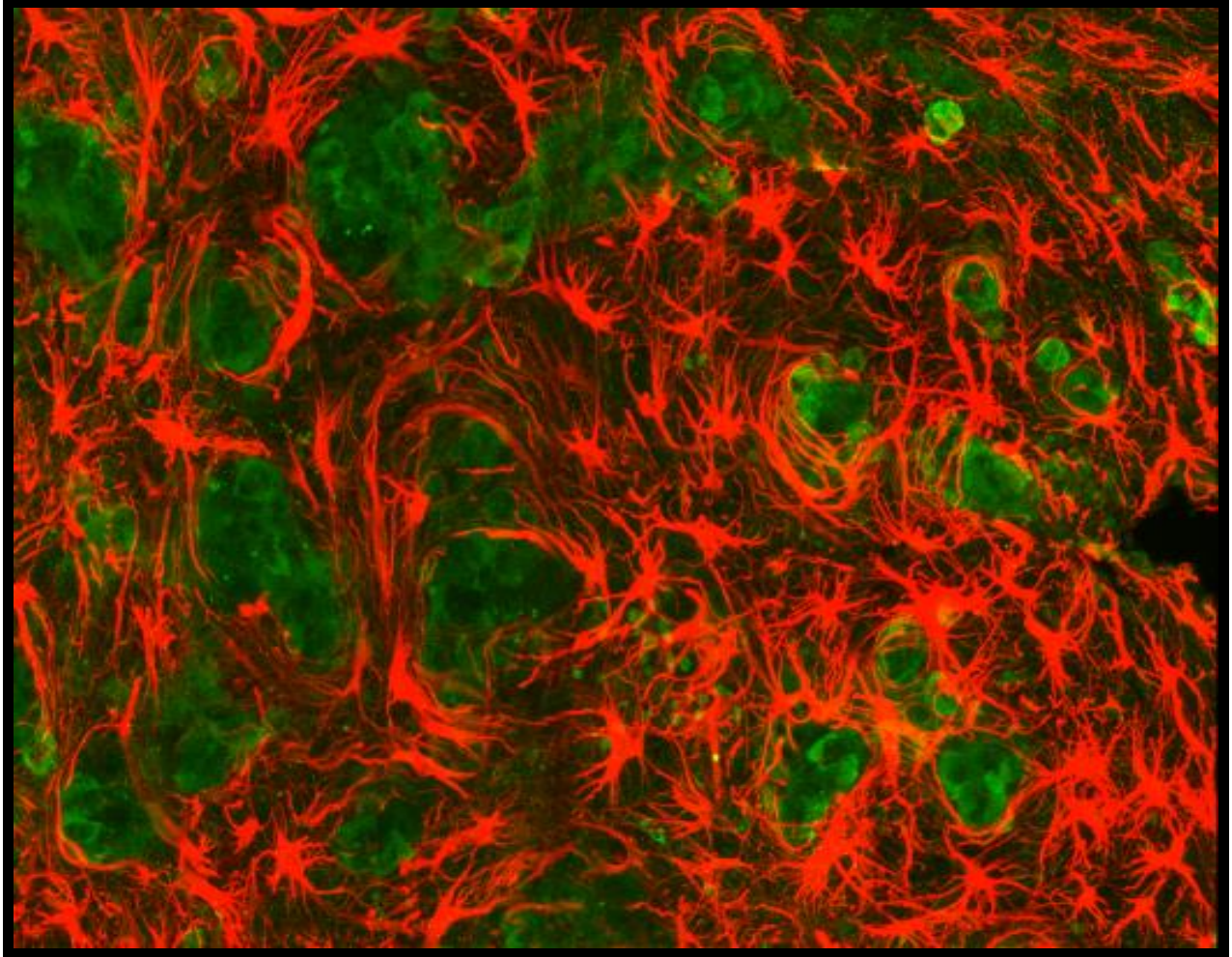
2019 BCMB Program Retreat



The cover photo presented is a snapshoot of inflamed/activated astrocytes surrounding breast cancer brain metastatic cells, credited to Bryan Ngo, from Dr. Lewis Cantley's lab and Chenu Jaywickreme, from Dr. Luis Parada's lab.

Weill Cornell Graduate School of Medical Sciences

Memorial Sloan Kettering Cancer Center

Weill Cornell Medicine
Graduate School of Medical Sciences
A partnership with the Sloan Kettering Institute

2019 BCMB Program Retreat:
Mohonk Mountain House
1000 Mountain Rest Road
New Paltz, NY 12561

THURSDAY – September 26

TIME	EVENT	DESCRIPTION/LOCATION
7:00 am	Departure	Depart from 1300 York Avenue
8:30 – 8:45am	Estimated Arrival to Mohonk	
8:50 – 9:30am	Continental Breakfast	Conference House
9:30 – 9:45am	Mary Baylies Introduction	Conference House
9:45am – 1:25pm	Plenary Sessions **Moderator:** Mary Baylies	Conference House
9:45 – 10:05am	Stewart Shuman	
10:05 – 10:25am	Lukas Dow	
10:25 – 10:45am	Kristy Brown	
10:45 – 10:55am	***Poster Teaser (10 minutes)***	
10:55 – 11:05am	***Coffee Break/Stretch (10 minutes)***	
11:05 – 11:25am	Mary Baylies and Kirk Deitsch Welcome to the First Year Class	
11:25am – 12:45pm	Stephen Long	
12:45 – 1:05pm	Thomas Vierbuchen	
1:05 – 1:25pm	Yariv Houvras	
1:25 – 1:35pm	***Poster Teaser (10 minutes)***	
1:35 – 2:35pm	Lunch	West Dining Room
2:35 – 4:35pm	Breakout Sessions	Conference House
2:35 – 3:35pm	**Activity 1:** How to Manage your 1st Yr. *(Second Year Students)*	Conference House

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THURSDAY – September 26

TIME	EVENT	DESCRIPTION/LOCATION
3:35 – 4:35pm	**Activity 2:** How to Pass the ACE/2nd Yr. *(Allie Dananberg, Mitchell Martin, Gina Tomarchio)*	Conference House
4:35 – 5:00pm	Check in at desk	Conference House/South Lobby
5:15 – 6:15pm	Meet and Greet the First Year Students *(All are invited)*	Pavilion/Skating Rink
6:15 – 7:45pm	Poster Session/Open Bar ***"Prizes for Best Faculty Judged Poster & People's Choice Award"***	Pavilion/Skating Rink
6:15 – 7:00pm	***Odd Number Posters***	
7:00 – 7:45pm	***Even Number Posters***	
7:55 – 9:25pm	Private Dinner	West Dining Room
9:30 – 11:00pm	Karaoke/Dancing	West Dining Room

FRIDAY – September 27

9:00 – 10:00am	Breakfast	Main Dining Hall
10:00am – 1:15pm	Concurrent Talks	Conference House
	BSB/MB (Rose/Dogwood Room)	**CDB (Azalea Room)**
	Moderators: Kalina Belcheva/ Ting-Wei Hsu	**Moderators:** Yining Jiang/ Xinran Tong
10:05 – 10:25am	Christopher Lima	Matthias Stadtfeld
10:25 – 10:45am	Tanya Schild	Reeti Sanghrajka
10:45 – 11:05am	David Simon	Jingli Cao
11:05 – 11:25am	Xiaolan Zhao	David Rickman

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FRIDAY – September 27

TIME	EVENT	DESCRIPTION/LOCATION
	BSB/MB (Rose/Dogwood Room)	**CDB (Azalea Room)**
	Moderators: Kalina Belcheva/ Ting-Wei Hsu	**Moderators:** Yining Jiang/ Xinran Tong
11:25 – 11:30am	***Coffee Break/Stretch (5 minutes)***	
11:50am – 12:10pm	David Eliezer	Qiao (Joe) Zhao
12:10 – 12:30pm	Bryan Ngo	Yujie Fan
12:50 – 1:10pm	Baran Ersoy	Vidur Garg
1:15 – 2:15pm	Box Lunch	Conference House
2:15pm	First Departure	Estimated time of arrival in NYC (4pm)
7:00pm	Second Departure	Estimated time of arrival in NYC (9pm)

FOR TRANSPORTATION RESERVATIONS

For trips to and from the New Paltz Bus Station or the Poughkeepsie Train Station, please contact the Mohonk Transportation Department at 845.256.2016. For airport service, door-to-door service, or other long-distance trips, please contact ALL Transportation Network at 800.258.9879. You may also call the Mohonk Transportation Department at 845.256.2016 to discuss your long-distance transportation needs. Please make your reservation at least 24 hours in advance.

LONG-DISTANCE TRAVEL

ALL Transportation Network will provide you with luxury transportation in a variety of vehicles including executive sedans, sports utility vehicles, limousines, vans, mini-buses and coaches. ALL Transportation Network chauffeurs are safety certified and are familiar with local and metropolitan areas. Services are available at any location, to and from Mohonk Mountain House. The needs of any size group can be readily accommodated. Please contact ALL Transportation Network at 800.258.9879, or visit ALL's website at www.alltransnet.com, for rates and information. Please make your reservation at least 24 hours in advance.

BILLING

For your convenience, transportation services may be billed to your room. Trips cancelled with less than 24 hours notice will be charged a fee equal to the trip cost. A fee will be charged for each additional pick-up or drop-off (the fee will vary depending on the location of the additional stop). A $65.00 "wait fee" will be charged per hour or portion thereof for extended wait time. For billing questions or pricing, please call the Mohonk Transportation Department at 845.256.2016. Quoted prices will include tolls, parking fees, and driver's gratuity.

Rates are subject to change without notice.

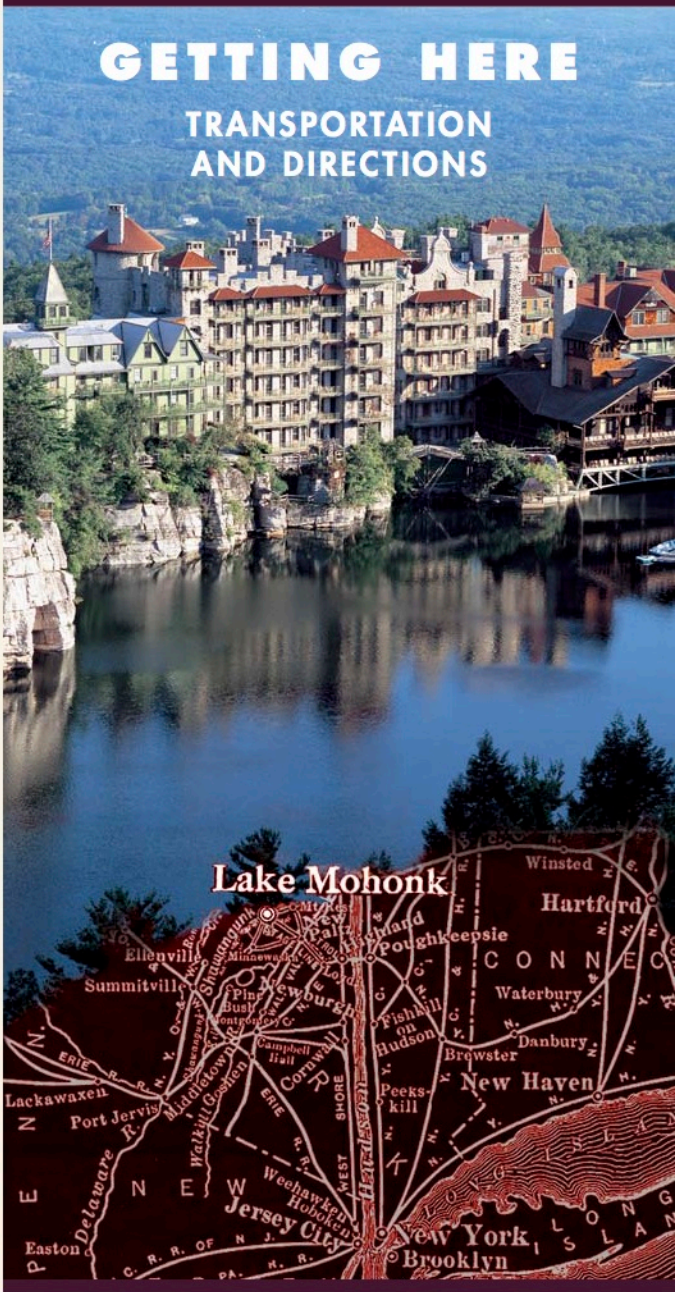
HISTORIC HOTELS of AMERICA
NATIONAL TRUST FOR HISTORIC PRESERVATION

Mohonk Mountain House
A NATIONAL HISTORIC LANDMARK
1000 Mountain Rest Road, New Paltz, NY 12561
www.mohonk.com
3-16

Mohonk Mountain House

GETTING HERE

TRANSPORTATION AND DIRECTIONS



A NATIONAL HISTORIC LANDMARK RESORT

Founded in 1869

www.mohonk.com

DRIVING DIRECTIONS

FROM NEW YORK CITY

West Side

Take West Street (West Side Highway and Henry Hudson Parkway) to the upper level of the George Washington Bridge. From the right-hand lane, turn onto the Palisades Parkway north. Take Exit 9W onto the NYS Thruway (Route 87) north. Exit the NYS Thruway at Exit 18, New Paltz.*

East Side

Take East River Drive (FDR Drive and Harlem River Drive) to the upper level of the George Washington Bridge. From the right-hand lane, turn onto the Palisades Parkway north. Take Exit 9W onto the NYS Thruway (Route 87) north. Exit the NYS Thruway at Exit 18, New Paltz.*

FROM SOUTHERN NEW YORK

East of the Hudson River

Follow main highways to Route 84 west. Follow Route 84 (cross over the Newburgh-Beacon Bridge) to the NYS Thruway. Go north on the NYS Thruway (Route 87) one exit to Exit 18, New Paltz.*

FROM SOUTHERN CONNECTICUT

Follow the New England Thruway (Route 95) west to Cross-Westchester Expressway (Route 287). Continue to the NYS Thruway to Exit 18, New Paltz.*

FROM NEW JERSEY

Follow Route 17 north or the Garden State Parkway north to the NYS Thruway north (Route 87). Take the NYS Thruway north to Exit 18, New Paltz.*

FROM BOSTON AND VICINITY

Follow the Massachusetts Turnpike (Route 90) west to the NYS Thruway. Go south on the NYS Thruway (Route 87) to Exit 18, New Paltz.*

FROM BINGHAMTON AND SOUTHERN CATSKILLS

Take NYS Route 17 to Liberty; then follow NYS Route 52 to Ellenville, and turn left onto U.S. Route 209. Continue on Route 209 north to Route 213 east to High Falls. In High Falls, turn right onto Mohonk Road (County Route 6A), across from the Egg's Nest Restaurant, and proceed five miles to Mohonk gate (on the right).

*FROM THE NYS THRUWAY EXIT 18

After leaving the NYS Thruway at Exit 18, turn left at the traffic light onto Route 299/Main Street. Follow Main Street through New Paltz. Immediately after crossing the Walkill River Bridge, turn right onto Springtown Road (there is a "Mohonk" sign). Bear left after a quarter mile onto Mountain Rest Road. Follow Mountain Rest Road and Mohonk signs to the Mohonk Gatehouse, on the left.

Mohonk is located west of New Paltz, six miles from the NYS Thruway.

BUS AND TRAIN TRAVEL

Bus service is available on Adirondack Trailways to the New Paltz Bus Station. (Please be sure to get off the bus at the New Paltz Bus Station in the middle of town, *not* at the Park and Ride by the Thruway.) Service departs from Port Authority in New York City, Albany Trailways terminal (360 Broadway), Route 17 in Ridgewood, New Jersey, and locations in southern New York and Long Island. Trailways can be reached at 800.858.8555 and online at www.trailwaysny.com.

Train service is available via Metro North and Amtrak trains to the Poughkeepsie Train Station. Metro North leaves from Grand Central in New York City; Amtrak leaves from Penn Station in New York City. Information is available at www.mta.info for Metro North, and www.amtrak.com for Amtrak trains.

BY AIR

Mohonk Mountain House is 25 miles from Stewart International Airport in Newburgh, 70 miles from Albany Airport, and less than 100 miles from New York City area airports.

Plenary Sessions

Stewart Shuman

Lukas Dow

Kristy Brown

Stephen Long

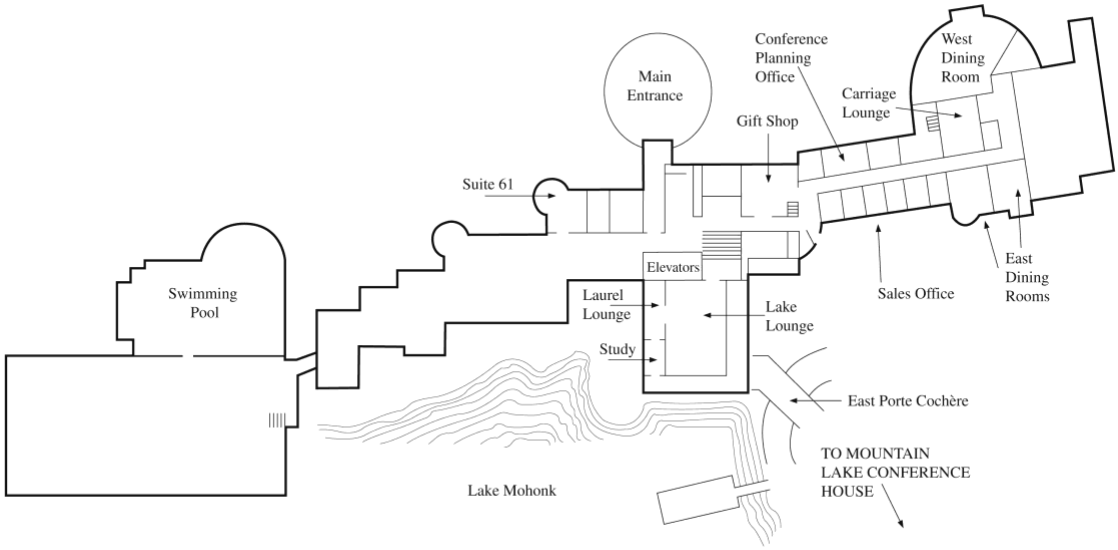
Thomas Vierbuchen

Yariv Houvras

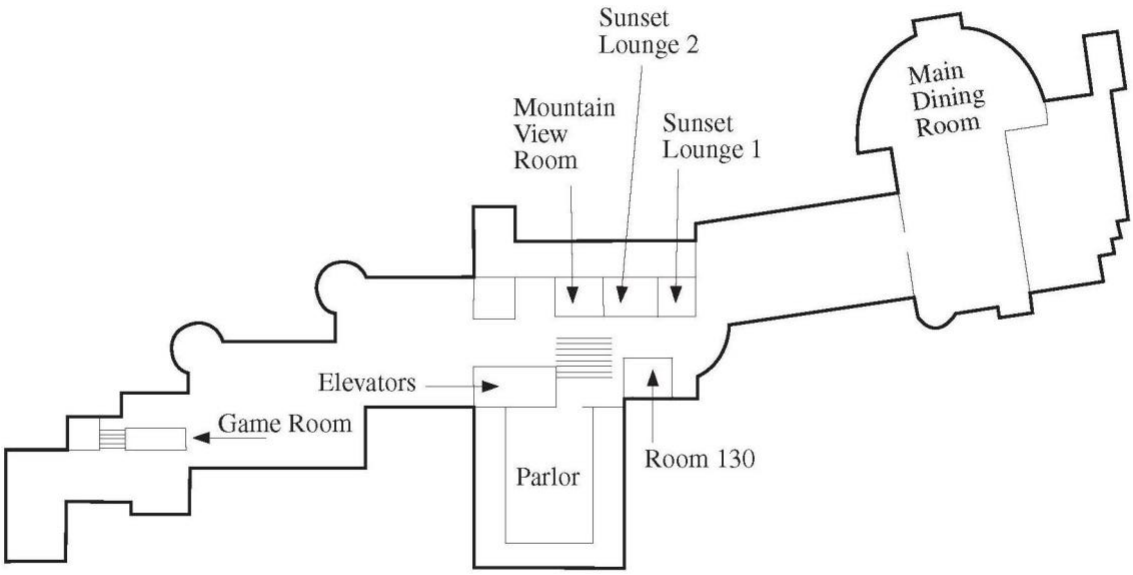
Main Dining Room

Pavilion - Private Dinner

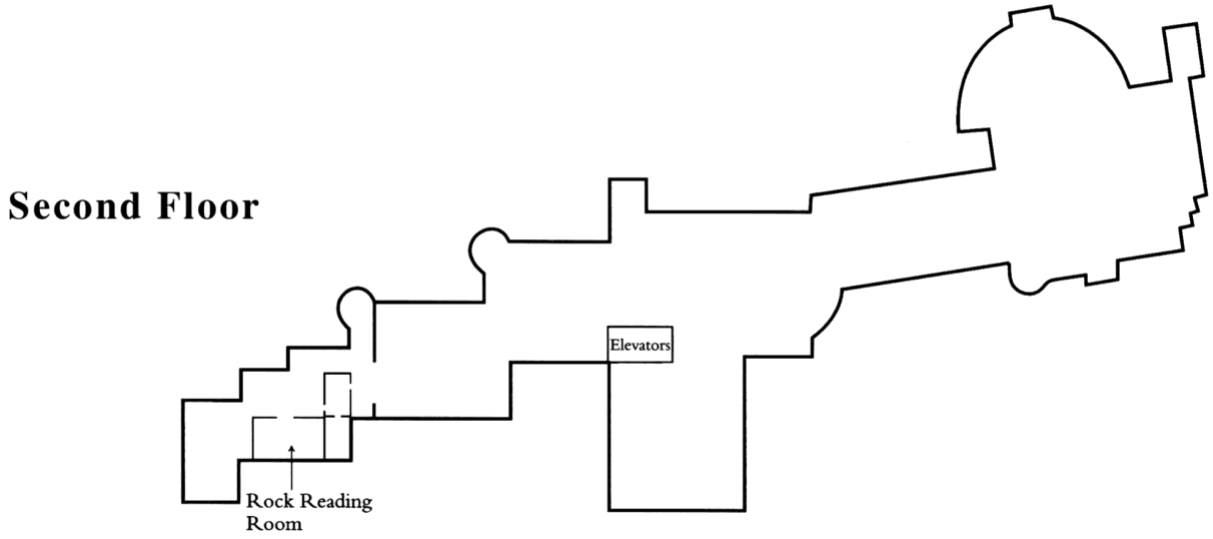
Mohonk Ground Floor Map



Mohonk First Floor Map



Mohonk Second Floor Map



Complimentary Amenities (seasonal availability):

- Golf Green Fees on our 9-hole Course
- Tennis
- Lawn Games
- Rowboats/Paddleboats/Canoes/Kayaks
- 85 Miles of Hiking Trails/Rock Climbing
- House History Tours
- Indoor Pool
- Fitness Center and Daily Fitness Classes
- Afternoon Tea and Cookies
- Ice Skating
- and many more

Golf

Indoor Pool

Archery

Tomahawk Throwing

Hiking

Canoeing

Rock Scramble

Additional Amenities (fees apply):

- Award-winning Spa
- Mountain Biking
- Rock Climbing
- Horseback Riding
- Carriage Rides

Spa

Guided Horseback Rides

Mountain Biking

Guided Hikes

Structure of the mycobacterial helicase-nuclease AdnAB: new insights into unidirectional translocation and Double-Strand Break end resection

Mihaela Unciuleac, Ning Jia, Dinshaw Patel, Stewart Shuman

Current models of bacterial homologous recombination posit that extensive resection of a DNA double-strand break (DSB) by a multi-subunit helicase-nuclease machine generates the 3' single-strand DNA substrate for RecA-mediated strand invasion. AdnAB, the helicase-nuclease agent of mycobacterial HR, consists of two subunits, AdnA (1045-aa) and AdnB (1095-aa), each composed of an N-terminal ATPase domain and a C-terminal nuclease domain. DSB unwinding by AdnAB depends on the ATPase activity of the "lead" AdnB motor translocating on the 3' ssDNA strand, but not on the "lagging" AdnA ATPase. It is proposed that translocation drives the 3' ssDNA strand through the AdnA motor and into the AdnB nuclease domain and that unwinding threads the 5' ssDNA strand into the AdnA nuclease domain.

To better understand how AdnAB works, we obtained cryo-EM structures of AdnAB in three functional states: as apoenzyme and in complex with forked duplex DNAs before and after cleavage of the 5' ssDNA tail by the AdnA nuclease. The structures reveal the path of the 5' ssDNA through the AdnA nuclease domain and the mechanism of 5' strand cleavage; the path of the 3' tracking strand through the AdnB motor and the DNA contacts that couple ATP hydrolysis to mechanical work; and the position of the AdnA iron-sulfur cluster subdomain at the Y-junction and its likely role in maintaining the split trajectories of the unwound 5' and 3' strands.

The genetics of tumor progression, response, and resistance to therapy

Lukas Dow

The past decade has produced a vast amount of clinical sequencing data that has comprehensively defined the genetic landscape of human cancers. We know with greater detail than ever, the number and type of mutation in any given gene, in a wide array of tumor types. Yet, despite this thorough catalogue of events, we have a very limited understanding of *how* this mutational heterogeneity impacts cancer initiation, progression, or therapeutic response. Thus, despite the enormous resources invested in genome sequencing, the vast amount of information gathered is uninformative as we simply do not know how most cancer-associated mutations dictate tumor behavior. Even extremely well-studied genes such as *KRAS* and *APC* harbor an array of mutational variants that may have significant prognostic and therapeutic relevance, but remain scarcely characterized. Further complicating the challenge is the number of mutational combinations that impact cancer behavior. To meet this challenge, my lab develops and exploits new genetic technologies to engineer organoid and animal model systems that faithfully represent the mutational events in human cancer. We use these tools to determine how specific mutational variants in major oncogenic pathways shape cancer progression, and influence cancer cell vulnerabilities and response to therapy. I will describe our efforts to understand the contribution of recurrent alterations in APC and KRAS, as well as our ongoing work to prospectively define mechanism of response and resistance to new WNT-targeted therapies now entering clinical trials.

Discovery of the unacylated ghrelin receptor: Implications for cancer treatment

CheukMan C. Au, John B. Furness, Kara Britt, Sofya Oshchepkova, Heta Ladumor, Kai Ying Soo, Brid Callaghan, Céline Gérard Giorgio Inghirami, Vivek Mittal, Yufeng Wang, Xin-Yun Huang, Jason A. Spector, Eleni Andreopoulou, Paul Zumbo, Doron Betel, Lukas E. Dow, Kristy A. Brown

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Breast cancer is the most common cancer in women. Despite significant advances, current treatments are associated with severe side effects and are not always effective. Ghrelin is a gut-derived hormone that stimulates appetite and growth hormone release. The unacylated form of ghrelin (UAG) does not bind to the cognate ghrelin receptor, GHSR1a, yet plays an important role in the regulation of energy homeostasis. Little is known of the mechanism of action of UAG at yet-to-be-identified UAG receptors. Previous work from our lab aimed to characterize effects of UAG on breast cancer cells and explore the therapeutic potential of UAG or analog AZP531. We demonstrate potent (pM) effects of UAG to suppress the growth of breast cancer cells, dependent on 3D culture and activation of Gαi. Suppression of MAPK signaling by UAG leads to cell cycle arrest and apoptosis, and accounts for resistance of cells carrying BRAF or KRAS mutations, or patient-derived breast cancer cells with activated MAPK signaling. AZP531, in clinical trials for Prader-Willi Syndrome, also suppresses the growth of breast cancer cells in vitro and in vivo. We have filed a patent related to this work. A CRISPR library screen was performed and a number of putative receptors identified. Going forward, we aim to identify the UAG receptor, characterize its function in a 3D organoid setting and initiate a clinical study in women with breast cancer. The identification of the UAG receptor will also lead to efforts to develop small molecules for the safe and effective treatment of breast cancer

Mechanisms of activation and inhibition in the ion channel bestrophin.

George Vaisey, Alexandria Miller, Stephen Long

The chloride channel bestrophin (BEST1) is activated directly by calcium and inhibited by peptide binding to a surface receptor. Mutations in BEST1 cause eye disease. Through a combination of electrophysiology and structural biology (cryo-EM and X-ray crystallography), graduate student George Vaisey and Alexandria Miller have discovered the mechanisms of gating and inhibition.

Their surprising findings, which point to novel physiological roles for BEST1, will be discussed along with possible future directions.

Genetic and Genomic Approaches to Deconstruct Enhancer Function

Emi Ling, Marty Yang, Christopher Cowley, Michael E. Greenberg, Thomas Vierbuchen

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Embryonic development requires the carefully orchestrated differentiation of thousands of cell types from the same set of genetic instructions. Enhancer elements are genomic regulatory sequences that direct the selective expression of genes so that genetically identical cells can differentiate and acquire highly specialized forms and functions. However, the mechanisms by which the DNA sequence of an enhancer ultimately controls its cell type-specific transcriptional regulatory activity remains incompletely understood. To approach this problem, we generated allele-specific maps of TF binding (AP-1, Tead1, CTCF), and chromatin state (H3K4me1/3, H3K27ac, ATAC-seq) from F1 hybrid fibroblast cultures derived from crosses between C57Bl/6J and four genetically divergent inbred strains (PWK/PhJ, MOLF/Ei, CAST/Ei, SPRET/Ei). This experimental design allowed us to compare the function and TF binding status of two closely related enhancer alleles in the exact same cell, and thus to identify thousands of instances in which subtle changes in enhancer sequence prevents TF binding and enhancer selection/activation. This rich dataset allowed us to examine hierarchical and collaborative relationships between TFs that work together to select enhancers from naïve chromatin. Our long-term interest is to understand transcriptional regulatory control of cell fate specification and differentiation in the mouse and human forebrain. In ongoing experiments, we are developing a panel of diverse F1 hybrid mouse ES cells and novel single cell epigenomic profiling techniques so that we can scale this approach to examine mechanisms of enhancer function in the developing brain using directed differentiation of pluripotent stem cells.

Zebrafish

Yariv Houvras

Zebrafish (Danio rerio) are a small freshwater species of vertebrate fish, commonly known to children from visiting pet stores. Although they are the most unremarkable fish in the pet store, they are the most remarkable vertebrate fish species to contribute new knowledge to biology. Although zebrafish science is a relatively young field, this organism has captivated scientists for its remarkable attributes in experimental biology. Zebrafish are highly fecund, their embryos are completely translucent, and the first cell of life is slow to divide. This slowly dividing first cell of life affords scientists with an opportunity to stick a needle in that cell and introduce a variety of different molecules including DNA, RNA, and proteins. Cancer research using zebrafish as a model organism is even younger than new BCMB students (!), yet in its short lifespan it has already made significant contributions to our understanding of cancer biology and genetics. In work from our lab I will highlight new cancer models we have created based on patients treated at Cornell. These zebrafish avatars of individual cancer patients are allowing us to explore strategies to bypass resistance to kinase inhibitors. I will also highlight our efforts to improve the efficiency of CRISPR/Cas9 genome editing in zebrafish. We are now able to read, write, and erase the zebrafish genome more or less like editing a word processor document. These new capabilities were essentially unimaginable a decade ago and are revolutionizing the ability of zebrafish to tackle problems in biomedical science.

Poster Session

First Name	Last Name	I am a	Poster #
Nohely	Abreu	BCMB/PhD Student	1
Abderhman	Abuhashem	BCMB MD/PhD Student	2
Amanda	Acosta	BCMB/PhD Student	3
Rhiannon	Aguilar	BCMB MD/PhD Student	8
Lissenya	Argueta	Non BCMB/PhD Student (IMP)	5
Seyeon	Bae	BCMB/Postdoc	31
Alyssa	Bagadion	BCMB/PhD Student	36
Maria Graciela	Cascio	BCMB/Postdoc	19
Emily	Costa	BCMB/PhD Student	12
Allie	Dananberg	BCMB/PhD Student	7
Tapojyoti	Das	BCMB/PhD Student	10
Maria	Falzone	BCMB/PhD Student	14
Sara	Haddock	BCMB/PhD Student	18
Pei-Ching	Huang	BCMB/PhD Student	13
Helene	Jahn	BCMB/PhD Student	11
Alyna	Katti	BCMB/PhD Student	16
Christina	Kling	BCMB/PhD Student	23
Hye In	Lee	BCMB/PhD Student	4
Mitchell	Martin	BCMB/PhD Student	15
Carolyn	Maskin	BCMB/PhD Student	21
Andrew	Minotti	BCMB/PhD Student	24
Pooja	Naik	BCMB/PhD Student	6
Leona	Nease	Non BCMB/PhD Student (PHARM)	25
Christopher	Noetzel	BCMB/PhD Student	17
Jonathan	Pai	BCMB/PhD Student	27
Elisa	Sanchez	BCMB/PhD Student	22
Avital	Shulman	BCMB/PhD Student	34
Gina	Tomarchio	BCMB/PhD Student	20
Joshua	Weiss	BCMB MD/PhD Student	26
Nicole	Weiss	Minkui Luo	35
Iestyn	Whitehouse	BCMB Faculty Member	28
Jiaqi	Xu	BCMB/PhD Student	30
Jenny	Xue	BCMB MD/PhD Student	29
Nevin	Yusufova	BCMB/PhD Student	9
Carolina	Zapater i Morales	BCMB/PhD Student	32
Sylvia	Zohrabian	BCMB/PhD Student	33

Defining the distinct regulation of metabotropic glutamate receptors 2 and 3

Nohely Abreu and Joshua Levitz

Precise regulation of glutamatergic signaling is imperative for normal brain function. In particular, metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors (GPCRs) that fine-tune synaptic transmission by initiating intracellular signaling cascades that lead to short and long-term forms of plasticity. Of particular interest are mGluR2 and mGluR3, which have 70% sequence identity, both couple to the $G_{i/o}$ pathway, and are commonly known as presynaptic autoreceptors that inhibit glutamate release. Accordingly, precise regulation of mGluR2/3 signaling is important to preserve synaptic function. GPCR signaling is typically regulated by phosphorylation of the C-terminal domain (CTD) by a GPCR kinase, subsequent β-arrestin binding and endocytosis of the receptor to prevent excessive surface signaling. Studies aiming to define the differential regulation of mGluR2/3 have been limited due to the lack of receptor subtype-specific drugs, antibodies, and methods with poor spatial resolution. We sought to determine whether mGluR2 and mGluR3 have distinct regulation by using a heterologous system to express SNAP-tagged mGluRs and specifically track surface receptor population. We found that unlike mGluR2, mGluR3 couples to β-arrestin and is endocytosed following stimulation with glutamate. Further analysis of the CTD revealed that mGluR3 has "phosphorylation codes" for optimal β-arrestin binding, and inserting these codes into the CTD of mGluR2 allows it to couple to β-arrestin. Moreover, we discovered that a melanoma-associated mutation in a mGluR3 phosphorylation code enhances β-arrestin binding. These results provide insight into the mechanism of β-arrestin coupling, and differential regulation between mGluR2 and mGluR3 suggests that they serve different functions at the synapse.

Dynamic spatial coupling between key lineage factors in the pre-implantation mouse embryo.

Abderhman A. Abuhashem, Gianna Mincone, Anna-Katerina Hadjantonakis

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Transcription factors play integral roles in determining cell identity. Competition and cooperativity between multiple transcription factors have been studied using genomic tools. However, the physical relationship between these factors in the nuclear 3D space during cooperativity and competition has been largely unknown. Here, we utilize high-resolution, single-cell based quantitative imaging to assess the spatial relationship between key lineage transcription factors using the pre-implantation mammalian embryo as a paradigm. During pre-implantation mammalian development, the unspecified inner cells mass (ICM) in the blastocyst expresses two key transcription factors: NANOG and GATA6. As the ICM specifies into Epiblast and Primitive Endoderm, the cells lose expression of GATA6 or NANOG, respectively, and maintain the other. We deployed high-resolution deconvolution microscopy to image single cells within the ICM during cell fate assignment into Epiblast and Primitive Endoderm. Subsequently, we utilized Statistical Object Distance Analysis (SODA), a recently developed algorithm to detect transcription factors puncta and analyze distances between them in 3D, to dissect the spatial relationship between NANOG and GATA6 during the ICM specification. The results show a previously unappreciated and dynamic spatial coupling between NANOG and GATA6 during the cell fate decision process of the ICM. Additionally, distinct coupling patterns may be associated with Epiblasts vs. Primitive Endoderm specification trajectories. Overall, our analysis offers a new perspective into understanding global transcription factors physical interactions at single-cell resolution *in vivo*.

Optogenetic Control of mGluR5 to Probe Spatiotemporal Signaling Dynamics

Amanda Acosta-Ruiz, Vanessa A. Gutzeit, Mary Jane Skelly, Samantha Meadows, Joon Lee, Puja Parekh, Anna G. Orr, Conor Liston, Kristen E. Pleil, Johannes Broichhagen, Joshua Levitz

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G protein-coupled receptors (GPCRs) control many cellular processes largely through the modulation of secondary messengers. G_q-coupled GPCRs, for example, drive the release of intracellular Ca^{2+} stores into the cytoplasm. One particular G_q-coupled receptor, metabotropic glutamate receptor 5 (mGluR5), is highly expressed in the central nervous system and drives long term forms of synaptic plasticity by initiating unique Ca^{2+} oscillations when activated by the major excitatory neurotransmitter glutamate. However, the spatiotemporal properties, mechanisms of regulation, and functional consequences of these Ca^{2+} oscillations remain unclear and limit our understanding of mGluR5 function in both neurons and astrocytes. To overcome the limitations of classical pharmacological techniques, we have developed photoswitchable tethered ligands that bind to tagged receptors to provide both genetic targeting and precise spatiotemporal control of receptor activation. These tools were first developed and characterized in HEK 293T cells where we probe the determinants of calcium oscillation frequency. In astrocytes, we used the spatial precision of this tool to identify unique Ca^{2+} dynamics based on whether mGluR5 activation was targeted to processes or the soma. Future work will employ these tools to dissect the dynamics of mGluR5 signaling within dendritic spines of hippocampal synapses, where mGluR5 is known to drive long term synaptic plasticity.

L-2-Hydroxyglutarate (L-2HG) maintains stemness in Hematopoietic Stem/Progenitor Cells

Sylvia Zohrabian

Somatic mutations in isocitrate dehydrogenase (IDH) enzymes are prevalent in acute myeloid leukemia (AML) and a variety of other cancers. IDH enzymes normally catalyze the NADP(H)-dependent interconversion of isocitrate and alpha-ketoglutarate (αKG). Mutant IDH enzymes lose wildtype activity and concomitantly gain the ability to efficiently catalyze reduction of αKG to the 'oncometabolite' D-2-hydroxyglutarate (D-2HG). D-2HG inhibits >70 different αKG-dependent enzymes that regulate chromatin modifications, stability of hypoxia inducible factor (HIF), and DNA repair, leading to impaired differentiation and a stem-like phenotype in IDH-mutant cells. Although cancer-associated IDH mutants specifically produce D-2HG, our research group discovered that normal and malignant cells without IDH mutations produce the mirror-image enantiomer L-2HG in response to hypoxia and decreased pH. Strikingly, L-2HG can function as a ~10-fold more potent inhibitor of many αKG-dependent enzymes. However, the biologic functions of L-2HG remain poorly understood. Our preliminary data demonstrate that L-2HG stabilizes HIF, induces repressive chromatin marks, and blocks differentiation of hematopoietic stem/progenitor cells (HSPCs). These findings suggest that L-2HG may account, at least in part, for the importance of hypoxic niches in maintaining the self-renewal of hematopoietic stem/progenitor (HSPCs). Therefore, the effect of L-2HG in hematopoietic cells was analyze in normal and stress hematopoiesis.

EGFL7 signaling in embryonic endothelium

Lissenya Argueta

Epidermal growth factor-like domain 7 (Egfl7) is an endothelial-restricted gene that encodes a secreted angiogenic factor. Functional studies in zebrafish and mice have shown that it is important during angiogenesis. Egfl7 is expressed during vasculogenesis and active angiogenesis. Secreted EGFL7 can act as a chemoattractant and has been shown to interact with the ECM proteins. To date, no cognate receptor has been found for EGFL7 and furthermore how EGFL7 signals within embryonic endothelium remains unclear. Previously, our lab has shown that EGFL7 functions as a Notch ligand to signal in endothelial cells. Despite EGFL7 containing two EGF-like domains, there is no evidence for its direct interaction with the EGFR. Embryonic and placental endothelial cell (EC) lines were generated in the lab by introducing a constitutively active myr-Akt in isolated primary EC cultures.

Preliminary *in vitro* studies indicate that EGFL7 signals indirectly through the EGFR and that this signal is mediated through ADAM17. To understand this indirect signaling mechanism, western blots to probe for the phosphorylation state of the EGFR and wound scratch assays to observe cell migration, under varying conditions, are being performed. Additionally, the expression of Egfl7 temporally and spatially aligns with endothelial-to-hematopoietic transition (EHT). Therefore, we are also investigating the role of Egfl7 in EHT. Together these studies will provide novel insight into how Egfl7 signals within the embryonic endothelium.

Patterned cell-surface receptors direct planar polarity during epithelial remodeling

Pooja Naik, Adam C. Paré, Jay Shi, Zachary Mirman, Karl H. Palmquist and Jennifer A. Zallen

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In the developing embryo, cells in the epithelia undergo highly coordinated changes in shape and position to form functional tissues and organs. These changes occur in response to signals which are conveyed by extracellular cell-surface receptors. During Drosophila convergent extension, the germband converges along the dorso-ventral axis and extends to almost twice its length along the anterior-posterior axis. This is a result of actomyosin contractility, by which localized cell behaviors cause elongation on a global scale. Tissue-level positional information for anterior-posterior patterning is provided by transcriptional factors such as Eve and Runt, but the cell-surface receptors which translate these gene expression patterns into organized cell behaviors were only recently discovered. Three Toll receptors were found to direct planar polarized cell behaviors which lead to elongation along the anterior-posterior axis. Now we show that the activity of Toll receptors is localized to specific columns of cells across the embryo, and other cell columns (at epithelial boundaries) still show residual polarity in the absence of Toll receptors. We found a new receptor system involving the leucine-rich receptor Tartan and its novel ligand which work together to direct planar polarity at epithelial boundaries. This new system works in parallel with the Toll receptor system to provide a high-resolution network of positional information which organizes cell behaviors in the epithelia.

Investigating the mechanism of episodic APOBEC3A mutagenesis

Allie Dananberg, Kevan Chu, John Maciejowski

Mutations arise in cancer genomes as a result of exogenous and endogenous sources. The anti-viral APOBEC3 (A3) family of cytosine deaminases is proposed to be a major endogenous source of mutagenesis in at least 22 cancer types. Using novel cancer cell line models and whole genome sequencing, we demonstrate that the A3A paralog is a source of mutations in breast cancer. A3A mutagenesis occurs in episodic bursts, however, the factors that trigger A3A activity, including the initiating factors for the bursts, are unclear. We hypothesize that episodic mutations are caused by transient, transcriptional upregulation of A3A in small subsets of cells. To test this hypothesis, I am developing monoclonal antibodies and A3A knock-in cell lines to assess A3A expression in a panel of breast cancer cell lines. I will also use single-cell RNA sequencing to query A3A expression and identify correlates that may trigger A3A expression. Finally, given A3A's canonical role in viral restriction, I will test the hypothesis that A3A transcriptional activation is caused by the engagement of anti-viral nucleic acid sensors in the cytosol with cancer-intrinsic cytosolic DNA species Collectively, this work will provide a mechanistic understanding of the factors that drive episodic A3A mutagenesis.

Analysis of chromatin assembly after DNA synthesis by CAF-1 in yeast

Rhiannon Aguilar, Mair Churchill, Jessica Tyler

Genomic processes are tightly regulated by the packaging of our DNA into chromatin. During every cell division, the newly-replicated genome must be rapidly assembled with histones, restoring the chromatin structure and reinstating regulation of genomic activities. Chromatin Assembly Factor 1 (CAF-1) is a histone chaperone that assembles new H3/H4 tetramers onto the DNA (tetrasomes) during replication and repair. Despite the vital role of CAF-1 in chromatin assembly, much of its structure and its mechanism of tetrasome assembly are still poorly understood. We are performing a structure-function analysis of CAF-1, combining *in vitro* structural and biophysical data with *in vivo* functional and phenotypic assays in *S. cerevisiae*. We are particularly interested in the DNA-binding regions of CAF-1's large subunit that have been shown to act cooperatively *in vitro* (Sauer et. al., *NAR 2018*), and how mutation of these regions affects the function of CAF-1 *in vivo*. We additionally will investigate how CAF-1's mechanism of tetrasome assembly relates to its DNA binding. It has been shown that full-length CAF-1 prefers to bind 80+bp pieces of DNA, markedly longer than the activity of the DNA-binding Winged-Helix Domain on its own (Sauer et. al., *NAR 2018*). These studies will make significant new contributions to current knowledge about the mechanism of CAF-1, the majority of which has been carried out only *in vitro*. Discoveries about the mechanism of this critical histone chaperone will contribute toward a unified understanding of how the epigenome is duplicated every cell division to ensure appropriate regulation of genomic processes.

Linker Histone Mutations Mediate Lymphomagenesis Through a Novel Chromatin Mechanism

Nevin Yusufova, Matthew R. Teater, Alexey A. Soshnev, Eftychia Apostolou, C. David Allis, Ethel Cesarman, Ari M. Melnick

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Somatic mutations in linker histone genes are identified in up to 30% of follicular and diffuse large B cell lymphomas. Taking advantage of the available H1c/e knock-out mouse model, we find that loss of H1 results in increased germinal center hyperplasia upon SRBC immunization by flow and PNA immunohistochemistry. Transcriptional analyses in sorted germinal center B cells reveal striking upregulation of genes typically repressed by Polycomb Repressive Complex, leading to the activation of canonical stem cell TFs (e.g. KLF4) and ESC and tumor stem cell signatures. To identify potential chromatin effects, we performed histone quantitative mass spectrometry and determined significant reduction of H3K27me3 and gain of H3K36me2 in H1c/e-/- GC B cells, consistent with upregulation of H3K27me3 silenced genes observed by RNA-seq. This was further validated on genome level by ChIP-seq-Rx, including upregulated genes such as Tet1 and Prdm5. To determine if a similar signature could be observed in primary human lymphomas, we analyzed a cohort of 322 DLBCLs and compared H1C/E mutant vs non-H1 mutant DLBCLs. Indeed, GSEA yielded significant enrichment for upregulation of the genes identified in the mouse H1c/e$^{-/-}$ GC B-cells as well as hematopoietic H3K27me3 signatures and PRC2 target genes. To determine whether H1C/E deficiency speeds lymphoma development, we crossed H1c/e$^{-/-}$ mice with the *VavPBcl2* strain. We observed shorter survival and acceleration of lymphoma disease in the VavPBcl2-H1c/e$^{+/-}$ mice. Together, these studies investigate the effects of H1 mutations, including role in regulation of transcriptional programs in B cell differentiation, reactivation of stem cell programs and malignancy.

Structure-function correlation of Parkinson's disease protein alpha-synuclein

Tapojyoti Das, Meraj Ramezani, Marcus Wilkes, David Holowka, Barbara Baird and David Eliezer

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Alpha-synuclein (aSyn), the principal protein forming toxic intraneuronal aggregates in Parkinson's disease, has a poorly defined physiological function related to regulation of neurotransmitter release. aSyn, a 140-residue protein, is classified as intrinsically disordered in solution. In presence of membranes, the N-terminal ~100 residues bind to membranes, whereas the C-terminal ~40 residues remain intrinsically disordered. Furthermore, the membrane-binding domain interconverts between an extended helix and a broken helix conformation (N-terminal Helix-1 and C-terminal Helix-2) on the surface of synaptic vesicle mimics, such as artificial lipid small uniform vesicles (SUVs) and detergent micelles, respectively. However, the correlation between the various observed conformational states and physiological function is less clear. Using an exocytosis inhibition assay in RBL-2H3 cells, we find that wild-type aSyn switches from an inhibitor of vesicle secretion at low expression level to a potentiator at a higher expression level, with a corresponding redistribution of vesicles from the perinuclear region to the plasma membrane-proximal zone. Furthermore, using solution-state nuclear magnetic resonance methods, we find that a perturbation in Helix-2 results in disruption of micelle-bound broken-helix conformation and loss of its exocytosis inhibitory function. In contrast, perturbation in Helix-1 results in an intact broken-helix structure on micelle binding and globally reduced SUV binding. Using our functional assay, this mutant shows inhibition of exocytosis at both low and high expression levels. Taken together, this suggests that the broken helix conformation is responsible for the exocytosis inhibitory function of aSyn, possibly by bridging two membranes at the vesicle-plasma membrane junction. In contrast, the extended helix conformation is relevant for exocytosis-potentiating function, possibly by binding to isolated vesicles causing de-aggregation of vesicle clusters and corresponding redistribution of vesicle pools.

Intra-Mitochondrial Lipid Transport – Phospholipid Flip-Flop Across Mitochondrial Membranes

H. Jahn, J.C.M. Holthuis, A.K. Menon

Mitochondria play crucial roles in cells, notably in cellular respiration. They have an outer and inner membrane (OM, IM), whose precise lipid compositions are critical. Most lipids are imported into the mitochondria from the endoplasmic reticulum (ER), requiring lipid transport events that are incompletely understood. The transbilayer movement (flip-flop) of lipids across the OM and IM is essential to this delivery system and presumably promoted by membrane proteins. As there are only a few reports about lipid flip-flop in the mitochondrial membranes, I will first characterize these processes in OM vesicles and mitoplasts using natural phospholipids and fluorescent lipid analogs, the latter offering better time resolution. Next, I will employ reconstitution-based approaches to identify the lipid flip-flop machineries of the OM and IM. A molecular dynamics study suggested that lipid flipping across the OM is facilitated by the Voltage-Dependent Anion Channel (VDAC). To test this idea, I reconstituted purified VDAC proteins into liposomes containing fluorescent phospholipid reporters and assayed flip-flop by monitoring the change in fluorescence upon extraction of the reporters by serum albumin. The experiments revealed that VDAC is able to scramble phosphatidylcholine. I propose next to validate this result in a yeast cell-based assay that provides a measure of lipid transport across the OM and into mitochondria. To identify the lipid machinery of the IM, I will employ proteomics derived data to generate a list of candidate proteins and validate their activity as described. My results will finally identify the lipid flip-flop machineries of the mitochondria.

Direct genome editing of patient-derived xenografts using CRISPR-Cas9 enables rapid *in vivo* functional genomics

Emily A. Costa, Christopher H. Hulton, Nisargbhai S. Shah, Álvaro Quintanal-Villalonga, Glenn Heller, Elisa de Stanchina, Charles M. Rudin, John T. Poirier

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Patient-derived xenograft (PDX) models are valuable tools for cancer research and are used extensively in preclinical studies. These models are advantageous over cell lines and cell line xenografts in this setting as they better maintain the intratumoral heterogeneity and histologic characteristics of primary tumors. However, CRISPR-Cas9 genome editing is rarely implemented in PDXs because poor transduction efficiency and requisite *in vivo* growth necessitate a high titer lentiviral vector and non-antibiotic selection methods. To overcome these limitations, we created an all-in-one doxycycline inducible Cas9 expression lentiviral vector with a constitutively expressed truncated CD4 epitope (pSpCTRE-CD4), enabling flow cytometry or magnetic bead enrichment of transduced PDX cells while avoiding prolonged *ex vivo* culture. Cas9 expression from pSpCTRE-CD4 is tightly regulated by doxycycline and produces editing as efficiently as constitutive Cas9 expression. We transduced 14 lung cancer PDXs with pSpCTRE lentivirus and successfully enriched five pSpCTRE-PDX models derived from multiple lung cancer subtypes. To functionally validate Cas9 expression and *in vivo* editing by pSpCTRE, we designed a clonal competition assay wherein the abundance of cells containing a targeting sgRNA are measured relative to cells containing a non-targeting sgRNA within a single tumor. In a KRAS mutant adenocarcinoma PDX, we observed Cas9 expression and significant depletion of cells containing a lethal sgRNA only in mice treated with doxycycline. Additionally, we used pSpCTRE in tandem with an AAV sgRNA vector containing a homology-directed repair template to introduce $EGFR^{C797S}$ point mutations and generate 3rd generation EGFR inhibitor resistance in a MEK-amplified lung adenocarcinoma PDX.

Role of Fun30 in double-strand break resection in meiosis

Pei-Ching Huang, Hajime Murakami, Eleni P. Mimitou, Shintaro Yamada, Scott Keeney

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Meiotic recombination initiated by Spo11-induced DSBs is required for proper segregation of homologous chromosomes. DSBs are exonucleolytically processed by Exo1 to generate 3′ single-stranded DNA (ssDNA) tails that invade the homologous chromosome, eventually leading to formation of a physical connection between the two chromosomes. How chromatin is altered in the vicinity of DSBs and how the resection machinery navigates through chromatin are still unresolved questions.

In a recent study, a sequencing assay (S1-seq) was developed to finely map meiotic resection endpoints by removing the long ssDNA tails with S1 endonuclease. S1-seq analysis of all natural hotspots in the yeast *Saccharomyces cerevisiae* exhibits heterogeneous lengths of resection tracts with reads showing peaks and valleys; overlay of S1-seq and nucleosome occupancy maps shows that the non-randomness of S1 signal is in register with nucleosome signal. These findings suggest that nucleosomes serve as a barrier to Exo1 and that chromatin structure affects resection endpoint distribution. However, computational modeling suggested that the speed of Exo1 *in vivo* is similar to degrading naked DNA *in vitro* and further indicated that Exo1 degrades the substrate with initially high processivity. These results create a paradox: Exo1 appears to stop preferentially at nucleosome boundaries *in vivo* and *in vitro*, yet it also appears to resect through several nucleosomes' worth of chromatin as if there's no barrier at all. Here, we test the hypothesis that nucleosomes are destabilized prior to resection and that chromatin remodelers slide or evict nucleosomes near DSBs. Preliminary data suggest that the ATPase Fun30 is involved in facilitating meiotic resection. Also, combining *fun30Δ* with an exonuclease-dead allele of *EXO1 (exo1-nd)* causes more severe resection defets than *fun30Δ* or *exo1-nd* single mutants. Epistasis analysis suggests that Fun30 might regulate resection at a step prior to Exo1-mediated resection, presumably Mre11/Sae2 nicking. Experimental progress and future plans will be presented

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Structural basis of lipid and ion transport by TMEM16 scramblases

Maria Falzone, George Khelashvili, Xiaolu Cheng, Byoung-Cheol Lee, Jan Rheinberger, Ashleigh Raczkowski, Edward Eng, Crina Nimigean, Harel Weinstein, Alessio Accardi

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The plasma membrane of eukaryotic cells is asymmetric, with polar and charged lipids sequestered to the inner leaflet at rest. Activation of phospholipid scramblases causes the collapse of this asymmetry and externalization of phosphatidylserine lipids. As a result, extracellular signaling networks, controlling processes such as apoptosis, blood coagulation, membrane fusion and repair, are activated. The TMEM16 family of membrane proteins includes phospholipid scramblases and Cl^- channels, all of which are Ca^{2+}-dependent. Many scramblase family members also have non-selective ion channel activity, the physiological relevance of which is unknown. The mechanisms underlying Ca^{2+}-dependent gating of the TMEM16 scramblases/non-selective channels remains poorly understood. Here we describe cryo-electron microscopy structures of a fungal scramblase/non-selective channel from *Aspergillus fumigatus*, afTMEM16, reconstituted in lipid nanodiscs in the presence and absence of Ca^{2+}. Differences between these two states reveal that Ca^{2+} binding induces an opening of the lipid permeation pathway by way of rearrangements of TM4 and TM6. Molecular dynamics simulations of the fungal nhTMEM16 scramblase/channel suggest that the lipid pathway can exist in multiple conformations, and that the insertion of lipid tails within the pathway favor its spontaneous closure by destabilizing a hydrophobic lock between TM4 with TM3. Parallel *in silico* and *in vitro* mutagenesis experiments show that destabilization of the TM3/TM4 hydrophobic lock via point mutations stabilize a novel conformation of the pathway that is permissive to ion movement but not to lipid transport. Indeed, the cryo-EM structure of a channel-only mutant shows a pathway that is closed to the membrane but sufficiently wide to accommodate ion passage.

GO: A functional base editing activated "Gene On-switch"

Alyna Katti, Miguel Foronda, Jill Zimmerman, Lukas Dow

Base editing is a powerful tool for engineering single nucleotide variants (SNVs) and has been used to create targeted mutations in cell lines, organoids, and *in vivo* models. Recent development of new base editing (BE) enzymes has provided an extensive toolkit for genome modification; however, little has been done to directly compare the efficiency, targeting range, and specificity of different editors. Here we report a "Gene On-switch" system that detects and reports base editing activity in situ with high sensitivity and specificity. We generate a collection of optimized high-fidelity and PAM variant enzymes and define the kinetics, efficiency, and fidelity of base editing variants using GO reporters in mouse and human cell lines as well as organoids. Further, the GO system is highly generalizable in its ability to report base editing activity by a wide variety of markers and functional proteins.

We demonstrate a diversity of applications to which GO can be employed including enriching edits at endogenous loci and linking base editing to activation of functional proteins including cre recombinase. GO has the power to quantitatively report and compare editing efficiencies and functionally tether base editing to enzymatic activities with numerous applications.

Exploring the role of Jumonji Histone Demethylases in *P. falciparum* blood stages

Christopher Nötzel, Xinran Tong, Krista A. Matthews, Elisabeth Martinez, Björn F.C. Kafsack

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While asexual replication of malaria parasites in red blood cells is responsible for pathogenesis, transmission to the mosquito vector requires the formation of non-replicative male and female sexual stages called gametocytes. Recent work has demonstrated a substantial upregulation of several regulators of chromatin organization during early gametocytogenesis, including a putative lysine-specific demethylase. Histone methylation is an important regulator of parasite gene expression and most of the parasite's ten protein lysine methyltransferases are essential for asexual growth. Intriguingly however, nearly all of the parasite's five putative histone demethylases (HDMs) have been shown to be dispensable for asexual growth. Given the small number of HDMs, as well as the functional and sequence diversity between them, we hypothesized that they may be important during parasite stage transitions (differentiation). To test this, we screened HDM inhibitors for their ability to inhibit asexual growth and sexual differentiation and found that parasites are highly susceptible to HDM inhibition during a defined window in early sexual development. Using epitope tagging and a conditional knockdown system, we show that at least two demethylases are expressed during gametocytogenesis and that knockdown of one of them impairs early sexual development. We also show that both of these HDMs are expressed in asexually replicating parasites and find evidence for a regulatory role in antigenic variation, a process known to be epigenetically regulated. Future efforts are focused on generating demethylase knockout lines, and on analyzing the effects of demethylase knockdown on global histone posttranslational modifications to characterize their enzymatic activity.

CD24 as a marker of stem-like tumor initiating cells in glioma

Sara Haddock, Yuxiang Wang, Sevin Turcan, and Timothy Chan

Gliomas are particularly devastating cancers that are considered incurable. Tumor cells diffusely infiltrate normal brain tissue, rendering complete surgical resection impossible. Surgery, chemotherapy, and radiotherapy provide a survival benefit, but fatal recurrence is inevitable. Cancer stem cells (malignant cells with stem-like features, including self-renewal, multipotency, and tumor initiation potential) have been isolated from gliomas. These cells are chemo- and radioresistant, suggesting that stem-like tumor subpopulations may underlie recurrence. The identification of markers specific to these stem-like cells is therefore crucial for the development of next-generation therapies intended to eliminate cells that resist conventional treatments.

The glycoprotein CD24 is upregulated in many cancers and is a predictor of poor prognosis and tumor aggressiveness. Although absent in mature brain, CD24 is expressed in the developing CNS and aberrantly expressed in many brain cancers and in glioma-derived progenitor cells. Unpublished data from our lab shows that the $CD24^+$ subpopulation of glioma tumor spheres self-renews, whereas the $CD24^-$ cells differentiate *in vitro*. In an orthotopic xenograft mouse model, $CD24^+$ cells are more efficient than $CD24^-$ cells at initiating highly aggressive tumors. Additionally, shRNA-mediated knockdown of CD24 in these cells is lethal *in vitro*, implicating CD24 as functionally relevant and critical for growth and survival, rather than simply as a "marker" that coincides with a malignant phenotype.

The goal of this project is to evaluate CD24 as a possible stem-cell specific target in glioma. Targeted killing of cancer stem cells has the potential to revolutionize the treatment of glioma and other malignancies.

The role of NAD Kinase isoform switching in melanoma metastasis

M. Graciela Cascio, Kelsey N. Aguirre, Elsbeth L. Kane, Leona A. Nease & Elena Piskounova

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Metastasis is responsible for more than 90% of cancer patient mortality yet there are no therapies that specifically target metastatic disease. Current chemotherapies target the primary tumor but are also cytotoxic to normal proliferating cells in the body, limiting treatment efficacy. A possible route to overcome this problem is to target specific vulnerabilities of metastasizing cells. Prior work from our lab using patient-derived melanoma xenografts (PDX) has shown that metastasizing melanoma cells experience high levels of oxidative stress both in circulation and in the visceral organs. Specifically, metastatic nodules have higher levels of $NADP^+$ as well as NADPH compared to primary tumors, suggesting that they may be producing more $NADP^+$ via NAD kinase (NADK) activity during metastasis in order to withstand oxidative stress.

Our preliminary data has found higher levels of NADK expression in metastatic nodules compared to primary tumor tissue. NADK has 3 isoforms with different enzymatic activities, with isoform 3 being the most active. We have found that human melanoma cells express different isoforms of NADK in primary tumor tissue vs metastatic nodules. In an *in vitro* system of oxidative stress, overexpression of NADK isoform 3 increased resistance and survival of melanoma cells to oxidative stress-induced death.We will investigate whether inhibition of NADK isoform 3 specifically blocks survival of melanoma cells and their metastatic spread. If metastasizing melanoma cells are more dependent on isoform 3 than normal cells, it has the potential to be a novel and effective therapeutic target and biomarker specific to metastatic melanoma.

Chemical genetics reveals a role for Aurora A kinase in early mitotic spindle nucleation but not in maintenance of the spindle assembly checkpoint.

Gina Tomarchio

Aurora kinase A (AurA) function is best understood in early mitosis, where it is involved in promoting centrosome maturation and centrosome mediated bi-polar spindle assembly. Though these AurA functions seem to be essential for mitotic progression, its exact substrates and mechanisms of action remain elusive. Current tools available make it difficult to accurately study AurA activity further, as most small molecule inhibitors are either not potent or specific. To address the gaps in understanding of AurA function, we have built a system for potent and specific inhibition of AurA activity using chemical genetics. We have inactivated endogenous AurA in human RPE-1 cells and complemented with an "analog-sensitive" allele which can be inhibited with non- hydrolysable purine analogs (PP1 analogs), leaving all other cellular kinases unaffected. This tool, alongside acute CRISPR-Cas9 induced knockouts, has provided novel insight into AurA function during mitosis.

Both acute depletion of AurA protein and inhibition using PP1 analogs have revealed defects in microtubule nucleation and organization in early mitosis. In addition, using chemical genetics, we present data to suggest that AurA is not essential for maintenance of the spindle assembly checkpoint (SAC), despite recent reports to the contrary. In addition to these phenotypic characterizations of AurA function, we hope to use our chemical genetics system in combination with quantitative phospho-proteomics to identify novel AurA substrates related to its established and previously unknown functions during mitosis.

A total synthetic approach to CRISPR/Cas9 genome editing and homology directed repair

Authors: Sara E. DiNapoli, Raul Martinez-McFaline, Caitlin K. Gribbin, Paul J. Wrighton, Courtney A. Balgobin, Isabel Nelson, Abigail Leonard, Carolyn Maskin, Darya Mailhiot, Clara Kao, Sean C. McConnell, Jill L.O. de Jong, Wolfram Goessling, Yariv Houvras

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CRISPR/Cas9 has become a powerful tool for genome editing in zebrafish that permits the rapid generation of loss of function mutations and the knock-in of specific alleles using DNA templates and homology directed repair (HDR). Using synthetic sgRNAs and dsDNA templates we optimized the efficiency of homology-directed repair (HDR) using a genetic assay in zebrafish. Utilizing these principles, we performed precision knock-in of fluorophores at multiple genomic loci.

We extended this approach to knock-in bacterial nitroreductase to a tissue specific gene, and demonstrate that the application of metronidazole leads to robust tissue specific lineage ablation. These edits have been transmitted through the germline, permitting line establishment. Our studies demonstrate the enhanced efficiency and utility of combining synthetic sgRNAs and DNA templates to perform gene knockout and homology directed repair in vivo.

Actin clearance promotes polarized dynein accumulation at the immunological synapse.

Elisa Sanchez, Xin Liu, Morgan Huse

T cell effector function is characterized by both actin clearance and centrosome reorientation at the immunological synapse (IS) following T cell receptor (TCR) activation. These events together enhance the specificity of T cell effector function through the direct secretion of cytokines or lytic granules toward the antigen-presenting cell. Centrosome polarization to the IS is driven by diacylglycerol (DAG) accumulation, which recruits novel protein kinase C (nPKC) proteins to the IS. Subsequently, DAG and nPKC recruit the molecular motor dynein to the synaptic membrane, where it pulls on microtubules to reorient the centrosome. Here, we used TCR photoactivation and imaging methodology to demonstrate that dynein accumulation to the IS is tightly linked to actin clearance. Disruption of actin dynamics in T cells with the actin stabilizer Jasplakinolide resulted in both delayed dynein accumulation and centrosome reorientation. Synaptic actin clearance also depended on DAG and PKC signaling, similar to what was shown previously for dynein. Interestingly, disruption of microtubules with taxol or nocodazole did not alter dynein accumulation, arguing against a role for microtubules in this process. Together, our findings reveal that dynein localization to the IS is driven by PKC dependent actin remodeling, rather than microtubule dynamics.

Determining the mechanism of suppression of FUS toxicity by hUPF1 in a yeast model of ALS

Christina Kling, He Feng, Robin Ganesan, Allan Jacobson, Jacqueline Burré, Gregory Petsko

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease for which there is no cure or significant disease modifying treatment. Mutations in the RNA-binding protein FUS can cause familial ALS and have also been linked to some cases of sporadic ALS. The phenotype of FUS toxicity can be recapitulated in *Saccharomyces cerevisiae* using an overexpression system. In the same yeast system, the human genes hUPF1 and hUPF2 were identified as potent suppressors of FUS toxicity. This activity was later validated in human neurons and rat models of ALS, but the mechanism by which these genes suppress ALS-model phenotypes is still unknown.

The work described here aims to identify the mechanism(s) of suppression by using the yeast model system to screen for hUPF1 mutants that display either enhanced or reduced suppression of FUS toxicity, and to investigate the ability of hUPF1 or hUPF2 to suppress FUS toxicity in specific yeast deletion strains. Additional work examines the functional output of a key UPF1/UPF2 regulated pathway, nonsense mediated mRNA decay (NMD) and aims to better understand the potential effect of FUS expression on translation in the cell and how that could be altered by hUPF1 or hUPF2.

Induced aging strategy for a human stem cell-derived model of Alzheimer's Disease

Andrew Minotti, Nathalie Saurat, and Lorenz Studer

Age-related diseases comprise the leading cause of death in the United States, with Alzheimer's Disease (AD) alone accounting for 4% of deaths annually. Developing treatments for AD has proven difficult due to limitations of animal models, poor early detection methods, and the complex genetic basis underlying disease onset. Human induced Pluripotent Stem Cells (iPSCs) hold tremendous promise for advancing the study of AD, as they offer an inexhaustible supply of human tissue that can form any cell type of the adult body while retaining the genetic background of patients. Unfortunately, the reprogramming process has been shown to erase age-associated phenotypes of the somatic cell source, thus creating a huge obstacle in accurately recapitulating the physiology of late-onset diseases. In this poster, we demonstrate a novel approach to model AD in cortical neurons derived from human Pluripotent Stem Cells (hPSCs) via expression of a progerin peptide, a fragment of the nuclear Lamin A protein whose accumulation has been associated with diseases of premature aging. Our data indicates progerin overexpression is capable of inducing several changes associated with both cellular aging and AD pathology. Therefore, our strategy is a promising method for inducing age-associated changes in vitro for applications in modelling human neurodegenerative and late-onset diseases in hPSC-derived cultures

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REGULATION OF THE SELENOCYSTEINE STRESS RESPONSE IN CANCER METASTASIS

Leona Nease, Kelsey Aguirre, Graciela Cascio, Elena Piskounova

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Metastatic disease is the leading cause of death from solid tumors and its suppression is an urgent therapeutic need. Recent work from our laboratory has shown that metastasis is limited by oxidative stress. Some of the major regulators of intracellular redox biology are selenoproteins – a family of 25 proteins that contain selenocysteine (Sec), the 21st amino acid. These proteins range from antioxidant enzymes (glutathione peroxidases, thioredoxin reductases) to ER protein folding chaperones (Selenoproteins S, K, M). Sec is structurally identical to cysteine; however, it contains a covalently bound selenium in place of sulfur. Unlike other amino acids, selenocysteine is synthesized on its own unique tRNA (tRNA^{Sec}) and both *cis*- and *trans*-acting factors are required for proper co-translational insertion at an in-frame UGA stop codon. Two isoacceptors of tRNA^{Sec} exist, differing only by a single 2'-O-ribose-methylation at the wobble uridine on the anticodon loop. This modification is influenced by both selenium status and oxidative stress and it induces a conformational change of the tRNA. This Um34 modification is unique to tRNA^{Sec} and is thought to selectively regulate a subset of stress response selenoproteins, however this has not been fully characterized and the methyltransferase remains unidentified. We hypothesize that tRNA^{Sec} Um34 wobble modification is upregulated in metastasizing cells and that this modification increases cell survival under oxidative stress by regulating stress-response selenoproteins. Our current work is focused on studying the role and regulation of selenoproteins in cancer metastasis. We aim to identify novel and targetable vulnerabilities that are specific to metastatic disease.

Using the Zebrafish as a Personalized Medicine Platform for Melanoma

Joshua Weiss, Theresa Simon-Vermot, Richard White

Thousands of cancer patients get their tumors sequenced every year. However, the extent to which this information is used clinically is still limited. There is a need to rapidly develop personalized in vivo models to better predict drug response and identify more effective therapies. We set do this for melanoma patients with a focus on acral melanoma, which is a rare subtype defined by its anatomic distribution on non-sun-exposed regions, such as the sole of the foot. We developed a “one shot” approach to rapidly create personalized zebrafish avatars, which involves simultaneous integration of over 6 plasmids into the zebrafish genome to model complex cancer genotypes. We developed a personalized zebrafish avatar of a single patient’s acral melanoma, referred to as patient “XYZ.” Whole genome and RNA-sequencing identified a unique combination of putative genetic drivers: NF1 deletion, CRKL amplification, GAB2 amplification, and TERT promoter mutation, which we predicted to synergize and drive overactivation of the MAPK pathway. Unlike BRAF and RAS mutant melanomas, we hypothesized that this genotype requires input through receptor tyrosine kinase to drive MAPK signaling and thus depends on microenvironmental factors. Using the XYZ zebrafish avatar, we identified insulin as a promising therapeutic target regulating the invasive properties of the patient’s disease. We hope that the combination of our “one shot” approach and our drug pipeline will enable personalized medical care for melanoma patients.

Analysis of gut endoderm morphogenesis in the mouse embryo at the single-cell level.

Jonathan Pai[1,2], Katie McDole[3], Ying-Yi Kuo[1], Philipp Keller[3], Sonja Nowotschin[1], Anna-Katerina Hadjantonakis[1,2]

Our lab has previously shown that the gut endoderm arises when epiblast-derived definitive endoderm (DE) cells emerge from the primitive streak at gastrulation, migrate in an anterior-ward direction, and then intercalate with the Primitive Endoderm (or extra-embryonic)-derived visceral endoderm (VE) epithelium. While these observations have shaped current models of mammalian gut endoderm morphogenesis, they also highlight a need to revise the previously held understandings of the cell behaviors that drive gut endoderm morphogenesis. To gain insight into the intrinsic behaviors of endoderm cells we are exploiting recent advances in microscopy techniques and image analysis methods. We are constructing a cellular resolution fate map of DE cells to detail the time and position at which they exit the primitive streak, their migration speeds and trajectories, and their spatial organization upon intercalation into the VE. To accomplish this, we use real-time, adaptive, multi-view, light-sheet microscopy to image mouse embryos with lineage-specific fluorescent reporters. The behaviors of individual endoderm cells are tracked in 4D using the Massive Multi-view Tracker (MaMut) Fiji plugin. In parallel we are characterizing the morphological changes within the endoderm epithelium before, during and after DE-VE intercalation. We use immunofluorescent labelling to mark cell and tissues features such as morphology, polarity, the cytoskeleton and extra-cellular matrix. As an example, we use Fiji to construct binary maps of cell junctions. We then quantify cell morphologies, determine how they change over time, and compare them between different regions of the embryo. Progress with these studies will be presented.

Heterogeneous adaptation of lung cancer cells to KRAS^{G12C} specific inhibition

Jenny Y Xue, Yulei Zhao, Jordan Aronowitz, Trang Thi Mai, Besnik Qeriqi, Elisa de Stanchina, Gregory J Riely, Linas Mazutis, Davide Risso, Piro Lito

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Allele-specific inhibitors targeting KRAS^{G12C}, one of the most frequent activating mutations in lung cancer and one that has historically been difficult to target, are now entering clinical trials. These drugs trap KRAS^{G12C} in its inactive conformation by suppressing nucleotide cycling, but whether cancer cells can adapt to overcome the effect of the drug is not understood. Here we used single cell RNA sequencing to investigated the effect of a KRAS^{G12C}-specific inhibitor (G12Ci) in ~10,000 lung adenocarcinoma cells. We identified distinct cell fates post treatment with G12Ci, where at a similar treatment time, some cells were sequestered in a quiescent state, while others rapidly adapted and resumed proliferation. Newly synthesized KRAS is required to restore transcriptional output and for escape from quiescence, and adaptive signals from epidermal growth factor receptor (EGFR) and aurora kinase (AURK) facilitate this process by shifting the new proteins toward an active/drug-insensitive state. Combined inhibition of these signaling pathways along with KRAS^{G12C} produced more potent antitumor effects in xenograft models. This work provides insight into the modulation of KRAS^{G12C} inhibition at the cancer cell population-level and a blueprint towards maximizing its therapeutic effect in lung cancer patients.

ANKRD31, a direct interactor of REC114, controls number, timing, and location of meiotic double strand breaks (DSBs)

Jiaqi Xu, Michiel Boekhout, Mehmet E. Karasu and Scott Keeney

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Meiotic recombination is required for proper homologous chromosome segregation. Recombination is initiated by developmentally programmed, SPO11-mediated double strand breaks. Our lab previously unveiled mouse ANKRD31 as a lynchpin governing multiple aspects of DSB formation. Spermatocytes lacking ANKRD31 have altered DSB locations and fail to target DSBs to sex chromosomes' pseudoautosomal regions (PAR). They also have delayed/fewer recombination sites but, paradoxically, more DSBs, suggesting DSB dysregulation. Unrepaired DSBs and pairing failures—stochastic on autosomes, nearly absolute on X and Y—cause meiotic arrest and sterility in males. Ankrd31-deficient females have reduced oocyte reserves. A crystal structure defines direct ANKRD31– REC114 molecular contacts. In vivo, ANKRD31 stabilizes REC114 association with the PAR and elsewhere. Our findings inform a model that ANKRD31 is a scaffold anchoring REC114 and other factors to specific genomic locations, promoting efficient and timely DSB formation but possibly also suppressing formation of clustered DSBs. To further investigate the mechanism and regulation of ANKRD31 during DSB formation, we generated ANKRD31-REC114 interaction deficient mice by inducing point mutation/ truncation at C-terminal of ANKRD31 by CRISPR. We will dissect the mechanism of ANKRD31 by characterizing ANKRD31-REC114 interaction deficient mice.

Modeling Nemaline Myopathy in *Drosophila* to identify causes and cures

Carolina Zapater, Mridula Balakrishnan and Mary K. Baylies

Nemaline myopathy (NM) is a hereditary muscle disorder that causes severe muscle weakness and can lead to death. There is currently no cure. One hallmark of NM is the presence of protein inclusions called nemaline bodies (NBs). NBs are composed of actin and Z-disc proteins, which are components of the sarcomere, the fundamental contractile unit of the muscle. Currently, there are 15 genes linked to NM and all appear associated with the sarcomere. How mutations in these genes contribute to the disease is not well understood. Our lab has shown that muscle specific knockdown of Twinstar (Tsr), the Drosophila homolog of the NM disease gene CFL2, leads to NM in Drosophila. Importantly, data from our lab show that a loss in muscle function correlates with specific alterations in muscle structure; we also found that enhancing proteasome function delayed disease progression in this model. To address whether these observations are generalizable to all forms of NM, we performed a selective RNAi screen in which we targeted the putative homologous genes known to lead to NM in patients. We have focused thus far on tropomodulin (tmod), the homolog of Tmod and Lmod, which are, respectively, actin capping and nucleator proteins. Muscle-specific knockdown of tmod in larvae resulted in muscle weakness and abnormal muscle structure: specifically, we detected disarrayed myofibrils, aberrant non-sarcomeric actin filaments and internalized nuclei. Since Tmod's vertebrate homolog interacts with an E3 ubiquitin ligase complex, we investigated whether tmod is involved in the Ubiquitin-Proteasome System (UPS), and specifically, whether there are changes in ubiquitin marks in tmod RNAi muscle. We find a significant increase in FK2 staining, which detects single and poly-ubiquitin, compared to controls. We hypothesize that there is improper protein homeostasis as a result of tmod RNAi, which in turn, leads to decreased muscle function. Our long-term goal is to use these different Drosophila models to identify general mechanisms underlying NM disease formation and new therapeutic targets.

MYC-dependent oxidative metabolism regulates osteoclastogenesis via nuclear receptor ERRα

Seyeon Bae and Kyung-Hyun Park-Min

Osteoporosis is a metabolic bone disorder associated with compromised bone strength and an increased risk of fracture. Inhibition of the differentiation of bone-resorbing osteoclasts is an effective strategy for the treatment of osteoporosis. Prior work by our laboratory and others has shown that MYC promotes osteoclastogenesis *in vitro*, but the underlying mechanisms are not well understood. In addition, the *in vivo* importance of osteoclast-expressed MYC in physiological and pathological bone loss is not known. Here, we have demonstrated that deletion of *Myc* in osteoclasts increases bone mass and protects mice from ovariectomy-induced (OVX-induced) osteoporosis. Transcriptomic analysis revealed that MYC drives metabolic reprogramming during osteoclast differentiation and functions as a metabolic switch to an oxidative state. We identified a role for MYC action in the transcriptional induction of estrogen receptor–related receptor α (ERRα), a nuclear receptor that cooperates with the transcription factor nuclear factor of activated T cells, c1 (NFATc1) to drive osteoclastogenesis. Accordingly, pharmacological inhibition of ERRα attenuated OVX-induced bone loss in mice. Our findings highlight a MYC/ERRα pathway that contributes to physiological and pathological bone loss by integrating the MYC/ERRα axis to drive metabolic reprogramming during osteoclast differentiation.

L-2-Hydroxyglutarate (L-2HG) maintains stemness in Hematopoietic Stem/Progenitor Cells

Sylvia Zohrabian

Somatic mutations in isocitrate dehydrogenase (IDH) enzymes are prevalent in acute myeloid leukemia (AML) and a variety of other cancers. IDH enzymes normally catalyze the NADP(H)-dependent interconversion of isocitrate and alpha-ketoglutarate (αKG). Mutant IDH enzymes lose wildtype activity and concomitantly gain the ability to efficiently catalyze reduction of αKG to the 'oncometabolite' D-2-hydroxyglutarate (D-2HG). D-2HG inhibits >70 different αKG-dependent enzymes that regulate chromatin modifications, stability of hypoxia inducible factor (HIF), and DNA repair, leading to impaired differentiation and a stem-like phenotype in IDH-mutant cells. Although cancer-associated IDH mutants specifically produce D-2HG, our research group discovered that normal and malignant cells without IDH mutations produce the mirror-image enantiomer L-2HG in response to hypoxia and decreased pH. Strikingly, L-2HG can function as a ~10-fold more potent inhibitor of many αKG-dependent enzymes. However, the biologic functions of L-2HG remain poorly understood. Our preliminary data demonstrate that L-2HG stabilizes HIF, induces repressive chromatin marks, and blocks differentiation of hematopoietic stem/progenitor cells (HSPCs). These findings suggest that L-2HG may account, at least in part, for the importance of hypoxic niches in maintaining the self-renewal of hematopoietic stem/progenitor (HSPCs). Therefore, the effect of L-2HG in hematopoietic cells was analyze in normal and stress hematopoiesis.

Novel Bedfellows: Primary Cilia and Cell-Cell Junctions

Avital Shulman, Bryan Tsou

Cilia grow strictly from centrioles and are typically described as hair-like structures projecting from a cell's surface. However, centrioles in certain animal lineages (including vertebrates) can function as the organizing center for microtubules and the golgi while simultaneously driving ciliogenesis, generating a mysterious, complex organelle we called submerged cilia. Unlike surfaced cilia, each submerged cilium is maintained in a deep membrane invagination, surrounded by a narrow extracellular cavity or pit (ciliary pit) whose functions and properties are unknown. To better understand the biogenesis and function of submerged cilia, we conducted a comparative genomics screen to identify novel cilia-associated proteins that co-evolved with submerged cilia.

At least two of the proteins identified, WDR54 and Kazrin, are found to specifically localize to submerged cilia. Intriguingly, in polarized epithelial cells naturally growing surfaced cilia, both WDR54 and Kazrin are seen at cell-cell junctions. Knockdown of WDR54 was found to destabilize not only the pit environment of submerged cilia in non-polarized cells but also cell-cell junctions of polarized epithelium. These results suggest possible functional and/or structural similarities between submerged primary cilia and cell-cell junctions.

A Noncanonical Histone Target of NSD2 and a Potential Role in DSB Repair

Nicole Weiss and Minkui Luo

The field of epigenetics has emerged relatively recently to be defined as a vital layer of regulation of the central dogma. Important players in maintaining and altering the epigenetic landscape are protein methyltransferases (PMTs), enzymes that add methyl groups to lysine and arginine residues of histone tails. NSD2 (MMSET, WHSC1) is a PMT that has been shown to dimethylate histone 3 at lysine 36 (H3K36me2). It is overexpressed in several cancers, such as multiple myeloma, and has a heterozygous gain-of-function point mutation (E1099K) in acute lymphoblastic leukemia (ALL). The canonical activity of NSD2 correlates with gene activation, which is augmented by the E1099K mutant. However, NSD2 has also been implicated in DNA repair and replication. Noncanonical histone sites have been reported for NSD2 but have yet to be confirmed due to the redundancy of activity across PMTs. Still, these novel sites suggest additional mechanistic roles for NSD2 supporting additional biological functions. Here, we show the NSD2 WT and E1099K are both capable of methylating H3K18 in vitro, a previously unknown activity for the enzyme. Published reports and preliminary experiments show that NSD2 is involved in DNA repair, although the mechanism remains unknown. We hypothesize that NSD2 mediates H3K18me to help recruit 53BP1 to sites of DNA damage to promote nonhomologous end joining (NHEJ). This activity may be increased by the E1099K mutant, contributing to its oncogenic role in ALL.

Concurrent Talks

	BSB/MB (Rose/Dogwood Room)	**CDB (Azalea)**
10:05 – 10:25am	Christopher Lima	Matthias Stadtfeld
10:25 – 10:45am	Tanya Schild	Reeti Sanghrajka
10:45 – 11:05am	David Simon	Jingli Cao
11:05 – 11:25am	Xiaolan Zhao	David Rickman
11:50 – 12:10pm	David Eliezer	Qiao (Joe) Zhao
12:10 – 12:30pm	Bryan Ngo	Yujie Fan
12:50 – 1:10pm	Baran Ersoy	Vidur Garg

A multi-subunit nucleoporin complex that promotes Ran GTPase activation and SUMO modification

Michael A. DiMattia and Christopher D. Lima

The nucleoporin RanBP2 is the main component of cytoplasmic filaments in nuclear pore complexes (NPC) where it contributes to nucleocytoplasmic transport by establishing the Ran GDP-GTP gradient at the nuclear pore complex (NPC) and at the kinetochore during mitosis. RanBP2 performs these functions via Ran-binding domains (RBDs) and by SUMO-dependent recruitment of the Ran GTPase activating protein RanGAP1 to RanBP2. SUMO proteins belong to the ubiquitin-like protein family and are attached to other proteins via conjugation to lysine residues, where they modulate many pathways including nucleocytoplasmic transport, the cell cycle, differentiation, apoptosis, and responses to stress. RanBP2 domains that bind SUMO modified RanGAP1 also catalyze SUMO E3 ligase activity at the NPC and at the kinetochore during interphase and mitosis, respectively. Intriguingly, Borealin is a SUMO substrate of this RanBP2 complex, and comprises part of the chromosomal passenger complex (itself a master regulator of mitosis), along with Survivin, INCENP, and Aurora B kinase. To determine how RanBP2 interacts with its cofactors and substrates, we reconstituted a multi-protein complex that includes RanBP2, SUMO1 modified RanGAP1, the SUMO E2 Ubc9, Ran[GTP] and the exportin CRM1 and determined its structure by single particle cryo-EM. Structural insights, combined with ongoing biochemical data, suggest that RanBP2 utilizes CRM1 to recruit substrates and to organize other RanBP2 domains to facilitate Ran GTPase activation and SUMO conjugation to substrates.

Harvesting Hepatic Maladaptations to Obesity for Novel Treatment Strategies

Baran Ersoy

The human body has evolved to sustain starvation and bouts of moderate food intake but not continuous excessive nutrition. Therefore, the maladaptation of our bodies to obesity often results in increased accumulation of sugar, fat and cholesterol, which in turn lead to type 2 diabetes, nonalcoholic fatty liver disease and cardiovascular disorders. However, the mechanisms linking excessive nutrition to the pathogenesis of metabolic disorders remain poorly understood. We use innovative comparative proteomics approaches to pinpoint the differences between the livers of obese patients with metabolic disorders and healthy obese controls. The rationale is that, the identification of aberrant responses to chronic obesity will provide novel targets for the management of increasingly prevalent metabolic disorders. We apply complementary use of biochemical and molecular biology techniques and state-of-the-art equipment including, *in vivo* and *in vitro* models, metabolic cages, thermal imaging, EchoMRI body composition analyzer, hyperinsulinemic euglycemic clamp, trafficking of isotope-labeled lipid and glucose substrates as well as mass spectrometry-based proteomics, lipidomics and metabolomics. The two main areas of interest include: 1) Improper utilization of excess fatty acids in biological pathways and 2) Impaired nuclear localization of metabolic regulators.

Interrogating the mechanisms driving the emergence of pluripotency using forward and reverse reprogramming strategies

Vidur Garg, Laurianne Scourzic, Boaz Eliezer Aronson, Effie Apostolou, Anna-Katerina Hadjantonakis

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Understanding how pluripotency is established and maintained is a cornerstone of developmental and stem cell biology, and has major implications for regenerative medicine. Pluripotency refers to the potential to give rise to all somatic tissues and germ cells, a behavior that is ascribed *in vivo* to the epiblast (EPI) lineage of the mammalian blastocyst. The pluripotent EPI is specified along with its sister lineage, the primitive (extra-embryonic) endoderm (PrE), from a common progenitor population in an irreversible process during blastocyst development. Despite the process being well studied, the molecular mechanisms underlying this cell fate decision, and the factors maintaining the barrier between sister lineages remain poorly understood. For deeper analyses, and to overcome the limited biological material available from blastocysts, we have leveraged embryo-derived embryonic stem (ES) and eXtraembryonic ENdoderm (XEN) cells – the *in vitro* stem cell counterparts of the EPI and PrE – to chart the sequence, and establish a roadmap to, or from, pluripotency. The goal of the project is to understand the mechanisms, identify the drivers and roadblocks, and establish the kinetics of EPI-to-PrE, and conversely PrE-to-EPI reprogramming at single-cell resolution. We show for the first time that XEN cells hold the potential to acquire a pluripotent identity (XEN-to-ES) using transcription factor-mediated lineage conversion by ectopic expression of *Oct3/4*, *Sox2* and *Klf4*. By contrast, *Gata4*-driven ES-to-XEN conversion represents an *ex vivo* model of EPI-to-PrE conversion. Furthermore, our data reveal novel routes to and from pluripotency, and open up new avenues to study these critical events at unprecedented resolution.

Developing novel regenerative medicine approaches to treat type 1 diabetes and digestive failure

Steve Xiaofeng Huang, Christoph Pertl, Jiaoyue Zhang, Wei Gu, Ying Lan, Yaohui Nie, Joe Q. Zhou

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Disease and injury strike every organ in our body, killing and damaging cells that perform vital functions. How to replenish the lost cells and repair organ function is the outstanding question in regenerative medicine. Our laboratory pioneered a new path for tissue regeneration. Based on principles of development, we identified master regulators that dictate the formation of specific cells (pancreatic beta-cells and intestine stem cells) and by modulating the expression of these master genes, one can remake, or reprogram existing adult cells from one type to another, thereby regenerating medically important cell types. I will describe two areas of interest in the laboratory. One is aimed at regenerating insulin-secreting cells that may be used in transplantation therapy to treat Type 1 diabetes.

We have discovered in mouse genetic models and human intestinal stem cell cultures that a defined set of master genes can reprogram specific gut cells into insulin-secreting cells. The second area of interest is colon stem cells, where we identified a master regulator which can convert large intestine into small intestine, thus endowing the large intestine with the ability to absorb nutrients. We are pursuing mechanistic studies with the hope of developing this approach towards a potential novel therapy to treat digestive failures.

Deriving human ENS lineages from sacral neural crest for the cell therapy of Hirschsprung disease

Yujie Fan and Lorenz Studer

The enteric nervous system (ENS) is the largest and most diverse component of the human autonomic nervous system. The ENS is derived from the vagal and sacral neural crest (NC) and represents a complex network of neurons with dozens of distinct neurotransmitter subtypes essential for gastro-intestinal (GI) function. Defects in ENS development are responsible for disorders including Hirschsprung disease (HSCR), a fatal congenital disease caused by the lack of ENS ganglia in the colon. Additional evidence suggests that the ENS is linked to various neurological disorders ranging from Parkinson disease (PD) to Alzheimer's disease. GI problems often precede onset of classic motor symptoms in PD. Braak hypothesis suggested that PD may initiate in the gut based on temporal anatomical analysis of α-synuclein accumulation. Despite the importance of the ENS in human disease, it remains poorly studied owing to the lack of an easily accessible model system. Over the past two years, I established the first protocol to generate human sacral NC from hPSCs. The sacral NC can be further differentiated into relevant ENS cell types including 5-HT+, GABA+ and NOS+ neurons. With the ability to generate both sacral and vagal NC, I directly compared molecular and functional properties of those two lineages to illuminate their role in generating the complete ENS. Finally, I assessed the potential of transplanting sacral, vagal and combined NC lineages for treating HSCR disease in mice model.

Structure/Function Studies of the Parkinson's Protein Alpha-Synuclein

David Eliezer, Tapojyoti Das, Meraj Ramezani, David Holowka, Barbara Baird

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The protein alpha-synuclein is linked to Parkinson's disease, yet little is known about its normal physiological functions. Alpha-synuclein aggregation is considered key to its role in PD. Despite this, the physiological context for pathological aS assembly remains poorly understood. The normal functional conformations of aS, preceding aggregation, likely constitute the best targets for preventing aggregation, will require a detailed understanding of the normal function of aS at the levels of both molecular structure and basic cell biology.

I will present results obtained utilizing cellular assays of synuclein function to enable structure-function studies and to reveal novel aspects of synuclein function in regulation of vesicle exocytosis.

NADK regulation by oncogenic KRAS signaling is essential for PDAC

Tanya Schild, Melanie McReynolds, Christie Shea, Vivien Low, Adam Rosenzweig, John Asara, Noah Dephoure, Joshua D. Rabinowitz, John Blenisand Ana P. Gomes

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RDiagnosis of pancreatic ductal adenocarcinoma (PDAC) carries a dismal prognostic outcome. In recent years, metabolic adaptations have been shown to be critical for PDAC maintenance and for its acquired resistance to therapies. Many of these adaptations rely heavily on NADPH availability—however our understanding of NADPH metabolism and how is it regulated to support PDAC is still in its infancy. NADPH is recycled through reduction of NADP+ by several enzymatic systems in cells. In conditions where NADPH availability is critical, cells have an evolutionarily conserved mode of increasing the global pools of NADPH through *de novo* synthesis of NADP+ catalyzed by the NAD+ kinase (NADK). Here we establish that NAD+ kinase (NADK) is a major contributor to NADPH pools in PDAC. NADK, which catalyzes the *de novo* synthesis of NADP+ from NAD+, becomes hyperactive when oncogenic KRAS is present and constitutes a vital mechanism for PDAC survival. We traced this hyperactivation to a KAS-induced and PKC-mediated phosphorylation of NADK, which results in an increase in the *de novo* NADP+ synthesis, and consequently to a rise in NADPH levels. For the first time, we show that NADK can be regulated by oncogenic signaling. This regulatory mechanism is essential for PDAC survival and highlights NADK as a much-needed new therapeutic target for PDAC.

Somatic mutations of chromatin regulator *Kmt2d* in cerebellar precursors influences SHH-medulloblastoma tumorigenesis

Sanghrajka, R.M., Tan I., Wojcinski A., Rallapalli H., Turnbull D., Ge K., Joyner, A.L.

Medulloblastoma (MB), the most common malignant pediatric brain tumor, is a classic example of dysregulation of developmental pathways leading to tumorigenesis. Despite advancements in multi-modal therapies, most patients suffer from disease-related and treatment-related neurocognitive and neuroendocrine sequelae. The Sonic Hedgehog subgroup of MB (SHH-MB) accounts for ~30% of all cases and originates from ATOH1+ cerebellar granule cell precursors (GCPs). Experimental data in mice has shown that activating mutations in the SHH pathway induce tumors only in rare GCPs, suggesting that additional mutations and epigenetic changes are required to influence tumor progression. The *KMT2D* gene, encoding the histone-lysine N-methyltransferase 2D, is amongst the ten most frequently mutated genes in MB, with somatic mutations seen in ~15% of all SHH-MB patients. We developed sporadic mouse models of SHH-MB with a low penetrance to enable studies of secondary mutations (Tan, *PNAS*, 2018).

Immunofluorescence staining for KMT2D on early-stage SHH-MB lesions, mid-stage and late-stage tumors revealed that a subset of lesions/tumors (16/98) do not express KMT2D and are negative for H3K4me3. Interestingly, P53 and NeuN expression showed a positive correlation with KMT2D in ~93% of tumors/lesions. Mice with a heterozygous or homozygous deletion of *Kmt2d*, in conjunction with mutations that result in the activation of the SHH pathway, showed aggressive tumors at high penetrance, with metastatic leptomeningeal spread in the brainstem and spinal cord, and decreased survival time, compared to mice with wildtype *Kmt2d* expression. Ongoing studies are determining how the chromatin landscape and gene expression are changed when *Kmt2d* is deleted in GCPs.

Maintenance of epigenetic integrity in pluripotent stem cells

Emily Swanzey, Simon Vidal, Alexander Polyzos, Ly-sha Ee, Sangyong Kim, Effie Apostolou and Matthias Stadtfeld

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Pluripotent stem cells have tremendous biomedical potential as they are capable of differentiating into all cell types. At the same time, pluripotent cells can be propagated for extended periods of time in culture, provided the opportunity for genetic engineering. Pluripotent cells can be derived either by explant culture of blastocyst-stage mammalian embryos (giving rise to embryonic stem cells; ESCs) or by reprogramming adult somatic cells by ectopic transcription factor expression (giving rise to induced pluripotent stem cells; iPSCs). The establishment of pluripotency, in particular via cellular reprogramming, requires extensive remodeling of chromatin and genome architecture, which has to occur without the introduction of detrimental epimutations. Similarly, epigenetic marks at developmental control loci have to be faithfully propagated during ex vivo expansion. Unfortunately, epigenetic abnormalities are common in pluripotent cells, suggesting failures in protective molecular mechanisms. My laboratory identifies and studies molecules involved in the maintenance of epigenetic integrity in pluripotent cells and attempts to understand their mode of actions using genetics, biochemistry and next generation sequencing. Beyond pluripotency, our approach should yield fundamental insight into human diseases characterized by epigenetic abnormalities, such as cancer. In my presentation, I will present the conceptual questions underlying the major projects in the lab. In particular, I will outline our interests in unexplored roles of repressive histone methyltransferases and in genetic factors predisposing to DNA hypermethylation at gene loci subject to genomic imprinting.

A hallmark of metastasis is the adaptation of tumor cells to new environments

Bryan Ngo, Eugenie Kim, Sophia Doll, Sophia Bustraan Ahmed Ali, Alba, Luengo, Gino Ferraro, Diane Kang Jing Ni, Roger Liang, Ariana Plasger, Edward R. Kastenhuber, Roozbeh Eskandari, Sarah Bacha, Roshan K. Siriam, Samuel F. Bakhoum, Edouard Mullarky, Adam Friedman, Vipin Suri, David M. Sabatini, Drew Jones, Min Yu, Jean J. Zhao, Matthew Vander Heiden, Matthias Mann Lewis C. Cantley, Michael E. Pacold

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A hallmark of metastasis is the adaptation of tumor cells to new environments. Although it is well established that the metabolic milieu of the brain is severely deprived of nutrients, particularly the amino acids serine and its catabolite glycine, how brain metastases rewire their metabolism to survive in the nutrient-limited environment of the brain is poorly understood. Here we demonstrate that cell-intrinsic *de novo* serine synthesis is a major determinant of brain metastasis. Whole proteome comparison of triple-negative breast cancer (TNBC) cells that differ in their capacity to colonize the brain reveals that 3-phosphoglycerate dehydrogenase (PHGDH), which catalyzes the rate-limiting step of glucose-derived serine synthesis, is the most significantly upregulated protein in cells that efficiently metastasize to the brain. Genetic silencing or pharmacological inhibition of PHGDH attenuated brain metastasis and improved overall survival in mice, whereas expression of catalytically active PHGDH in a non-brain trophic cell line promoted brain metastasis. Collectively, these findings indicate that nutrient availability determines serine synthesis pathway dependence in brain metastasis, and suggest that PHGDH inhibitors may be useful in the treatment of patients with cancers that have spread to the brain.

Balancing Pro-Survival and Pro-Degenerative Cues to Promote Axon Survival

David J. Simon

How do some neurons survive a lifetime while others succumb to neurodegeneration? We take a 'bottom-up' approach to this question by first understanding the molecules and pathways that control neuronal survival, and then working upstream to ask how cellular stresses including misfolded proteins, physical injury, and loss of neurotrophic support disrupt these pathways to promote degeneration. This has led us to focus on core metabolic and apoptotic pathways and the discovery of several surprising parallels between neurodegenerative mechanisms and signaling events that drive tumorigenesis. A particular focus has been on survival of the axon, the long cable-like structures that connect neurons and degenerate earliest in many neurodegenerative diseases. We found that axon degeneration is controlled by bi-directional signaling to and from the cell body, leading us to now ask how the cell body controls the localized death of the axons. We further seek to identify proteins that sense the kind of cellular stresses that are not, in themselves, damaging and determine how these proteins signal within a neuron to promote degeneration. Finally, we are interested how neuronal survival is affected by nearby inflammation, in particular in identifying endogenous protective mechanisms that allow the neuron to withstand chronic low-level inflammation without degenerating. Our ultimate goal is understanding how a neuron reacts to its environment and how environmental stimuli affect the balance between survival and death. In support of these questions we employ a range of molecular, genetic, biochemical, and imaging techniques.

Fishing for Cues for Heart Regeneration

Yingxi Cao, Sierra Duca, Jingli Cao

The adult human heart shows minimal regeneration of cardiac muscle after a heart injury like myocardial infarction (MI). There remains no regenerative therapy for human heart disease; thus, continued basic research and diverse approaches are necessary. Zebrafish possess a remarkable capacity for cardiac regeneration. Understanding how natural heart repair is regulated in zebrafish will help to shape strategies for human heart repair. In zebrafish, the epicardium, which covers all vertebrate hearts, is activated by cardiac injury and enables muscle regeneration through paracrine effects and as a multipotent cell source. Our recent work revealed that the presence of epicardium is required for muscle regeneration. However, how epicardial cells respond to heart injury and further exert effects on muscle regeneration are poorly understood, and our recent finding of the cellular heterogeneity of the zebrafish epicardial cells added complexity to this question. This deficiency of knowledge represents a major barrier for harnessing epicardium for therapeutic goals. To address this, using a combination of zebrafish model, explant tissue culture, single-cell RNA sequencing, chemical screening, live imaging, and genome editing approaches (such as CRISPR/Cas9), we are dissecting how epicardial cells are molecularly regulated at the single-cell level to engage in the heart muscle regeneration. The outcome of this work will inspire new approaches for enhancing the limited regenerative capacity of the human heart after MI.

Genome guardians that enable faithful genome duplication and cell cycle progression

Nalini Dhingra and Xiaolan Zhao

In human development is a daunting task but absolutely critical to prevent many forms of genomic instability underlying diseases such as cancer. While a general understanding of genome replication and cell cycle progression has been established, many important questions await to be addressed. Some of the questions that our laboratory is pursuing include how replisome components are assembled into an amazing machinery capable of synthesizing mega-bases of DNA in one run with few mistakes, how replisomes collaborate with a myriad of regulators to overcome all sorts of template blockades, and how signal processes can adjust cell cycle progression according to DNA replication and genome stress status. In our lab we take advantage the powerful yeast model system that allows us to combine genetic, biochemical, genomic, proteomic, and cell biological approaches to address many questions in a short period of time.

Using these approaches, students and postdocs in the lab have elucidated important mechanisms that support replisome assembly, identified a protein network key for synthesizing high-risk genomic loci, and uncovered a SUMO-based regulatory system promoting genome duplication and repair. One of our current research directions addresses how genome stress and DNA repair status are sensed by the SUMO system and the DNA damage checkpoint pathway. I will describe recent findings that suggest a new mechanism allowing the resumption of cell cycle after DNA stress is removed.

Drivers of lineage plasticity in advanced prostate cancer

Nicholas J. Brady, Adeline Berger, Alyssa M. Bagadion, Rohan Bareja, Brian Robinson, Vincenza Conteduca, Michael A. Augello, Loredana Puca, Adnan Ahmed, Xiaodong Lu, Inah Hwang, Andrea Sboner, Olivier Elemento, Jihye Paik, Jindan Yu, Christopher E. Barbieri, Noah Dephoure, Himisha Beltran and David S. Rickman

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Despite recent therapeutic advances, prostate cancer remains a leading cause of cancer related death. A subset of castration resistant prostate cancers become androgen receptor (AR) signaling-independent and develop neuroendocrine prostate cancer (NEPC) features through lineage plasticity. These NEPC tumors are associated with aggressive disease and poor prognosis. In the last decade, we and others have identified several candidate drivers of plasticity including, but not limited to, loss of TP53 and RB1, upregulation of MYCN (encodes N-Myc), EZH2, or SOX2. The mechanisms that underlie this lineage plasticity as a mechanism of treatment resistance remain unclear. Integrative analysis of the N-Myc transcriptome, cistrome and interactome using in vivo including genetically engineered mice), in vitro and ex vivo models (including patient-derived organoids) identified a lineage switch towards a neural identity associated with epigenetic reprogramming. N-Myc and known AR-co-factors (e.g. FOXA1) overlapped, independently of AR, at genomic loci implicated in neural lineage specification. Moreover, histone marks specifically associated with lineage defining genes were reprogrammed by N-Myc. We also demonstrated that the N-Myc-induced molecular program accurately classifies our cohort of patients with advanced prostate cancer. Finally, we revealed the potential for EZH2 inhibition to reverse the N-Myc-induced suppression of epithelial lineage genes. Most recently, we have found that N-Myc overexpression and RB1 loss accelerates progression to NEPC tumors, castration resistance and onset of metastasis to lungs, lymph nodes, and liver. Altogether, our data provide insights on how N-Myc regulates lineage plasticity and epigenetic reprogramming associated with lineage-specification. The N-Myc signature we defined could also help predict the evolution of prostate cancer and thus better guide the choice of future therapeutic strategies.