFP which retains its functional inhibition of thrombin. We are also developing AT-GYPB and validating its ability to form inhibitory complexes. Efforts are underway to separate variants in *SERPINC1* associated with poor secretion from reference and other functional variants. Our next steps also involve full library cloning and screens. Any variants found to affect AT secretion or function will be used to make a functional map to find mutational clustering patterns.

#45

Ankyrin-G in Developmental and Epileptic Encephalopathy

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Developmental and epileptic encephalopathies (DEEs) are a group of disorders in which infants and children have seizures and developmental impairments. Ion channel gene mutations and GABAergic interneuron dysfunction have both been implicated in DEE etiology. Interestingly, DEE-associated ion channels are binding partners of ankyrin-G, a scaffolding protein encoded by the *ANK3* gene critical for the proper localization of these ion channels to neuronal excitatory domains, such as the axon initial segment (AIS) and nodes of Ranvier. Additionally, ankyrin-G is essential for the stabilization of inhibitory domains, including GABAergic synapses. However, it remains unknown how ankyrin-G dysfunction at each of these domains may contribute to DEE etiology. We have identified three DEE patients with compound heterozygous *ANK3* variants of interest (VOIs). Understanding the impact of these VOIs on ankyrin-G-dependent ion channel localization and GABAergic signaling could provide a mechanistic link between ankyrin-G dysfunction and abnormal neuronal activity seen in DEE. To determine how these *ANK3* VOI's may impact ion channel localization and GABAergic synapse formation, we will knock out ankyrin-G in cultured mouse hippocampal neurons and rescue expression with a wild-type or variant form of ankyrin-G. Immunocytochemistry will be used to stain for ankyrin-G-dependent ion channels and key inhibitory synaptic components. Additionally, to determine the impact of ankyrin-G loss-of-function at inhibitory and/or excitatory domains on seizure susceptibility, we will utilize mouse models which abolish ankyrin-G function at individual domains in a febrile seizure induction experiment. These studies may reveal a novel pathway underlying DEE etiology, paving the way for the development of effective DEE treatments.

#46

Combatting Cholesterol Accumulation in Niemann-Pick Type C Disease

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Lysosomal storage disorders (LSD's) are a heterogenous group of more than 50 diseases that are characterized by the accumulation of substrates in the lysosomes due to organelle disfunction. These diseases are present in about 1 in 8,000 live births with a median age of onset of 2.7 years, representing a common health problem and large cause of infant mortality. One such disorder is Niemann-Pick type C, an autosomal recessive disease that leads to progressive neurodegeneration and early death, often in childhood. Niemann-Pick C is caused by loss-of-function mutations in one of the lysosomal cholesterol efflux proteins, NPC1 or NPC2, leading to the accumulation of unesterified cholesterol in late endosomes and lysosomes of all cells. The accumulation of unesterified cholesterol in the diseases lead to neurological deterioration, foamy macrophages, neuron loss, cerebellar ataxia, hepatosplenomegaly, and ultimately death.

Currently, there are no FDA-approved treatments for most LSDs, including Niemann-Pick C, and all treatments have focused on treating the symptoms of patients and not the cause. While the disease is characterized by the accumulation of cholesterol in cells, it remains unclear if the clearance of cholesterol is sufficient to prevent neurodegeneration. A new potential therapeutic using the biological High-Density Lipoprotein pathway has been a proposed solution for effluxing the accumulated cholesterol from cells. Former studies with a preliminary formulation of the sHDL have shown the ability to reduce cholesterol accumulation in human fibroblasts containing mutated Niemann-Pick C. Brain slices from the NPC mouse model recapitulated this reduction when treated with the sHDL.

While our lab has identified an sHDL formulation with the capacity to efflux cholesterol, we want to take it further and optimize an sHDL that will work congruently when being injected into peripheral tissue as well as the brain. These experiments will be conducted in isogenic neuronal cultures containing mutated NPC. Once an optimal formulation has been determined, the sHDL will be further tested in the NPC mouse model to determine if it is able to reduce cholesterol accumulation and rescue disease phenotype. This study anticipates that reducing the high accumulation of cholesterol in the brain and organs through substrate-reduction therapies will have potential therapeutic benefits of alleviating disease phenotypes in patients with mutations causing the Niemann-Pick Type C disease.

#47

Targeting DNA Double Strand Break Repair in Homologous Recombination Proficient High Grade Serous Ovarian Cancers

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Objectives:
Ovarian cancer is the fifth highest cause of cancer deaths among woman in the United States, with a 5-year survival rate of less than 50%. Most women develop recurrent disease that is resistant to standard treatments, thus it is imperative that we develop new treatment strategies to improve clinical outcomes. While approximately 50% of ovarian cancers demonstrate homologous recombination deficiency (HRD) and respond platinum and PARP inhibitor therapies, tumors with HR proficiency (HRP) are less responsive to current treatment. We therefore sought to characterize DNA damage repair (DDR) pathway function across a HRD and HRP ovarian cancers, and to develop rational new therapeutic approaches to treat HRP tumors.

Methods:
HRD cell lines OVCAR3 and OVCAR8 plus HRP cell lines OVCAR5 and ES2 were studied. Specific DDR proteins targeted include ATM, ATR, DNA-PK, and PARP. We established fluorescence reporter assays to characterize the relative engagement of the NHEJ and HR pathways in the absence and presence of DDR inhibitors. The utilization of the canonical NHEJ and POLθ-mediated end-joining (TMEJ) pathways was also quantified. Based on single agent effects and relative engagement of DDR pathways, the combinations of PARP inhibition plus ATM inhibition (Olaparib + KU60019) as well as platinum plus ATR inhibition (cisplatin + AZD6738) were studied. Cell viability assays were performed and synergy was assessed.

Results:
In the absence of DDR inhibition, HR is the predominant pathway of DDR in HRP cell lines. In HRD cell lines, both NHEJ and HR pathways are active, with higher NHEJ than HR activity. Furthermore, HRP cell lines utilize the canonical NHEJ pathway more than the TMEJ pathway, in contrast to HRD cell lines in which TMEJ predominates. Relative pathway engagement shifts following DDR inhibitor therapy, with both ATM and ATR inhibition decreasing HR activity with a concomitant increase in TMEJ most commonly. The two combination therapies tested both resulted in synergistic cell killing in HRP cell lines in addition to HRD cell lines.

Conclusions:
DDR is a complex process in high grade serous ovarian cancer cell lines, with engagement of both the HR and NHEJ cells in not only HRP but also HRD cell lines. DDR inhibitor therapy alters the relative engagement of repair pathways. This presents opportunities for new treatment combinations for ovarian cancer patients including those with HRP tumors, by inducing synergistic cell death to overcome compensatory shifts in DDR pathway activation following single agent therapy. Next steps include extending these findings to both in vivo animal models and patient samples, with careful consideration of potential toxicity with clinical translation.

#48

CRTC1-MAML2 fusion translocation and transcription regulation in mucoepidermoid carcinoma

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Mucoepidermoid carcinoma (MEC) is a rare heterogeneous salivary gland carcinoma, primarily comprised of mucosal, epidermoid, and intermediate cells. MEC tumors are characterized by a high frequency of somatic chromosomal translocations, including gene fusions. The most common translocation is the CRTC1-MAML2 fusion, a driver mutation found in approximately 60% of MEC tumors; however, the mechanism of this rearrangement is not known. Therefore, we document the mechanism of CRTC1-MAML2 fusion formation in NCI-H292, a MEC cell line, using genome wide Nanopore long read sequencing. We describe a chain of translocation events leading to the CRTC1-MAML2 fusion, as well as novel rearrangements, including a *TERT* rearrangement with a potential role in tumorigenesis. Our findings support that the structural variants common to MEC are caused by complex chromothripsis rearrangement mechanisms. Next, we characterize gene expression dysregulation caused by the CRTC1-MAML2 fusion. CRTC1-MAML2 is known to be a rogue activator of several transcription factors, but the cell-type specific effect of the fusion throughout the heterogeneous tumor is unknown. Therefore, we perform scRNAseq and scATACseq of NCI-H292, coupled with spatial RNAseq of primary MEC tumors. We determine a gene expression signature associated with CRTC1-MAML2 expression across individual cells. Via spatial RNAseq we identify spatial expression patterns of CRTC1-MAML2-regulated genes. Our findings support a rearrangement pattern for CRTC1-MAML2 formation in MEC and support a potential pattern of intra-tumoral transcription regulation associated with the fusion.

#49

Ribosomal quality control in repeat-associated non-AUG translation of GC rich repeats

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Nucleotide repeat expansions cause multiple neurodegenerative disorders including C9orf72-associated amyotrophic lateral sclerosis and frontotemporal dementia (C9 ALS/FTD) and Fragile X-associated tremor/ataxia syndrome (FXTAS). C9 ALS/FTD results from a GGGGCC (G_4C_2) hexanucleotide repeat expansions within an intron of *C9orf72* while FXTAS is caused by CGG repeat expansions in the 5'UTR of *FMR1*. These repeat-containing RNAs elicit toxicity at least in part by triggering repeat-associated non-AUG (RAN) translation, a non-canonical initiation process that generates toxic proteins from GC rich repeats. As repetitive RNA elements form strong RNA secondary structures might impact translational elongation, we explored the impact of ribosome-associated quality control (RQC) pathways on RAN translation. RQC rescues stalled ribosomes and prevents translation of aberrant transcripts, with specific roles for both the mammalian nuclear export mediator factor (NEMF, homologous of Rqc2 in yeast) and the E3 ubiquitin ligase Listerin. Here we show that depletion of NEMF markedly increases the production of RAN products from both G_4C_2 and CGG repeats. This effect appears to be mediated post transcriptionally and does not involve the nuclear-cytoplasmic transport functions of the protein. Ongoing studies are characterizing how loss of Listerin impacts RAN translation and repeat RNA stability while exploring whether NEMF meditated CAT-tailing of RAN translation products impacts their turnover and toxicity.

#50

MDM2 inhibition as a non-hormone dependent radiosensitizing strategy in p53 wild-type breast cancer models

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Background: Radiation therapy (RT) is standard in the treatment of most women with breast cancer (BC), though efficacy of RT remains inadequate for women with locally advanced BC. Thus, more effective radiosensitization strategies are needed. We performed a radiosensitizer screen paired with transcriptomic and proteomic data to identify potential mediators of RT resistance and identify novel therapeutic targets.

Methods: Microarray and RPPA data were used for gene/protein expression and pathway analysis. Clonogenic survival assays were performed to assess radiosensitization and calculate relative enhancement ratios (rERs) with MDM2 inhibitors navtemadlin (AMG-232) and alrizomadlin (APG-115) in p53 wild-type (WT) and mutant (MT) models of estrogen receptor positive (p53 WT: MCF-7, p53 MT: T47D) and triple-negative BC (p53 WT: CAL-51, p53 MT: MDA-MB-231). AlamarBlue was used to determined IC-50 concentrations of MDM2 inhibitors. Flow Cytometry with Annexin V staining was used to evaluate apoptosis. Light microscopy with β-gal staining was used to evaluate senescence.

Results: An MDM2 inhibitor (JNJ-26854165) was nominated as an effective drug in treatment for RT-resistance BC cell lines ($R^2 = 0.43$, p value <0.01) in our novel radiosensitizer screen. Differential gene expression and pathway analysis in non-overlapping p53 WT BC cell lines treated +/-RT identified apoptosis, cell cycle, and p53 signaling as the top pathways induced in p53 WT cell lines by RT. MDM2 was significantly overexpressed after RT compared to no RT in p53 WT cells. MDM2 inhibition radiosensitized p53 WT cells (rERs: 1.81-2.85) but not p53 MT cells (rERs: 1.00-1.03). This phenotype was reversed in p53 CRISPR knockout cells (rERs: 1.06-1.12). Combination RT with navtemadlin or alrizomadlin demonstrated increase apoptosis after 72 hours in the p53 WT CAL-51 cell line. This increase in apoptosis was abgrogated with p53 CRISPR knockout of p53. Combination MDM2 inhibition with navtemadlin and RT demonstrated an increase in senescence after 72 hours in the p53 WT CAL-51 cell line similar to positive control Palbociclib treatment. This increase in senescence response was abrogated by CRISPR knockout of p53.

Conclusions: These results demonstrate the combination of RT and MDM2 inhibition may be an effective therapeutic strategy in patients with p53-wild type breast cancer, regardless of hormone receptor status.

#51

Microbiota metabolic exchange is critical for colorectal cancer redox homeostasis and growth

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Intestinal microbiota play a fundamental role in human health and disease. Microbial dysbiosis is a hallmark of colorectal cancer (CRC) as tumor stage-specific shifts potentiate cancer growth, influence the inflammatory microenvironment, and alter response to therapy. Recent work has demonstrated a critical role for microbial metabolite exchange in host response. However, the role of most microbial metabolites in colon cancer growth is unclear. To better understand how metabolic exchange between the microbiota and tumor epithelium alter CRC growth, a screen of the most abundant bacterially derived metabolites was assessed. Several metabolites were found to alter CRC growth, and reuterin most significantly suppressed CRC cell proliferation. Reuterin is a bifunctional metabolite containing both hydroxy and aldehyde functional groups. Reuterin is primarily synthesized from glycerol by *Lactobacillus reuteri*, a commensal bacterium found throughout the gastrointestinal tract. Interestingly intra-tumoral reuterin levels are significantly lower compared to normal tissues from mouse models and CRC patients. Our previous work demonstrated that reuterin was a direct inhibitor of the hypoxic response via binding to the transcription factor hypoxia-inducible factor (HIF)2a. To test if reuterin altered CRC growth via HIF2a, knockdown and knockout cells were tested. We found that reuterin suppressed growth independent of HIF2a. Through unbiased metabolomics and RNA-seq analysis we found that reuterin altered the redox balance of CRC cells. Mechanistically, reuterin potentiates reactive oxygen species (ROS) which leads to irreversible cysteine oxidation and enhanced cell death. These studies demonstrate the potential of reuterin to suppress colorectal cancers.

#52

FIC-1/FICD-mediated AMPylation of HSP70 family chaperones modulates polyglutamine toxicity

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Neurodegenerative diseases (NDs) are characterized by a failure of cellular proteostasis machinery and the subsequent accumulation of misfolded protein aggregates. Regulation of key protein quality control machinery thus represents one avenue for attenuating protein aggregation and toxicity. Fic AMPylases are enzymes that catalyze the post-translational addition and removal of adenosine monophosphate (AMP) to Ser/Thr/Tyr residues of target proteins. Recent work from our lab identified the human AMPylase, FICD, and its *C. elegans* ortholog, FIC-1, as potent regulators of the ER-resident HSP70 family chaperone, BiP, and its worm orthologs, HSP-3/4, respectively. Using *C. elegans* models of polyglutamine (polyQ) diseases, a family of inherited NDs caused by the aberrant expansion of polyQ tracts, we previously reported that FIC-1-mediated AMPylation/deAMPylation of target proteins directly alters polyQ aggregate number, size, solubility, and toxicity. Yet, little is known about the mechanism(s) through which FIC-1 modulates polyQ aggregation dynamics.

To this end, we asked if changes in AMPylation levels are detrimental during larval development, when proteostasis is burdened by both rapid cell division and aggregation-prone polyQ proteins. Using an RNAi-based knock-down approach, we identified *hsp-3* and *hsp-4* as essential for the survival of Q40::YFP worms in a polyQ length-dependent manner, while this requirement was eliminated in *fic-1* null animals. From this, we hypothesized that the unfolded protein response in the ER (UPR^{ER}) downstream of HSP-3/4 may be involved in mediating this effect. Indeed, we demonstrate here that the beneficial effect of *fic-1* loss is eliminated by knock-down of the UPR^{ER} mediators PERK and ATF-6, but not IRE-1 – suggesting that the protective effect of *fic-1* loss is at least partially mediated through these arms of the UPR^{ER}. In line with previous findings, we further show that *fic-1* loss perturbs polyQ aggregation dynamics in developing animals. Ongoing work is focused on characterizing additional genes involved in the *fic-1*-mediated regulation of polyQ aggregation dynamics using mutants identified in an unbiased chemical mutagenesis screen. In parallel, we are exploring the translational impact of FICD-mediated AMPylation using induced pluripotent stem cell (iPSC)-derived models of human polyQ expansion diseases. The ultimate goal of this project is to identify conserved mechanisms by which AMPylation regulates the aggregation and toxicity of disease-linked polyQ expansion proteins.

#53

Evaluating shared molecular features and potential vulnerabilities of low-H3K27me3 pediatric brain tumors

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Brain tumors have the worst survival rates of all childhood malignancies, and a better understanding of their biology is needed for therapeutic development. Two especially devastating subtypes of pediatric brain tumors—diffuse midline gliomas, H3K27M-mutant (DMGs) and posterior fossa ependymomas, group A (PFAs) are both characterized by a hallmark of global reduction of histone 3 lysine 27 trimethylation (H3K27me3), a mark associated with transcriptional repression. Most DMGs harbor mutations of histone 3 (H3K27M) that inhibit the function of the H3 methyltransferase enhancer of zeste homolog 2 (EZH2). Most PFAs overexpress EZH inhibitory protein (EZHIP), resulting in a similar effect on EZH2 activity. Intriguingly, a small subset of DMGs found to be lacking H3K27M mutations express EZHIP, and a fraction of histological PFAs lacking EZHIP expression harbor H3K27M mutations. Given the highly similar putative driving alterations and epigenetic features in these two tumor types, we undertook a systematic evaluation of the clinical and molecular features of each tumor class to elucidate additional shared features and potential targetable vulnerabilities. We performed detailed analyses of genomic aberrations, gene expression, and epigenomic landscapes to identify key similarities and differences in tumor biology using published tumor datasets from our group and others. We first found shared recurrent copy number gain of the long arm of chromosome 1 (1q) and identified several 1q genes whose expression correlated with survival differences in cohorts of both tumors. Additionally, our findings demonstrate similar H3K27me3 landscapes with convergence of residual tri-methylated loci between PFA and DMG tumors. Together, these findings better define the commonalities and differences of these highly aggressive, low-H3K27me3 pediatric brain tumors and will provide a framework for understanding which therapeutic strategies may translate from one tumor to the other.

#54

RNA methylation influences TDP43 binding and disease pathogenesis in models of amyotrophic lateral sclerosis and frontotemporal dementia

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Methylation of RNA at the N6 position of adenosine (m6A) is one of the most common RNA modifications, impacting RNA stability as well as its transport and translation. Previous studies uncovered RNA destabilization in models of amyotrophic lateral sclerosis (ALS), in association with accumulation of the RNA-binding protein TDP43, which is itself mislocalized from the nucleus in >95% of those with ALS. Here, we show that TDP43 recognizes m6A-modified RNA, and that RNA methylation is critical for both TDP43 binding and autoregulation. We also observed extensive hypermethylation of coding and non-coding transcripts in ALS spinal cord, many of which overlap with methylated TDP43 target RNAs. Emphasizing the importance of m6A for TDP43 binding and function, we identified several m6A factors that enhance or suppress TDP43-mediated toxicity via a single-cell CRISPR/Cas9 candidate-based screen in primary neurons. The most promising genetic modifier among these—the canonical m6A reader YTHDF2—accumulated within spinal motor neurons in ALS postmortem sections, and its knockdown prolonged the survival of human neurons carrying ALS-associated mutations. Collectively, these data show that m6A modifications modulate RNA binding by TDP43, and that m6A is pivotal for TDP43-related neurodegeneration in ALS.

#55

Exogenous UPF1 corrects TDP-43-directed NMD-sensitization of the transcriptome in neuronal models of ALS

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ALS is a relentless neurodegenerative disease affecting upper and lower motor neurons (MNs). Nearly all individuals share the histological hallmark of TDP-43 pathology in affected neurons. TDP-43, a nuclear RNA-binding protein, is essential for life and influences the RNA splicing and metabolism. We and others have found that co-expression of the nonsense mediated decay (NMD) pathway member and RNA helicase UPF1 reduces TDP-43 toxicity in primary rodent neurons and in several other in vitro and in vivo models of ALS. Taken together, these data suggest that impaired NMD is a convergent component of ALS disease.

To quantify global canonical NMD (the degradation of transcripts containing a prematurely terminating codon) in existing models and human specimens, a next-generation sequencing based approach was employed. For each dataset, a de novo transcriptome was assembled and used for transcript-level quantification. Transcripts were categorized into "NMD" or "non-NMD" classes according their predicted sensitivity to NMD. Abundances (TPM) were summarized with two approaches: 1) Global NMD burden (GNB), or the cumulative abundance of NMD-sensitive transcripts divided by total abundance, and 2) Differential NMD-class usage (DNU), or the gene-level ratio of NMD transcript abundance to total transcript abundance.

NMD-sensitive and -insensitive isoforms were quantified in 14 datasets. Significant NMD-impairment is detectable in NMD-null models regardless of the summary statistic used. In ALS datasets, however, GNB is only detectable in cells and tissues in which TDP-43 is directly perturbed. DNU is present in all models and tissues to some degree, but distinctly greater than respective controls in TDP-43-opathy backgrounds. Moreover, providing TDP-43 over expressing MNs with exogenous UPF1, abrogates expression of PTC-containing isoforms that contribute to TDP-43-dependent DNU. These data suggest that the rescue effect of UPF1 operates not by enhancing global metabolism of NMD-sensitive transcripts, but through the surveillance of a subset of genes that undergo TDP-43-directed NMD-sensitizing splicing anomalies.

#56

Investigating the Mechanisms of Estrogen Induced Thrombosis

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Estrogen has been associated with thrombosis (VTE) for over a half-century and estrogen therapy, including the use of oral contraceptives, is considered a bona fide thrombosis risk, yet the precise mechanism remains elusive. Over 200 million women worldwide use estrogen-containing contraceptives. Estrogen is prescribed to treat and alleviate symptoms associated with numerous conditions, including menopause and endocrine disorders, and the therapeutic role of estrogen is rapidly expanding into even seemingly unrelated fields like psychology and neurology. Estrogen induced thrombosis causes morbidity and mortality in thousands of exposed women every year, but due to our ignorance of the mechanism behind it, there is little that can be done to prevent it.

While its widespread, pleiotropic effects on the cardiovascular system are partially responsible for our gap in understanding of how estrogen causes thrombosis, the lack of an adequate animal model has also been a severe hinderance (e.g. mice do not develop VTE in response to estrogen). We have turned to the freshwater teleost *Danio Rerio* (zebrafish) to resolve this deficiency. Importantly, the zebrafish hemostatic system, endogenous estrogens, and estrogen receptors are almost entirely conserved with mammals. Using a line that produces circulating, fluorescently labeled fibrinogen (*Tg(fabp:fgb-eGFP)*), we found that zebrafish larvae reliably and rapidly develop VTE in response to estrogen.

Estrogen modulates coagulation factor expression inducing a hypercoagulable state, but our data indicate that this regulation alone doesn't adequately explain estrogen induced thrombosis. The coagulation cascade culminates in fibrin clot formation, and the precursor of fibrin – fibrinogen – is cleaved by thrombin following its activation by Factor Xa (FXa). **Surprisingly, we found that estrogen induced thrombosis persists despite treatment with anticoagulants and in the absence of thrombin and FX, suggesting that it may occur outside of the canonical coagulation cascade.** According to the prevailing paradigm of coagulation, thrombin is solely responsible for cleaving fibrinogen to fibrin, and fibrinogen is not actively involved in thrombosis, except as the precursor to fibrin. However, our data suggest that fibrin formation in response to estrogen does not rely on thrombin cleavage, or that fibrinogen is primarily responsible. To determine whether fibrin or fibrinogen is involved, an uncleavable form of fibrinogen was developed and tested. To further delineate the cell types involved in this currently undescribed mechanism we are investigating the effect of estrogen on RBCs, neutrophils, thrombocytes and vessel endothelial cells using qualitative and quantitative techniques.

#57

The role of PTEN in epigenetic and metabolic regulation of IDH-mutant gliomas

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Astrocytic tumors of the central nervous system (CNS) are intractable tumors and harbor dismal outcomes due to a lack of targeted treatment options. More than 70% of astrocytic tumors bear mutations in the TCA cycle-related gene, isocitrate dehydrogenase 1 (IDH1). Wildtype IDH1 catalyzes the oxidative decarboxylation of isocitrate to generate α-Ketoglutarate (αKG), while mutated IDH1 (IDH1m) metabolizes αKG into the oncometabolite D-2-hydroxyglutarate (D2HG). Several epigenetic dioxygenases including histone and DNA demethylases require αKG to demethylate histone residues and DNA CpG islands. D2HG competitively inhibits these enzymes due to its structural similarity to αKG. This results in an increase in histone and DNA methylation referred to as the glioma-CpG island methylation phenotype (G-CIMP). However, DNA methylation G-CIMP levels are not uniform in IDH1m astrocytomas. IDH1m astrocytomas with low *versus* high levels of G-CIMP bear a particularly grim prognosis. My preliminary data identifies PTEN loss of heterozygosity (LOH) as the top genetic alteration in G-CIMP low versus G-CIMP high tumors. Moreover, PTEN LOH independently related with a poor prognosis. However, it remains unknown how *PTEN* LOH drives tumorigenicity in IDH1m astrocytomas. Our premise is based on the observation that PTEN is a critical tumor suppressor. In other cancers, *PTEN* LOH activates PI3-kinase (PI3K)/AKT-mTOR signaling to drive tumor growth through various mechanisms including metabolic reprogramming. My preliminary data demonstrates increased cell proliferation in IDH1m astrocytic cell lines with partial PTEN knockdown (KD) accompanied by a reduction in D2HG levels. Based on my premise and preliminary data, I hypothesize that PTEN LOH activates PI3K/AKT-mTOR signaling to metabolically reprogram IDH1m astrocytomas to reduce D2HG levels and thereby mediate lowered DNA methylation and G-CIMP levels. Subsequently, targeting PI3K/AKT-mTOR signaling represents a promising candidate for therapeutic development. To test this hypothesis, I propose two specific aims. Aim 1 will map alterations in metabolic pathways that generate D2HG in human and murine-derived IDH1m astrocytomas with or without PTEN reduction using a novel *in-utero* electroporation (IUE) *Pten* $^{+/-}$ IDH1m model. In parallel, I will assess corresponding genome wide epigenetic alterations including DNA methylation using next generation sequencing-based epigenetic assays in relation to changes in gene expression. Aim 2 will determine if targeting *PTEN* LOH-driven PI3K/AKT-mTOR activation is therapeutic *in vitro* and *in vivo* in animal models. Together, my work will address a critical gap in our knowledge by defining how *PTEN* LOH drives a subset of aggressive mIDH1 astrocytomas *via* metabolic reprograming and leverage our novel genetically engineered mIDH1 animal model to lay the groundwork for developing targeted and effective therapies for malignant mIDH1 astrocytomas.

MICROBIOLOGY, VIROLOGY, IMMUNOLOGY AND INFLAMMATION

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#58

HPV is a novel cargo for the COPI retrograde sorting complex during virus entry

Mara Calypso Harwood[1,2], Tai-Ting Woo[1], Yuka Takeo[3], Daniel DiMaio[3], and Billy Tsai[1]

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During entry, human papillomavirus (HPV) traffics from the cell surface to the endosome and then the Golgi apparatus. The virus must escape the Golgi to reach the nucleus to cause infection, although how this is accomplished is unclear. Combining cellular fractionation, unbiased proteomics, and siRNA-mediated gene knockdown strategies, we identified COPI—a multi-subunit protein complex that sorts cellular cargos out of the Golgi for retrograde transport—as a host factor required for HPV infection. Upon Golgi-arrival, HPV binds directly to COPI via a conserved non-canonical di-arginine motif in the capsid protein L2. COPI inactivation traps the virus in the Golgi, similar to the fate of a COPI binding-defective HPV L2 mutant pseudovirus, suggesting the L2-COPI interaction drives Golgi-escape of HPV during virus entry. Our findings demonstrate that HPV exploits COPI to exit the Golgi *en route* to the nucleus for infection and reveal an incoming virus as a novel cargo for this retrograde sorting complex.

#59

Elucidating the Role of Osteoclasts on T cell differentiation and Systemic Inflammation

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Osteoclasts are multinucleated, bone-resorbing cells, derived from a diverse pool of myeloid progenitors. Similar to other myeloid cells, osteoclasts can phagocytose pathogens, process peptides, and activate T cells, to induce adaptive immune responses. Previous literature suggests that osteoclasts directly interact with and prime T in inflammatory disease models. However, the evidence showing a direct interaction between osteoclasts and T cells has never been shown in-vivo, and our lab has previously shown that multinucleated osteoclasts plated on bovine bone chips in-vitro are unable to uptake pathogens and antigens that they are capable of doing on other, less-biologically relevant substrates. Thus, we sought to determine if osteoclasts play a direct role on T cell function in-vivo. Using an osteoclast knock-out mouse model, Ctsk-DTA (*Ctsk*$^{CreERT2/+}$ Diphtheria Toxin Fragment A), we were able to ablate functional osteoclasts in mice and determine if osteoclasts are essential for proper T cell differentiation and inflammation. This Ctsk-DTA model has been verified in our lab, and T cells isolated from bone marrow were processed for flow cytometry to phenotype differentiated T cells. We hypothesized that ablating osteoclasts increases inflammatory T cell differentiation, influencing systemic inflammatory effects. Our data suggest that ablating osteoclasts using the Ctsk-DTA model, decreases regulatory, anti-inflammatory, CD3+CD4+FoxP3+ regulatory Treg populations from bone marrow, and increases pro-inflammatory CD8+ populations in both male and female mice. Inflammatory cytokine levels will be quantified from serum from these mice and complete blood count (CBC) will be obtained. To further observe this osteoclast ablation model during inflammatory diseases, our lab will also be utilizing a murine rheumatoid arthritis (RA) model (KbXN) as well as an aged mouse model, where osteoclasts will be ablated while they have RA and also in mice aged 12+ months. We hypothesize that inflammatory T cell differentiation, and overall inflammatory response will be augmented by these inflammatory models lacking osteoclasts, indicating a systemic homeostatic role for osteoclast presence and function. Osteoclast ablation is physiologically relevant, as nearly all current therapeutics for treating bone loss pathologies function by either inducing osteoclast apoptosis or inhibit osteoclast maturation/differentiation. The successful completion of this project will further support the need for better therapeutics to be used for patients suffering from bone loss, using an approach to modulate osteoclast resorptive function, rather than eliminating osteoclasts in treating bone-degrative diseases.

#60

Mucus Hungry Microbes: An arsenal of M60 proteases equips *Bacteroides caccae* for life in the gut

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The mucus layer lining the intestine is the first line of defense against opportunistic pathogens; its integrity is critical for maintaining a healthy bowel. Excessive microbial degradation of the mucus barrier is linked to several of the most common gastrointestinal diseases, such as inflammatory bowel disease (IBD) and colonic cancer. Intestinal mucus is composed of glycoproteins termed mucins, which are often extensively modified to provide a wide variety of glycoconjugates. The human gut commensal *Bacteroides caccae* encodes an arsenal of specialized mucin degradation enzymes, including 16 M60 proteases theorized to be responsible for completing the cleavage of the mucin backbone. This cleavage may result in the release of various mucin byproducts for consumption by other microbial community members. In our preliminary studies, we have observed the vast upregulation of several *B. caccae* M60 proteases when grown with mucin *O*-glycans. Thus, these enzymes may be a crucial part of *B. caccae's* adaptation to the intestine. We are currently conducting a multiscale investigation of the regulation, substrate specificity, and ecological importance of four key M60 proteases. This work will directly inform the identification of precision therapeutic targets for conditions involving excessive gut mucus degradation, such as IBD.

#61

The impact of HIV-1 Vpr on host transcription in macrophages

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Vpr is a highly conserved HIV accessory protein that is necessary for optimal replication in macrophages, but its mechanism of action is poorly understood. Studies using human lymphoid tissue (HLT), which are rich in both T cells and macrophages, have found that loss of Vpr decreases virus production but only when the virus strain used is capable of efficiently infecting macrophages. These studies provide evidence that Vpr enhances infection of macrophages and increases viral burden in tissues where macrophages reside. Because Vpr is packaged into the virion and localizes to the nucleus, it may enhance early viral replication events. However, *vpr*-null virus in which Vpr protein is provided by trans-complementation in the producer cells replicates poorly compared to wild-type virus in mononuclear phagocytes. Thus, there is genetic evidence that Vpr's role in the HIV replication cycle continues into late stages. Work by our group has demonstrated that Vpr counteracts mannose receptor, a macrophage specific restriction factor that targets Env and Env-containing virions for lysosomal degradation. Mannose receptor protein levels are inversely proportional to the amount of viral output from macrophages, affecting spread to neighboring cells including CD4+ T cells in co-culture conditions. Importantly, Vpr reduces transcription of the gene encoding mannose receptor, *MRC1*, as well as other innate immune genes including *IFNA1* through an unknown mechanism. Vpr's ability to specifically alter transcription levels of these genes, led us to investigate the role of Vpr on global transcription in macrophages from three independent donors using single-cell RNA sequencing. Differential gene expression analysis between *vpr*-WT, and *vpr*-null infected cells revealed transcriptional difference between the two cell types. Closer examination of the genes downregulated in the presence of Vpr revealed patterns of transcriptional control mechanisms. Studies are ongoing to determine whether Vpr targets specific host transcription factors to alter pathways that influence viral infection and spread.

#62

Investigating the extracellular degradation of HIV-1 genomic RNA

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HIV-1 and other retroviruses are unique in packaging two identical copies of their positive-sense single-stranded RNA genome, which associate with one another in viral particles via a non-covalent dimer linkage. Although the dimeric nature of retroviral genomes has been well established, the functional role dimerization plays in the viral life cycle remains unknown. One explanation is that encapsidated retroviral RNA appears to be extracellularly degraded, and the inclusion of two copies of the viral genome allows for reverse transcriptase to successfully generate viral DNA from fragmented genomic RNA through a template switching mechanism. This model is supported by several independent observations that retroviral genomes exhibit marked degradation. We have performed a series of northern blot experiments to determine the time frame over which genomic RNA is degraded as well as whether degradation is uniform throughout the genome. The viral genome increasingly fragments over time, and this degradation tapers off approximately twenty-four hours post-budding. Furthermore, different regions of the genome are differentially degraded. It has recently been demonstrated that the ALU retrotransposon is capable of self-cleavage. The signal recognition particle RNA, 7SL, contains an Alu domain and is packaged in seven-fold molar excess of the viral genome. To determine whether 7SL contributes to the degradation of HIV-1 genomic RNA in viral particles, we performed a depletion experiment and found that exclusion of 7SL from viral particles has a minimal effect on extracellular genomic degradation. Our lab has additionally observed that the poly(A) tails of encapsidated RNAs shorten over time, and that CNOT7, a component of the CCR4-Not deadenylase complex, is present in virions. Based on this, we tested whether components of CCR4-Not facilitate the deadenylation of encapsidated viral genomes. Further work is being conducted to elucidate the effects of this complex on HIV-1 genomic integrity. Finally, our model predicts that the more fragmented a genome is, the more template switching it will undergo. We tested this hypothesis by measuring repeat deletion as a proxy for template switching. Preliminary results suggest that reverse transcriptase engages in more repeat deletion with degraded genomes than with relatively intact genomes.

#63

A specific EMC subunit supports Dengue virus infection by promoting virus membrane fusion essential for cytosolic genome delivery

Parikshit Bagchi[1,5,*], Kaitlyn Speckhart[1,2,*], Andrew Kennedy[3], Andrew W. Tai[3,4], and Billy Tsai[1,2, #]

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Dengue virus (DENV) represents the most common human arboviral infection, yet its cellular entry mechanism remains unclear. The multi-subunit endoplasmic reticulum membrane complex (EMC) supports DENV infection, in part, by assisting the biosynthesis of viral proteins critical for downstream replication steps. Intriguingly, the EMC has also been shown to act at an earlier step prior to viral protein biogenesis, although this event is not well-defined. Here we demonstrate that the EMC subunit EMC4 promotes fusion of the DENV and endosomal membranes during entry, enabling delivery of the viral genome into the cytosol which is then targeted to the ER for viral protein biosynthesis. We also found that EMC4 mediates ER-to-endosome transfer of phosphatidylserine, a phospholipid whose presence in the endosome facilitates DENV-endosomal membrane fusion. These findings clarify the EMC-dependent DENV early entry step, suggesting a mechanism by which an ER-localized host factor can regulate viral fusion at the endosome.

#64

Modeling *Vibrio cholerae* Infections in Zebrafish, a Natural Host

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Vibrio cholerae is an aquatic bacterial species that causes the human disease cholera. *V. cholerae* is associated with numerous animals in the aquatic environment, including vertebrate fish. Zebrafish (*Danio rerio*) are an attractive model organism for a variety of scientific studies, including host-microbe interactions. Over the past 10 years, the Withey lab has established zebrafish as a unique *V. cholerae* model, in which the entire infectious cycle can be studied. This is particularly useful for examining host-pathogen interactions. On the bacterial side, it is unclear what colonization factors *V. cholerae* uses in the fish intestine. *V. cholerae* can successfully compete with mature, intact zebrafish intestinal microbiota for a colonization niche, and recent studies have examined this process. Zebrafish get diarrhea from the infection, and it is unclear what toxin(s) are responsible for this. *V. cholerae* also becomes hyper-infectious when transiting a fish, and the bacterial genes responsible for this are not well understood. Current studies are underway to identify genetic factors and mechanisms responsible for these aspects of the life cycle. On the host side, we have found genetic differences in *V. cholerae* correlate with changes in the timeline of infection. It is likely that some *V. cholerae* are cleared by strictly innate responses, whereas other strains require adaptive responses for clearance. Overall, zebrafish present an ideal model for studying the life cycles of aquatic enteric pathogens.

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GRADUATE PROGRAM IN CELLULAR AND MOLECULAR BIOLOGY

NEW FACULTY FOR 2021-2022

Joshua Emrick, Ph.D.
(Assistant Professor of Biologic and Materials Sciences & Prosthodontics)

Kurt Hankenson, DVM, Ph.D.
(Assistant Professor of Orthopedic Surgery)

Adam Helms, Ph.D.
(Assistant Professor Internal Medicine, Division of Cardiovascular Medicine)

Costas Lyssiotis, Ph.D.
(Associate Professor of MIP)

Shoji Maeda, Ph.D.
(Assistant Professor of Pharmacology)

Hayley McLoughlin, PhD.
(Assistant Professor of Neurology)

Randy Seeley, PhD.
(Professor of Surgery)

Lauren Surface, PhD.
(Assistant Professor of Dentistry)

Chase Weidmann, Ph.D.
(Assistant Professor of Biological Chemistry)

Sethu Pitchiaya, Ph.D.
(Assistant Professor of Urology)

Jason Miller, MD, Ph.D.
(Assistant Professor Department of Ophthalmology and Visual Sciences)

GRADUATE PROGRAM IN
CELLULAR AND MOLECULAR BIOLOGY

INCOMING GRADUATE STUDENTS FOR 2021-2022

PIBS/CMB-Interest Students (First Year)
for 2021-2022

Rachel Benedeck
(Mentor: TBD)

Michael Cadigan
(Mentor: Nicole Koropatkin)

Faith Carranza
(Mentor: Scott Leiser)

Caroline Hsieh
(Mentor: Sami Barmada)

Amelia Lauth
(Mentor: TBD)

Kayla Lenshoek
(Mentor: TBD)

Lwar Naing
(Mentor: Marilia Cascalho)

Rachael Powers
(Mentor: Hank Paulson)

Nicholas Rossiter
(Mentor: Yatrick Shah)

Ziad Sabry
(Mentor: Zhong Wang)

Siara Sandwith
(Mentor: John Moran)

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CELLULAR AND MOLECULAR BIOLOGY

INCOMING GRADUATE STUDENTS FOR 2021-2022

New CMB Students (Second Year/G1)
for 2021-2022

Collie, Sam
(Mentor: Carole Parent)

Correia, Adele
(Mentor: Lieberman, Andrew)

Dean, Kendall
(Mentor: Paul Jenkins)

Elkahlah, Najia
(Mentor: Josie Clowney)

Forson, Jacqueline
(Mentor: Ryan Baldridge)

Gordian, Desiree
(Mentor: Martin Myers)

Machlin, Jordan
(Mentor: Ariella Shikanov)

Orosco, Amanda
(Mentor: Pierre Coulombe)

Royzenblat, Sonya
(Mentor: Peter Freddolino)

Shay, Madeline
(Mentor: Nicole Koropatkin)

Vangos, Nicholas
(Mentor: Michael Cianfrocco)

Gordian, Desiree
(Mentor: Martin Myers)

Machlin, Jordan
(Mentor: Ariella Shikanov)

Orosco, Amanda
(Mentor: Pierre Coulombe)

Royzenblat, Sonya
(Mentor: Peter Freddolino)

Shay, Madeline
(Mentor: Nicole Koropatkin)

Vangos, Nicholas
(Mentor: Michael Cianfrocco)

Machlin, Jordan
(Mentor: Ariella Shikanov)

Nino, Charles*
(Mentor: Corey Speers)

Pourmandi, Narges*
(Mentor: Costas Lyssiotis)

Williams, Jonathan*
(Mentor: Jayakrishnan Nandakumar)

***CMB MSTP Students**

GRADUATE PROGRAM IN
CELLULAR AND MOLECULAR BIOLOGY

Ph.D. GRADUATES
(since the last CMB symposium)

Student	Mentor	Defense Date	Title
McGrath, Brian	Bielas	5/21/21	Histone H2A Mono-Ubiquitination in Neurodevelopmental Disorders: Molecular Insights from Rare Genetic Variants
Valesano, Andrew	Lauring	5/25/21	Using intrahost genetic diversity to understand RNA virus evolution and transmission
Warrington, Hillary	Leiser	6/3/21	Regulation of Stress-Induced Longevity
Kunselman, Jenny	Puthenveedu	6/29/21	Regulation of Opioid Receptor Trafficking and Signaling by Opiod Peptides
Kuo, Molly	Antonellis	6/30/21	Defining the Role of Cysteinyl-tRNA Synthetase (CARS1) in Human Recessive Disease
Kinnear, Hadrian	Shikanov	12/2/2021	Reproductive Impact of Testosterone Therapy in a Transgender Mouse Model
Moritz, Lindsay	Hammoud	12/8/2021	Revisiting the role of sperm protamine proteins in organismal development and fertility
Crilly, Stephanie	Puthenveedu	12/14/2022	Membrane Trafficking and Signaling of the Delta Opioid Receptor within the Biosynthetic Pathway
Ravichandran, Vani	Westfall	1/18/2022	The Contribution of Chronic Protein Kinase C-Mediated Troponin I Phosphorylation to Cardiac Dysfunction
Gingerich, Morgan	Soleimanpour	5/2/2022	Intrinsically disordered protein regions encoded by the diabetes gene *CLEC16A* regulate mitophagy