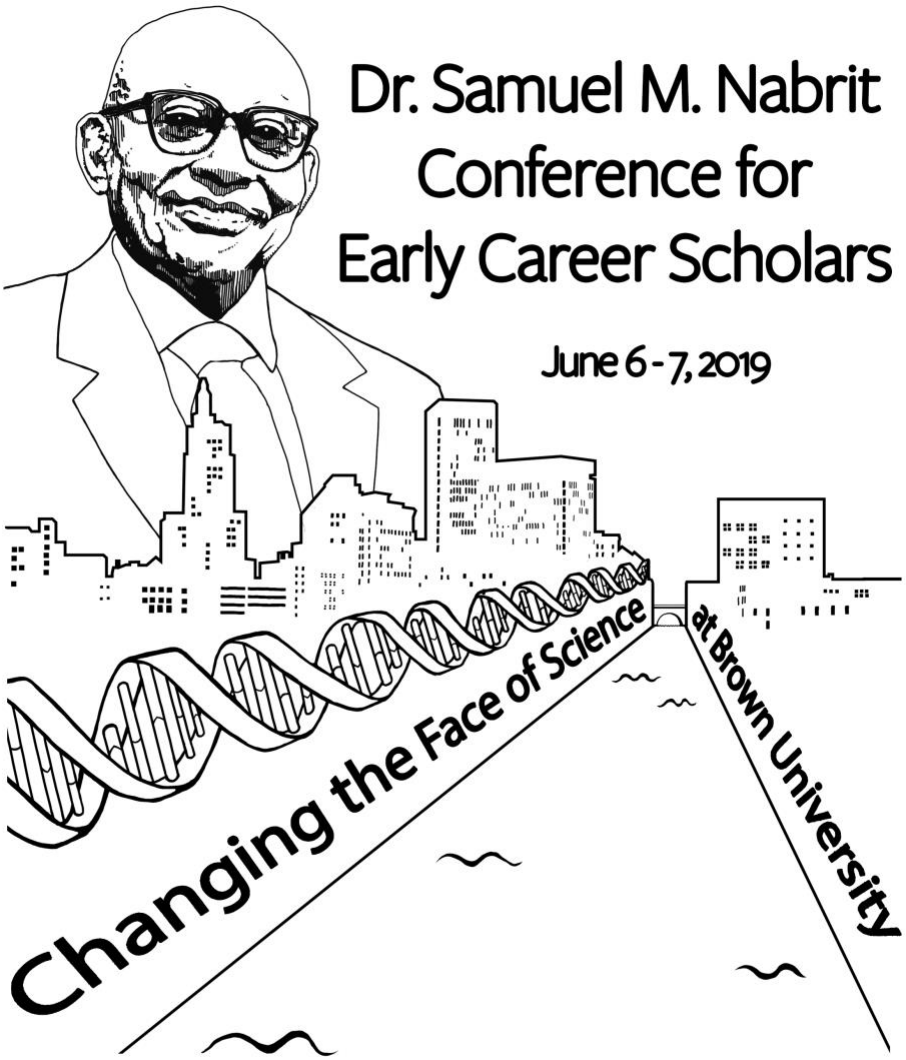
Abstracts of the presentations at the



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Thursday, June 6 – 8:05pm

Keynote Speaker

Karine Gibbs
Harvard University

Surface-surfing with a few million friends: how identity formation influences group behaviors of a bacterial opportunistic pathogen

Friday, June 7 – 9:00am

Short Talks Session I

Session Chair: Alison DeLong, Brown University

NAD+ metabolism during aging: Insight from quantitative flux analysis
Melanie R. McReynolds, Karthikeyani Chellappa, Connor Jankowski, Qingwei Chu, Sarmistha Mukherjee, Yashaswini Rajendra Bhat, Joshua D. Rabinowitz, Joseph A. Baur
Presenter affiliation: Princeton University

Ubiquitin-dependent modulation of cell fusion
Fernando Rodriguez Pérez, Michael Rape, Andrew Manford, Angela Pogson, Brenda Martinez Gonzalez
Presenter affiliation: University of California, Berkeley

Pro-proliferative role of Polo-like Kinase 4 in non-melanoma skin cancers
Debra Garvey, Mary A. Ndiaye, Nihal Ahmad
Presenter affiliation: University of Wisconsin-Madison

Neural pre-conditioning underlying oxygen deprivation in *C. elegans*
Heather Bennett, Robert G. Kalb
Presenter affiliation: Bard College

Friday, June 7 – 11:30am

Poster Session A

The high mobility group transcription factor Sox9b is necessary for great vessel and neurovascular development
Layra Cintron-Rivera, Catherine Seitz, Nathan Martin, Jessica S. Plavicki
Presenter affiliation: Brown University

How do cells track chemical signals? Evidence for an exploratory polarization model
Manuella R. (Rossie) Clark-Cotton, Nicholas Henderson, Daniel J. Lew
Presenter affiliation: Duke University School of Medicine

Elucidating BRPF1's role in regulating chromatin dynamics in Glioblastoma Multiforme
David Johnson, Alea A. Mills
Presenter affiliation: Cold Spring Harbor Laboratory

Mesenchymal-Amoeboid Transitions in Composite 3D Silk-Collagen Hydrogels
Amanda Khoo, Thomas Valentin, Susan Leggett, Dhananjay Bhaskar, Elisa Bye, Ian Wong
Presenter affiliation: Brown University

Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism
Vayu M. Rekdal, Elizabeth N. Bess, Jordan E. Bisanz, Peter J. Turnbaugh, Emily P. Balskus
Presenter affiliation: Harvard University

Differential integration of synaptic inputs by distinct classes of neurons in the thalamic reticular nucleus
Rosa I. Martinez-Garcia, Bettina Voelcker, Saundra L. Patrick, Barry W. Connors, Scott J. Cruikshank
Presenter affiliation: Brown University

Development of a novel 3D organoid model to study epithelial ovarian cancer growth and matrix invasion
Carolina Mejia Pena, Thomas Skipper, Jeffrey Hsu, Michelle R. Dawson
Presenter affiliation: Brown University

Friday, June 7 – 1:30pm

Keynote Speaker

Enrique De La Cruz
Yale University

How cells use chemistry and physics to break the bones that power their movement

Friday, June 7 – 2:30pm

Poster Session B

Evaluating calcium sensitivity in human induced pluripotent stem cell (hiPSC) derived cardiomyocytes
Alicia Minor, Kareen Coulombe
Presenter affiliation: Brown University

Calpain 1 as potential therapeutic target for Sickle Cell Disease
Farha Mithila, Toshihiko Hanada, Yunzhe Lu, Lidija Covic, Robert Flaumenhaft, Chao Fang, Glenn Merrill-Skoloff, Athar Chishti
Presenter affiliation: Tufts University School of Medicine, Beth Israel Deaconess Medical Center

Human nonvisual opsin 3 regulates pigmentation of epidermal melanocytes through functional interaction with MC1R
Lauren Olinski, Rana N Ozdeslik, Melissa M Trieu, Danield D Oprian, Elena Oancea
Presenter affiliation: Brown University

Calcium oscillations provide a molecular signature for barriers to interspecific hybridization
Nathaniel Ponvert, Mark A. Johnson
Presenter affiliation: Brown University

Exploring the role of MYP through the WNT and BMP pathways in the developing embryo of the sea star, *Patiria miniate*
Gerardo Reyes, Vargas, H., Foster, S., Onorato, T.M., Veras, I., Oulhen, N., Wessel, G.M.
Presenter affiliation: Brown University

Ferlin proteins in Toxoplasma gondii organelle biogenesis and Ca2+-dependent secretion
Daniel Tagoe, Allison A. Drozda, Bradley I. Coleman, Emily Stoneburner, Isabelle Coppens and Marc-Jan Gubbels
Presenter affiliation: Boston College

BRSK2 is a novel regulator of the oxidative stress responsive transcription factor NRF2
Tigist Tamir, Megan Agajanian, Brittany Bowman, Priscila Siesser, Dennis Goldfarb, Seth Weir, Ryan Murphy, Dahval Bhatt, Kyle Lepak, Ben Major
Presenter affiliation: University of North Carolina at Chapel Hill

Friday, June 7 – 3:15pm

Short talks Session II

Session Chair: Phyllis Dennery, Brown University

Is dynamic neuronal signaling necessary for developmental CNS myelinatin?
Sonia Mayoral, Ainhoa Etxeberria, Yun-An Shen, Jonah R. Chan
Presenter affiliation: University of California, San Francisco

A massively parallel reporter assay to investigate the contribution of noncoding variation in autism spectrum disorder
Tomás Lagunas, Stephen Plassmeyer, Joseph Dougherty
Presenter affiliation: Washington University in St. Louis

Crucial role of SH2B1 PH domain function for the control of energy balance and metabolism
Anabel Flores, Lawrence S. Argetsinger, Alvaro Malaga, Paul Vander, Lauren C. DeSantis, Erik S. Clutter, Martin G. Myers Jr., Christin Carter-Su
Presenter affiliation: University of Michigan

Engineered stem cell models for mechanistic studies of human kidney disease
Samira Musah
Presenter affiliation: Duke University

Neural pre-conditioning underlying oxygen deprivation in *C. elegans*

Heather Bennett, Robert G. Kalb.

Bard College, Department of Pediatrics, Division of Neurology, Research Institute, and Children's Hospital of Philadelphia, Pennsylvania, and the Department of Neurology, Perelman School of Medicine, University of Pennsylvania, and Department of Neurology, Northwestern University Feinberg School of Medicine

Neurons, like all cells, require oxygen for proper operation and complete lack of oxygen (anoxia) causes neuronal dysfunction and death. Sub-lethal stress prior to anoxic insult ("pre-conditioning") can protect neurons from subsequent oxygen deprivation, suggesting that mild cellular stress can enhance survival. However, the molecular mechanisms, and overall role of nervous system activity in the pre-conditioning response to oxygen deprivation have not been characterized. We are using Caenorhabditis elegans, to study the molecular pathways and neuronal circuitry underlying the pre-conditioning response to oxygen deprivation. In initial experiments, we find L4 stage animals are vulnerable to a 48 hour anoxic insult. To test the effects of pre-conditioning on anoxia, we temporarily hyperpolarized neurons by expressing the histamine gated chloride channel in all or select populations of neurons prior to a hypoxia survival assay. Interestingly, animals with complete loss of nervous system activity for 3 hours prior to anoxic insult have increased survival to 48 hours of anoxia. To determine the relevant neuronal populations that mediate resistance, we inactivated different neuronal subtypes and found that only loss of cholinergic and GABAergic activity confers a survival benefit. This result suggests that inactivation of neuromuscular signaling may underlie protection to anoxia. To test this we inhibited muscle activity and also observed a survival benefit. In summary, these results suggest that there is a novel and specific circuit between subsets of neurons to muscle causing lethality during anoxia, and blocking these circuits prior to oxygen deprivation is beneficial to the animal's survival.

The high mobility group transcription factor Sox9b is necessary for great vessel and neurovascular development

Layra Cintron-Rivera, Catherine Seitz, Nathan Martin, Jessica S. Plavicki

Brown University

SOX9 is an essential transcription factor for development of the central nervous system, cardiac valves, and chondrocytes. Human mutations in SOX9 are associated with great vessel and congenital heart defects (CHD). These clinical findings suggest SOX9 is involved in great vessel development; however, no systematic animal studies have examined the cellular and molecular basis of the observed phenotypes. Zebrafish are a great model for studying vascular development because embryos obtain oxygen through passive diffusion from water. Therefore, embryos can survive with severe vascular malformations which would be otherwise embryonically lethal in mammals. Zebrafish have two SOX9 orthologues, sox9a and sox9b. To determine sox9b's role in vascular development, we developed a dominant negative sox9b (dnsox9b) construct that competitively inhibit endogenous Sox9b from binding to its target genes. To manipulate Sox9b function, we generated transgenic fish with the dnSox9b construct fused to an "upstream activating sequence" (UAS) and then used cell-type specific Gal4 lines to drive expression of the dnSox9b. We used a ubiquitous Gal4 promoter to study the effects of global loss of Sox9b function and found great vessel development was severely disrupted. Since a subset of the neural crest gives rise to the cardiovascular system, we examined the effects of sox9b loss in neural crest derived cells. We used a sox10 Gal4 promoter to drive dnsox9b expression in the neural crest and found vessel development was disrupted and the brain was avascular. Together, these findings elucidate novel functions for sox9b and could ultimately further our understanding of CHD etiology.

How do cells track chemical signals? Evidence for an exploratory polarization model

Manuella R. (Rossie) Clark-Cotton, Nicholas Henderson, Daniel J. Lew

Duke University School of Medicine

Cells track chemical signals during feeding, development, and immune responses, but the mechanisms by which they locate the source of a signal remain elusive. Haploid yeast secrete a pheromone that binds receptors on the opposite mating type, triggering upregulation of mating-specific genes, concentration of polarity proteins into a "polarity patch", and polarized growth toward a mate. Counter to conventional models, live-cell imaging of mating cells recently revealed that clusters of polarity proteins transiently localize to different regions of the cell cortex during an "indecisive" period before entering a "committed" phase when the two partners' patches are oriented toward each other. To evaluate the role of clustering in partner selection, we characterized the behavior of wildtype cells attempting to mate with mutants defective in clustering. We found that wildtype cells did not commit to mutants that couldn't cluster. Other experiments indicated that coincident orientation of polarity clusters towards each other was necessary for commitment. Co-localization studies showed that both pheromone secretion and sensing occur at the transient clusters. These results provide evidence for an exploratory polarization model of partner detection, in which transient polarity clusters act as both mobile pheromone sources and sensors, and the co-orientation of two patches delivers a mutually-reinforcing concentrated pheromone signal that stabilizes both patches and initiates the commitment of both partners.

Crucial role of SH2B1 PH domain function for the control of energy balance and metabolism

Anabel Flores[1], Lawrence S. Argetsinger[2], Alvaro Malaga[2], Paul Vander[2], Lauren C. DeSantis[2], Erik S. Clutter[2], Martin G. Myers Jr. [1,2,3], Christin Carter-Su[1,2,3]

[1]Cell and Molecular Biology Graduate Program, [2]Department of Molecular and Integrative Physiology, [3]Department of Internal Medicine, University of Michigan

Mice lacking the adaptor protein, SH2B1, display severe early onset obesity and insulin resistance, similar to human patients containing SH2B1 mutations. Because several obesity-associated human SH2B1 mutations (including P322S) lie in the PH domain, we examined the role for the SH2B1 PH domain in mice. P322S/P332S homozygote mice exhibited substantial prenatal lethality; thus, we examined the metabolic phenotype of P322S/+ heterozygotes, revealing their late-onset glucose intolerance. To circumvent the lethality of P322S and permit the study of mice homozygous for PH domain mutations, we studied mice containing a 2-amino acid deletion within the SH2B1 PH domain (ΔP317,R318; ΔPR). Mice homozygous for ΔPR were born at the expected Mendelian ratio and exhibited obesity plus insulin resistance and glucose intolerance beyond that attributable to their increased adiposity. At the cellular level, the ΔPR deletion impaired the ability of SH2B1 to enhance neurite outgrowth in PC12 cells and changed the localization of SH2B1 from being primarily in the cytoplasm and plasma membrane to being primarily in the nucleus. Together, these results suggest that the PH domain may be a key regulator of SH2B1 subcellular localization, which in turn affects the ability of SH2B1 to enhance neuronal function and regulate energy balance and glucose homeostasis.

Pro-proliferative role of Polo-like Kinase 4 in non-melanoma skin cancers

Debra Garvey, Mary A. Ndiaye, Nihal Ahmad

University of Wisconsin-Madison

Nonmelanoma skin cancers (NMSC), including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are the most common cancers in the United States. Current therapeutic strategies are not highly effective to manage this neoplasm. Therefore, better understanding of NMSC biology may provide novel targets for management of these cancers. Polo-like kinase 4 (PLK4) is a serine/threonine kinase, known to play a role in cell division by regulating centriole duplication. As cancers regularly exhibit centrosome aberrations, PLK4's role in centriole duplication indicates that it may be a target for cancer management. Employing in vitro and ex vivo approaches, we found that PLK4 is significantly overexpressed in NMSCs. Further, employing multiple PLK4 small molecule inhibitors we found that PLK4 inhibition resulted in dose- and time- dependent decreases in growth and viability in vitro. Moreover, the inhibition of PLK4 resulted in a marked reduction in the clonogenic survival of A431 cells. Treatment with one inhibitor resulted in an induction of apoptosis, as detected using the RealTime-Glo Annexin V Apoptosis Assay, and immunoblot analyses of PARP cleavage, which was accompanied by a G1-phase cell cycle arrest of A431 cells. Interestingly, treatment with specific inhibitors resulted in a G2/M phase arrest, possibly due to increased mitotic errors. Immunofluorescence staining revealed irregular microtubule formation, an indicator of mitotic errors, along with notable centrosome amplification that may be attributable to partial PLK4 inhibition. This data suggests that PLK4 plays a pro-proliferative role in NMSC and warrants further study as a target and/or biomarker for NMSC management.

Elucidating BRPF1's role in regulating chromatin dynamics in Glioblastoma Multiforme

David Johnson, Alea A. Mills

Cold Spring Harbor Laboratory

Glioblastoma (GBM) is an aggressive form of brain cancer that is believed to originate from glial and neural stem cells. A myriad of proteins have been implicated in gliomagenesis, some of which, when dysregulated, revert these differentiated glial cell types to a "stem cell-like" state. Using a CRISPR-based genetic screen to deplete chromatin regulators in a panel of 7 human GBM lines our lab prioritized top hits that are both essential for survival and specific to GBM when compared to other cancers. Based on these findings and published literature, Bromodomain and PHD Finger Containing 1 (BRPF1) is a potentially novel therapeutic target that has yet to be studied in glioblastoma. BRPF1 is a chromatin reader that facilitates histone acetyltransferase activity, specifically promoting H3K23ac. Since BRPF1 appears to be a vulnerability and is specific to GBM, I hypothesize that deregulation of BRPF1 and the pathway(s) it regulates sets the stage for gliomagenesis. Using a combination of genetic, cellular, biochemical, and molecular techniques, I propose to: i) further define BRPF1 as a novel target for GBM by demonstrating that its depletion is detrimental to GBM survival, ii) determine the underlying mechanism by identifying downstream targets and interacting partners of BRPF1, and iii) assess its potential as a therapeutic target by evaluating the effects of depleting BRPF1 on gliomagenesis in vivo. Ultimately, this project will identify molecular mechanisms by which BRPF1 maintains the fitness of GBM, and assess the utility of inhibiting BRPF1 as a therapeutic target for GBM.

Mesenchymal-Amoeboid Transitions in Composite 3D Silk-Collagen Hydrogels

Amanda Khoo[1], Thomas Valentin[1], Susan Leggett[1,2], Dhananjay Bhaskar[1], Elisa Bye[1], Ian Wong[1,2]

[1]Center for Biomedical Engineering, School of Engineering. Brown University, [2]Pathobiology Graduate Program

Malignant cells can exhibit heterogeneous phenotypes when escaping the primary tumor into the surrounding stroma during the beginning of metastasis (Chaffer, 2011; Friedl, 2009). Invasive single cells, for example, can disseminate and migrate using mesenchymal or amoeboid motility, among other mechanisms (Pandya, 2016). Investigating these transitions may be relevant in our understanding of the persistence of the metastatic cascade. • Silk and collagen composite hydrogels allow for independent tuning of ligand density and mechanical properties. • We observe mesenchymal and amoeboid migration phenotypes in MDA-MB-231 breast cancer cells as a function of silk concentration in composite silk collagen hydrogels. • We observe MDA-MB-231 cancer cells invade across various matrix conditions, with decreasing invasion as a function of increasing silk concentration. • Sublethal dose of taxol does not stop invasion of MDA-MB-231 breast cancer cells, but does dramatically change the cell morphology in all matrix conditions.

A massively parallel reporter assay to investigate the contribution of noncoding variation in autism spectrum disorder

Tomás Lagunas, Stephen Plassmeyer, Joseph Dougherty

Department of Genetics at Washington University School of Medicine in St. Louis

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects ~1.7% of the population which leaves families and affected individuals with substantial lifetime costs. Current research has made significant investments in sequencing the genomes of ASD families as a diagnostic method and to further understand the genetic architecture of this complex disease. Whole Genome Sequencing (WGS) studies have revealed an enrichment of mutations in the untranslated regions (UTRs), which are noncoding regulatory regions, of ASD genomes. However, assessment of these variations poses a challenge since these regions do not follow the triplet code and, even with prediction algorithms for RNA secondary structure or motif loss, these mutations must be defined experimentally. To address these challenges, I have used a Massively Parallel Reporter Assay (MPRA) to functionally assay several hundred mutations in parallel. MPRAs are a novel molecular genetic tool for assaying hundreds to thousands of predefined sequences for functional effects in a high-throughput manner. From a completed assay that looked at 650 3' UTR mutations from ASD genomes, I have reported 12 candidates 3' UTR variations that appear to have functional effects on mRNA stability – indicating that some of these mutations may contribute to disease. These findings will justify the significant investments in sequencing patients with ASD by reporting on the burden of noncoding disease mutations and contribute to our understanding of ASD genetic architecture and UTRs.

Differential integration of synaptic inputs by distinct classes of neurons in the thalamic reticular nucleus

Rosa I. Martinez-Garcia [1,2], Bettina Voelcker[1], Saundra L. Patrick[1], Barry W. Connors[2], Scott J. Cruikshank

[1]Department of Neuroscience, [2]Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University

Most sensory information destined for the neocortex is relayed there from the thalamus, where considerable transformation occurs. One powerful means of transformation involves interactions between thalamocortical neurons that carry information to cortex and inhibitory neurons of the thalamic reticular nucleus (TRN) that regulate the flow of those data. Here, we describe a novel organizational and operational framework for somatosensory circuits in the mouse TRN. We show that there are two groups of genetically defined TRN neurons that are topographically segregated, physiologically distinct, and innervated by independent thalamic nuclei. These two structurally and functionally distinct cell types make discrete subcircuits in somatosensory TRN. Functionally, the two subcircuits have different dynamics determined by the two neurons' intrinsic physiology and the short-term dynamics of their thalamic input synapses. Our results suggest that the inhibitory output of each TRN subcircuit appears to be tuned to the temporal characteristics of the signals it preferentially processes.

Is dynamic neuronal signaling necessary for developmental CNS myelinatin?

Sonia Mayoral, Ainhoa Etxeberria, Yun-An Shen, Jonah R. Chan.

University of California, San Francisco

Myelin is important for proper nervous system function. In the CNS, specialized glial cells known as oligodendrocytes (OLs) differentiate from precursor cells (OPCs) and produce myelin by wrapping their plasma membranes around axons. Myelin's main function is to insulate axons and allow for the fast conduction of electrical impulses, and myelin damage can lead to permanent neurological disability. Currently no therapy exists for repairing this damage, but promoting OL differentiation and myelination is a promising strategy. It is therefore important to understand how these processes are regulated. Emerging evidence suggests that neuronal signaling is crucial for OL myelination, however the necessity of this signaling during development is unclear. By eliminating dynamic neuronal signaling along the developing optic nerve we find that OL differentiation and the initiation of myelination are not dependent on neuronal signaling. However, we find that myelination is dependent on a permissive substrate, namely supra-threshold axon caliber. We propose that OL differentiation is regulated by non-neuronal factors during optic nerve development, whereas myelination is sensitive to the biophysical properties of axonal diameter.

NAD+ metabolism during aging: Insight from quantitative flux analysis

Melanie R. McReynolds[2,3,4*], Karthikeyani Chellappa[1*], Connor Jankowski[2,3,4], Qingwei Chu[1], Sarmistha Mukherjee[1], Yashaswini Rajendra Bhat[1], Joshua D. Rabinowitz[2,3,4*#], Joseph A. Baur[1#]

[1]Department of Physiology and Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine, University of Pennsylvania, [2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, [3]Department of Chemistry, Princeton University, [4]Diabetes Research Center, University of Pennsylvania

Decline of NAD+ is associated with aging and disease; hence this critical metabolite is proposed as a master regulator of age-dependent pathology. NAD+ is found in all living cells and is an essential coenzyme that broadly impacts metabolism and protein covalent modification. NAD+ depletion contributes to mitochondrial dysfunction, which is a classic hallmark of aging. Redox state and the rates of synthesis and degradation of the NAD+ chemical backbone govern the cellular concentration of NAD+. NAD+ decline during aging reflects depletion of the total NAD(H) pool, rather a shift in the NADH/NAD+ ratio, suggesting defective NAD+ synthesis and/or over-consumption of NAD+ by cleaving enzymes. Concentration measurements are ill-suited to distinguish these possibilities. Thus, there is a critical need to examine NAD+ metabolic flux, i.e. the rates of production and consumption of the NAD+ chemical backbone. To this end, we used isotope-labeled NAD+ precursors, mass spectrometry, and quantitative modeling to determine NAD+ fluxes in young and aged mice. We first established an in-depth repertoire of NAD metabolism across 20 tissues in aged mice and found that NAD(H) and NADP(H) levels are decreased in multiple tissues. This reduction in NAD metabolites occurs without any limitation in abundance of NAD precursors in tissues. Tissue-specific measurements of NAD+ labeling enabled organ-specific assessment of NAD+ fluxes. These experiments support increased NAD consumption across several tissues rather than defective synthesis as the cause of reduced NAD levels with age. Our study will lay the foundation for understanding how NAD+ homeostasis is achieved and deranged during aging.

Evaluating calcium sensitivity in human induced pluripotent stem cell (hiPSC) derived cardiomyocytes

Alicia Minor, Kareen Coulombe

Center for Biomedical Engineering, Brown University

The movement of calcium ions across the membrane of muscle cells allow electrical stimuli to be translated into mechanical force. In the heart, the synchrony of electrical activation, calcium flux, and contraction of cardiomyocytes sustains cardiac output and heart function. The concentration of calcium in the heart averages about 1.8mM and facilitates cell activity. As human induced pluripotent stem cells (hiPSCs) become a popular cell source for cardiac tissue engineering applications, it is important that these cells resemble the native structure and function of cardiomyocytes. In this study, we cultured hiPSC-cardiomyocytes in a physiological concentration of calcium to determine how greater calcium levels affect the genetic and mechanical maturation of these cells. HiPSC-CMs were differentiated according to a well-established protocol. On day 7, the experimental group was cultured with media supplemented to 1.8mM calcium. Cells were cultured in these conditions for an additional 7 days then harvested to make engineered tissues. Mechanical assessment and real-time quantitative polymerase chain reaction (RT-qPCR) were used to quantify force production and gene expression changes. We found that the amount of force generated at a given length was not significantly different between control and experimental tissues. Control tissues had a significantly greater Young's modulus and were relatively more compact, suggesting that calcium may influence cell-matrix interactions. Most notably, tissues in the experimental group displayed a greater sensitivity to calcium in their environment producing greater force at a given calcium concentration when compared to controls. This sensitivity was also reflected in gene expression changes as there was an increase in L-type calcium channel expression and a significant increase ryanodine receptor expression. Our results support the use of physiologically relevant concentrations of calcium in cell media to enhance the growth and maturation of hiPSC-derived cardiomyocytes.

Calpain 1 as potential therapeutic target for Sickle Cell Disease

Farha Mithila, Toshihiko Hanada, Yunzhe Lu, Lidija Covic, Robert Flaumenhaft, Chao Fang, Glenn Merrill-Skoloff, Athar Chishti

Tufts University School of Medicine, Beth Israel Deaconess Medical Center

Sickle cell disease (SCD) is a genetic hematological disorder characterized by sickle shaped red blood cell and hemolytic anemia. SCD patients often possess inherently hyperactive platelets exhibiting increased vaso-occlusion and acute pain crisis ,Äì two major characteristics of SCD pathophysiology. Ubiquitous calpain 1 (CAPN1) is a calcium activated cysteine protease abundantly expressed in both platelets and red blood cells. Previously, our lab generated CAPN1 knock-out mouse model (CKO) in C57BL/6 genetic background. CKO platelets exhibit reduced platelet activity and accompanying hemostasis functions. More recently, our lab has generated CAPN1 null in Townes sickle cell disease mouse model (SSCKO) to evaluate function of CAPN1 in SCD. Here, we show that genetic knock out of CAPN1 in SSCKO rescues agonist induced hyperactive platelet aggregation of Townes sickle cell mouse model (SS). Interestingly, CKO platelets show both reduced overall cytosolic Ca2+ concentration and reduced Ca2+ release from the sequestered Ca2+ compared to WT upon agonist induced platelet activation. Similar experiment revealed significantly decreased calcium flux in SSCKO platelets compared to SS, mirroring in vitro platelet aggregation trends. Finally, CAPN1 knock out significantly reduced laser induced in vivo thrombosis in SSCKO compared to age and sex matched SS despite proportionate level of fibrin presence in both model. We conclude that CAPN1 might be attenuating platelet hyperactivity in SCD via reducing Ca2+ flux in platelet signaling cascade. Taken together, we propose that CAPN1 is a potential therapeutic target in SCD attributing to alleviated platelet hyperactivity.

Engineered stem cell models for mechanistic studies of human kidney disease

Samira Musah.

Departments of Biomedical Engineering & Medicine Duke University

More than 10% of the world's population suffer from kidney disease, and targeted therapeutic strategies are still needed. Functional in vitro models of the human kidney could facilitate therapeutic discovery and enable mechanistic studies of renal disease. Efforts to develop such models are limited by the lack of human kidney cells such as podocytes, which are the specialized epithelial cells that regulate selective permeability in the glomerulus. We developed a highly efficient method for differentiation of human induced pluripotent stem (iPS) cells into kidney glomerular podocytes. The iPS-derived podocytes express markers consistent with mature phenotype and exhibit primary and secondary foot processes. By using Organ Chip microfluidic devices, we developed an in vitro model of the human glomerular capillary wall that supports podocyte differentiation and recapitulate the normal tissue-tissue interface and selective permeability of the glomerulus. Our results demonstrate the feasibility of generating mature podocytes in a robust manner, providing an opportunity to engineer a functional human kidney model. These results could advance current understanding of developmentally regulated events in kidney pathophysiology and provide a robust platform for biomarker identification and the development of novel therapeutics for human kidney disease.

Human nonvisual opsin 3 regulates pigmentation of epidermal melanocytes through functional interaction with MC1R

Lauren Olinski[1], Rana N. Ozdeslik[1], Melissa M. Trieu[2], Danield D. Oprian[2], Elena Oancea[1].

[1]Brown University, [2]Brandeis University

Opsins form a family of light-activated, retinal-dependent, G protein-coupled receptors (GPCRs) that serve a multitude of visual and nonvisual functions. Opsin 3 (OPN3 or encephalopsin), initially identified in the brain, remains one of the few members of the mammalian opsin family with unknown function and ambiguous light absorption properties. We recently discovered that OPN3 is highly expressed in human epidermal melanocytes (HEMs)—the skin cells that produce melanin. The melanin pigment is a critical defense against ultraviolet radiation (UVR), and its production is mediated by the Gαs-coupled melanocortin 1 receptor (MC1R). The physiological function and light sensitivity of OPN3 in melanocytes are yet to be determined. Here, we show that in HEMs, OPN3 acts as a negative regulator of melanin production by modulating the signaling of MC1R. OPN3 negatively regulates the cyclic adenosine monophosphate (cAMP) response evoked by MC1R via activation of the Gαi subunit of G proteins, thus decreasing cellular melanin levels. In addition to their functional relationship, OPN3 and MC1R colocalize at both the plasma membrane and in intracellular structures, and can form a physical complex. Remarkably, OPN3 can bind retinal, but does not mediate light-induced signaling in melanocytes. Our results identify a function for OPN3 in the regulation of the melanogenic pathway in epidermal melanocytes; we have revealed a light-independent function for the poorly characterized OPN3 and a pathway that greatly expands our understanding of melanocyte and skin physiology.

Development of a novel 3D organoid model to study epithelial ovarian cancer growth and matrix invasion

Carolina Mejia Pena, Thomas Skipper, Jeffrey Hsu, Michelle R. Dawson

Department of Molecular Biology, Cell Biology & Biochemistry, Brown University, School of Engineering, Brown University

Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer with a mortality rate of over 60% (1). The lethality of this disease is due in part to the fact that 70% of all cases are diagnosed at advanced or metastatic stages, thus highlighting the incomplete understanding of the cues present in the tumor microenvironment promoting EOC metastasis. While two-dimensional (2D) in vitro studies of ovarian cancer have provided fundamental insight, three-dimensional (3D) culture systems that more accurately mimic the in vivo tissue microenvironment, are critical research tools in studying the progression and metastasis of ovarian cancer. A crucial characteristic that 3D models must incorporate is the well-established biophysical stimuli present in the tumor microenvironment that play a key role in tumor progression. Therefore, I am proposing to develop a robust 3D tissue culture model that allows for the long-term culture of EOC cells in a microenvironment which is rich in fibroblasts and cross-linked collagen. This 3D organoid model will be used to monitor the growth of EOC cells encapsulated in alginate microspheres and the invasion by the EOC cells of the matrix facilitated by the degradation of the alginate. This work is based on the central hypothesis that fibroblasts remodel the microenvironment of developing tumors to promote tumor growth and matrix invasion. This poster describes the optimization of the 3D organoid model.

Ubiquitin-dependent modulation of cell fusion

Fernando Rodriguez Pérez, Michael Rape, Andrew Manford, Angela Pogson, Brenda Martinez Gonzalez

University of California, Berkeley

Metazoan development is tightly dependent on the robust spatiotemporal execution of stem cell fate determination programs. Although changes in transcriptional and translational landscapes are well characterized throughout many differentiation paradigms, modulation of other regulatory processes during differentiation are poorly understood. Post-translational modification of proteins with ubiquitin has recently been found to be a key modulating step of developmental programs. Using a high-content imaging screen, we identified the E3 ubiquitin ligase CRL3-KCTD10 as a novel regulator of the late stages of myogenesis. Through an unbiased mass spectrometry approach coupled to genetic screening, we identified the BAIAP2-EPS8 complex, two key modulators of actin cytoskeleton morphogenesis, as a substrate of CRL3-KCTD10. CRL3-KCTD10 regulates the subcellular localization of the BAIAP2-EPS8 complex, thereby allowing for faithful myoblast fusion, an essential step in the generation of terminally differentiated muscle fibers. Our work highlights the importance of ubiquitin modifications in modulating the molecular underpinnings of cellular differentiation processes. Furthermore, we elucidate a new regulatory mechanism of muscle cell fusion, paving the way for possible therapeutic avenues for muscle degenerative diseases and myopathies.

Calcium oscillations provide a molecular signature for barriers to interspecific hybridization

Nathaniel Ponvert, Mark A. Johnson

Brown University

After landing on the stigma, pollen grains germinate a tube through floral tissue, and shuttle their cargo of sperm cells toward the female gametes. After reaching the female gametophyte, the pollen tube signals its arrival and identity to the receptive synergid cell. If successful, the pollen tube releases sperm for fusion with female gametes. Interspecific pollination results in signaling failure, and sperm is not released. Inactivation of the synergid cell-expressed FERONIA/LORELEI receptor complex results in similar pollen tube reception failure even during same-species pollination, and also attenuation of calcium ion oscillations that co-occur with successful pollen tube-synergid cell signaling. Given the similarity in pollen tube reception defects, we asked if interspecific pollen tubes fail to activate the FERONIA/LORELEI receptor complex. Strikingly, we find that interspecific pollen tubes are able to elicit calcium ion oscillations in the cytoplasm of wildtype synergid cells in a FERONIA/LORELEI-dependent manner. Our results suggest that genetic identity is communicated downstream of FERONIA/LORELEI activation. We also show that conspecific pollen tubes lacking a MYB-dependent gene regulatory network also elicit calcium ion oscillations despite an ultimate failure to release sperm, suggesting that MYB-dependent genes define a portion of the pollen tube's genetic identity.

Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism

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The human gut microbiota metabolizes the Parkinson's disease medication Levodopa (L-dopa), potentially reducing drug availability and causing side effects. However, the organisms, genes, and enzymes responsible for this activity in patients and their susceptibility to inhibition by host-targeted drugs are unknown. Here, we describe an interspecies pathway for gut bacterial L-dopa metabolism. Conversion of L-dopa to dopamine by a pyridoxal phosphate-dependent tyrosine decarboxylase from Enterococcus faecalis is followed by transformation of dopamine to m-tyramine by a molybdenum-dependent dehydroxylase from Eggerthella lenta. These enzymes predict drug metabolism in complex human gut microbiotas. While a drug targeting host aromatic amino acid decarboxylase does not prevent gut microbial L-dopa decarboxylation, we identify a compound that inhibits this activity in Parkinson's patient microbiotas and increases L-dopa bioavailability in mice.

Exploring the role of MYP through the WNT and BMP pathways in the developing embryo of the sea star, *Patiria miniate*

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Most oviparous animals store yolk proteins within their eggs. These yolk proteins are the source of nourishment during embryogenesis. For most vertebrates the yolk proteins are the vitellogenins. However, some echinoderms have a different yolk protein, called the major yolk protein (MYP). MYP was first observed in the sea urchin yolk platelets of the animal,Äôs oocyte migrating at a ~180 KDa size. The MYP protein is present during early stages of development until the larval stage when its abundance diminishes. MYP is also found in the sea star Patiria miniata. An alignment of the sea urchin and sea star shows that MYP protein sequences are 38% identical and contain the major domains of iron binding, originally seen in the sea urchin MYP. Surprisingly though, the MYP mRNA and protein of Patiria miniata are present in select structures of the larva, the right anterior pouch, the posterior enterocoel (the site of germ line formation), and the anal opening. The expression of MYP in a feeding larva is uncharacteristic to that of a yolk protein, which raises a question about its role as a true yolk protein, and its function. We are currently investigating its expression and function. How is MYP expressed in the germline and what does it do there so early in the formation of the new germ line?

Ferlin proteins in Toxoplasma gondii organelle biogenesis and Ca2+-dependent secretion

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The obligate intracellular parasite Toxoplasma gondii infects a third of the world's population and is responsible for birth defects and opportunistic infections. Pathology is driven by the lytic cycle made up of replication, egress, motility and invasion. In the sequential secretion of three organelles, micronemes, rhoptries, and dense granules Ca2+ signaling plays a pivotal role. Proteins with Double C2 (DOC2) domains are known Ca2+ sensors mediating protein secretion across eukaryotes. Of the five known DOC2 protein families, only two are represented in the Toxoplasma genome: three Ferlin family proteins (FER1-3) and one unconventional protein we named TgDOC2. To decipher the functions and mechanism of the Ferlins we generated a set of mutants. Overexpression of a dominant negative FER1 allele led to a defect in trafficking of fully processed microneme proteins in an apical compartment. On the other hand, overexpressing of full length FER1 led to a phenotype consistent with constitutive microneme secretion. Both these phenotypes cause a growth defect. FER2 is essential for secretion of the rhoptries. Interestingly, a gene knock-out of FER3 resulted in increased proliferation and accumulation of material in the parasitophorus vacuole. Together, these data indicate that Ferlin proteins have pivotal roles in trafficking, tethering and/or secretion.

BRSK2 is a novel regulator of the oxidative stress responsive transcription factor NRF2

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NRF2 is a transcription factor and master regulator of cellular antioxidant response essential for cell survival. NRF2 serves a cytoprotective role against oxidative stress, and is misregualted in neurodegenerative disorders, aging, and cancer. When the cell is healthy, the protective roles of NRF2 are not required, which leads to its degradation via the ubiquitin proteasome system. However, under conditions of oxidative stress, NRF2 is ubiquitylation is inhibited, allowing for NRF2 accumulation and subsequent transcriptional activation of ~200 cytoprotective genes. NRF2 target genes promote cellular fitness and significantly contribute to chemoresistance. This is important in cancers with aberrant NRF2 expression, such as lung, kidney, and bladder cancers. There is a continued effort to elucidate regulators and downstream effectors of NRF2 signaling. We addressed this problem by using a high throughput gain-of-function (GOF) screen of the kinome to identify activators and inhibitors of NRF2. Identifying kinase regulators of NRF2 is essential to our understanding of the pathway and its role in diseases. In addition, kinases are tractable drug targets with numerous small molecules in clinical trials for the above-mentioned cancers. Our initial results indicated that Brain Selective Kinase 2 (BRSK2) was a potent repressor of NRF2-mediated transcription. BRSK2, similar to NRF2, plays a major role in regulating endoplasmic stress; however, it is unknown if these two proteins affect each other. Our results show that overexpression of kinase active BRSK2 inhibits NRF2 function. Specifically, BRSK2 downregulates NRF2 protein levels. Continued experiments are focusing on the mechanism(s) of BRSK2 control over NRF2.