2016 Joint Biology Research Retreat

Abstracts

Table of Contents

Pippa Marrack

Chair, Biomedical Research
National Jewish Health

“T cell recognition of antigens”

In retrospect, the means by which T cells recognize foreign antigens has turned out to be just about as confusing as it possibly could have been. How could evolution have come up with such an unexpected way for T cells bearing alpha/beta receptors to realize that foreign invaders have entered the body? Antibody molecules, produced by B cells are relatively straightforward, they bind directly to the foreign invader, whether it is, for examples, influenza virus or tetanus toxin. T cells, on the other hand usually react only with fragments of the invader, usually peptides but sometimes lipids or even vitamin metabolities. these are not recognized on their own by T cells but rather only if they are bound to a major histocompatibility complex protein. This talk will describe how T cell receptors manage this unexpected task, and the ramifications of the phenomenon, for immune responses to invaders and in autoimmunity.

Teal Brechtel

MCB

Tricia Serio

"[*PIN*$^+$]ning down the relationship between variant characteristics and [*PSI*$^+$] inducibility"

Prions confer new phenotypes to cells by adopting an alternative fold that changes their function and by templating the conversion of normally folded protein to the same state. While we understand how prions in yeast maintain themselves in cells and colonies once they arise, the mechanism by which prions first appear is poorly understood. Our studies suggest that one prion, called [*PIN*$^+$] encourages the induction of another prion, called [*PSI*$^+$], by titrating away chaperones that would otherwise resolve the alternative protein folds as they initial arise and thereby inhibit [*PSI*$^+$] induction. My studies reveal additional evidence of chaperone titration by demonstrating that [*PIN*$^+$] interferes with the accumulation of [*PSI*$^+$] in cells already propagating the two prions when chaperone levels are reduced. We hypothesize that differences in [*PSI*$^+$] inducibility can be linked to differences in the degree of chaperone titration by distinct alternative forms of [*PIN*$^+$].

Nicolette Brown

MCB

Timothy Bolger

“Translational regulation in DDX3/Ded1 medulloblastoma mutants”

Many of the molecular mechanisms driving cancer progression remain unclear. Medulloblastoma is the most common pediatric malignant brain tumor. Although the overall survival rate is 70-80%, the current treatment protocol causes serious cognitive and endocrine long-term side-effects. Multiple concurrent genome-wide studies of medulloblastoma tumors identified the DEAD-box helicase, DDX3, as a significantly mutated gene within multiple subtypes of medulloblastoma tumors. DEAD-box proteins are the largest family of RNA helicases with critical and generally essential roles in multiple aspects of RNA metabolism. Proposed functions of DDX3/Ded1 include repression and activation of translation; however, the cellular mechanisms remain largely unknown. In particular, the relationship between Ded1’s biochemical activities and its translation or gene regulation role(s) is unclear. In order to examine the biochemical and cellular defects caused by the medulloblastoma-associated mutations (mam) identified in DDX3, we made equivalent, conserved mutations in the yeast gene DED1. To our intrigue, although ded1-mam cells exhibit growth defects, most do not display substantial defects in general translation. Alternatively, we have observed particular mRNA in yeast, such as subclasses of 5’ UTR structure, to be specifically affected, which may also be reflected in humans. Ultimately, our goal is to identify defects in the Ded1 molecular mechanism that will elucidate possible consequences of the DDX3 mutations in medulloblastoma.

Nichole Eshleman

MCB

Ross Buchan

“Determining the role of the TORC1 signaling pathway in regulation of mRNA stability during a stress response”

Regulation of gene expression is a crucial means by which cells adapt to changes in their environment. In yeast, a dramatic decrease in ribosome biosynthesis (Ribi) and ribosomal protein (RP) mRNAs is seen during the response to osmotic stress. This decrease depends upon the Target of Rapamycin Complex 1 (TORC1) signaling pathway, which current models suggest acts at the level of transcription. However, transcriptional shut off alone cannot explain completely the decrease in levels seen for these mRNAs. In addition, other studies suggest Ribi mRNAs are destabilized during osmotic stress. We therefore hypothesize that TORC1 is able to control not only transcription of mRNA but also mRNA decay. By having control over both mRNA synthesis and decay, the TORC1 pathway may be able to more quickly and efficiently regulate cellular adaptation to osmotic stress, as well as other changes in cellular environment.

Krysta Felix

IMB

Joyce Wu

“Segmented Filamentous Bacteria Confer Protection Against a Lung Infection Through Innate Mechanisms”

Segmented Filamentous Bacteria (SFB), commensal bacteria that colonize the small intestine under normal, healthy conditions, profoundly impact the host immune system. In particular, they induce protection against intestinal pathogens such as *Citrobacter rodentium* and *Entamoeba histolytica*, potentially in an IL-17-dependent manner. Immune responses initiated in the intestines can extend to other mucosal sites, such as the respiratory system. Because of this, we hypothesized that SFB colonization would increase resistance to a lung infection caused by *Streptococcus pneumoniae*. We found that SFB colonization does not make a difference in disease susceptibility in WT mice. We further identified B cells as key players in protecting mice against *S. pneumoniae* infection. This B cell-mediated protection requires B cell-intrinsic MyD88, as in contrast to mice receiving WT B cells, mice receiving MyD88-/- B cells are unable to maintain normal temperature after infection, a phenotype comparable to total lack of lymphocytes. In the absence of B cells, mice that lack SFB develop more severe disease, while those that are colonized by SFB maintain resistance, as demonstrated by protection against body temperature decrease and weight loss, as well as decreased lung bacterial load and lower neutrophil infiltration. These data suggest that SFB protect the host against *S. pneumoniae* infection in a manner dependent on the innate immune system. Our data demonstrate the significant impact of the gut microbiota on infections even in gut-distal locations such as the lungs.

Neng Ke

CBC

Katrina Miranda & Craig Aspinwall

“Detection of HNO production and the effect of HNO on cells”

Nitroxyl (HNO) donors induce a range of key pharmacological responses including vasoconstriction, enhancement of myocardial contractility, preconditioning against ischemia/reperfusion injury, induction of apoptosis and suppression of tumor angiogenesis, and inhibition of alcohol metabolism. Due to a rapid self-consumption reaction, it is difficult to directly detect HNO, hence various markers have been employed to trap HNO. We use glutathione (GSH), which is an abundant cellular species to trap HNO forming a stable adduct, glutathione sulfinamide (GS(O)NH2). This species can be quantified by a highly sensitive, high resolution technique called capillary zone electrophoresis-laser induced fluorescence (CZE-LIF). This enables us to validate the effect of HNO on cell, and to investigate the formation of HNO from various sources. Our lab is specifically interested in endogenous HNO production mechanisms/pathways.

Iris Ma

IMB

Maggie So

“A mouse model for *Neisseria* asymptomatic colonization and persistence”

A major roadblock to understanding the biology of commensal and pathogenic *Neisseria* is the lack of a suitable animal model. We have developed a mouse model for studying two important aspects of *Neisseria* biology (asymptomatic colonization and persistence) by pairing a new commensal species from a healthy wild mouse, *Neisseria musculi* (Nmus), with the well-studied lab mouse. We found that the susceptibility of the mouse to Nmus colonization depends on its genetic background and an intact innate immune system. In addition, the presence of a functional Type IV pilus also determine the susceptibility of Nmus colonization.

Ernesto Manzo

MCB

Daniela Zarnescu

“Uncovering cellular energetics at the neuromuscular junction in a Drosophila model of ALS”

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's Disease, is a fatal neurodegenerative disorder affecting upper and lower motor neurons (MNs). TAR DNA-binding protein 43 (TDP-43) is found in cytoplasmic inclusions in almost all non-SOD1 mediated ALS cases and is thought to play a major role in pathogenesis of the disease. Our lab has previously shown that overexpression of either wild type or mutant human TDP-43 in MNs of *Drosophila melanogaster* induces motor deficits and reduces lifespan. Using this model we have performed global metabolomics profiling and identified several significant changes consistent with alterations in glucose and lipid metabolism seen in the plasma of ALS patients.

Our preliminary data indicate that a high glucose diet suppresses toxic locomotor effects caused by TDP-43, SOD1, or C9ORF72 Drosophila models of ALS. Additionally, the genetic expression of either human glucose transporters 3 or 4 (hGlut3 or hGlut4), rescues impaired locomotor function caused by TDP-43. Using immunostaning techniques, we find alterations in hGlut3 expression both in Drosophila transgenics and induced pluripotent stem cells (iPSC) MNs derived from patients. To further test whether the expression of TDP-43 affects glucose transporter dynamics, we have used total internal reflection fluorescence (TIRF) microscopy, and found hGlut4 localization defects in primary MNs expressing TDPWT. This data suggests that hGlut3/hGlut4 expression and dynamics are altered, in both both fly and iPSC MNs, and are consistent with defects in glycolysis identified through metabolomics. Indeed, pfk mRNA, a key indicator of glycolytic activity is significantly upregulated in iPSC MNs with TDP-43 pathology. Taken together, our findings indicate specific metabolic alterations in ALS and highlight the predictive power of *Drosophila* as a model organism for human disease.

Michael Marty

CBC

New Faculty!

“Nanodiscs as a Launch Pad for Membrane Protein Mass Spectrometry”

Membrane proteins play critical biochemical roles. However, their poor solubility makes analysis challenging, and it is especially difficult to study interactions between membrane proteins and their surrounding lipid environment. Noncovalent or native mass spectrometry (MS) has proven to be a powerful tool for measuring lipids bound directly to membrane proteins, but conventional approaches using detergents for solubilization are only able to resolve a small number of bound lipid species.

Nanodiscs, which are discoidal lipid bilayers encircled by two amphipathic membrane scaffold proteins (MSP), offer an attractive alternative to detergent micelles due to their relative monodispersity. I will present the development of Nanodiscs as a vehicle for native MS, from early work with simple systems to large membrane protein oligomers.

Initial spectra of Nanodiscs without embedded membrane proteins demonstrated that they could be maintained intact in the mass spectrometer. Combining breakthroughs in instrumentation and data analysis has allowed us to count the number of lipids bound to membrane protein oligomers within the Nanodisc. From this, we discovered that membrane proteins ejected from Nanodiscs retain a large number of lipids bound in several distinct states. The largest distributions measured by MS agreed with the stoichiometry of the lipid annular belt predicted by molecular dynamics simulations.

Looking ahead, the techniques developed on bacterial membrane proteins are now being applied to more complicated mammalian membrane protein transporters. We are moving beyond homogenous lipid systems by using mixed-lipid Nanodiscs for studying lipid stoichiometry and composition. These experiments demonstrate the unique ability of native MS with Nanodiscs to measure a large number of protein-lipid interactions and explore the chemical interface between membrane proteins and local lipid environment.

Cynthia Miranti

CMM

New Faculty!

"The Tetraspansin CD82 is Required for OC and OB Differentiation and Bone Homeostasis"

Bone is a dynamic tissue, striking a balance between degradation and re-synthesis. Osteoblasts are derived from mesenchymal stem cells and terminally differentiate to build calcified bone. Whereas osteoclasts, derived from hematopoietic stem cells through the monocytic lineage, degrade bone. Soluble signals released from osteoblasts stimulate the differentiation of osteoclasts, and vice versa in a continuous cycle. Disruption of the cycle results in thinner or thicker bones which are more susceptible to fracture or improper healing. Direct adhesion to bone markedly enhances osteoclast differentiation *in vitro*. Differential gene expression analysis comparing isolated osteoclasts plated on bone fragments versus plastic and induced to differentiate identified several up-regulated genes, including tetraspanin CD82 which was up-regulated ~9-fold. Therefore, we hypothesized that mice deficient in CD82 expression would have bone abnormalities resembling those seen in mice with known defects in osteoclasts. CD82 is inducibly expressed in differentiating osteoclasts (OCs). We assessed OCs from global CD82-/- and LysM-Cre monocyte-specific KO mice. Abnormal TRAP+ OCs were seen in CD82-/- bones and CD82-/- OCs had several in vitro defects. CD82-/- OCs were super-fused, improperly polarized, the underlying actin cytoskeleton was disrupted, and bone matrix degradation was reduced. Classic OC differentiation markers were unchanged indicating differentiation per se was normal. These CD82-/- OC phenotypes are similar to those from Src, Syk, and Vav3 deficient mice. Correspondingly, Src, Syk, and Vav3 activation were lost in CD82-/- OCs. Syk-Vav3 signaling regulates actin cytoskeletal dynamics; CD82 loss resulted in reduced Rac activation. Integrin αvβ3, the ITIM receptor Clec-2, and its ligand podoplanin, were inducibly expressed during OC differentiation. All three proteins were decreased in CD82-/- OCs; but mRNAs were unaltered. Despite defective OCs, bone density in vivo was unaltered in the global KO mice; however, the total bone diameter was reduced and there were more adipocytes in the bone marrow suggesting additional defects in osteoblasts (OB). Correspondingly, CD82-/- mice had a reduced capacity to generate differentiated OBs in vitro. The commitment of bone mesenchymal cells to the OB lineage is dependent on adhesion and cell spreading. Thus, global loss of CD82 disrupts both OCs and OBs, leading to a mild bone defect. In both cases, this is likely due to defects in adhesion-induced signaling and actin cytoskeletal dynamics.

Marvin O'Ketch

IMB

Dominik Schenten

“Rig-I-like receptors (RLRs) directly regulate adaptive immune responses to WNV infection.”

The activation of pattern recognition receptors is a major regulatory checkpoint for the generation of adaptive immunity. Recognition of microbial RNA in the cytosol by Rig-I-like Receptors (RLRs) induces an antiviral state in infected cells and leads to the release of proinflammatory cytokines and interferons. RLRs are therefore important mediators of the innate immune response to many viral infections. However, the role of RLRs in the regulation of adaptive immunity is less well understood. Infection of mice deficient of the essential RLRs signaling adaptor MAVS, with West Nile Virus (WNV) results in a defective adaptive immune response. While this finding suggests a direct role for RLRs in the regulation of adaptive immunity to WNV, it is difficult to interpret due to the high viral titers in the absence of an IPS-1-dependent innate immune response. In order to overcome this caveat, we have infected IPS-1-deficient mice with a replication-incompetent mutant of WNV. In the absence of IPS-1, we find elevated levels of both CD4+ and CD8+ T cells in the draining lymph nodes and also an enlarged germinal center (GC) B cell compartment. Furthermore, despite the normal production of WNV-specific antibodies in the absence of MAVS, we observe a defect of these antibodies to neutralize WNV. These findings suggest that RLR-dependent signals indeed directly modify T and B cell responses to WNV. We are currently in the process of elucidating the molecular and cellular mechanisms responsible for the RLR-mediated control of adaptive immune response to WNV. The identification of these mechanisms will provide fundamental conceptual insights that will support the design of novel vaccine strategies to WNV in particular and RNA viruses in general.

Marco Padilla-Rodriguez

CMM

Gus Mouneimne

“Regulation of Metastasis by the Estrogen Receptor in ER+ Breast Cancer”

Estrogen receptor positive (ER+) breast tumors account for 75% of diagnosed breast cancer in the United States. These breast tumors are defined by their dependency on estrogen for continued growth. Paradoxically, clinical data has shown that invasive ER+ tumors are most commonly found in post menopausal women, when estrogen levels are at their lowest. Additionally, many studies have suggested that estrogen may protect against tumor invasion, however, how ER activity influences invasion remains poorly understood. During cancer cell invasion, the actin cytoskeleton undergoes a highly-coordinated reorganization, allowing cancer cells to migrate away from the primary tumor and spread to secondary tissues. How ER activity influences the actin cytoskeleton is not well understood. However, our lab has determined the actin elongation factor EVL as a transcriptional target of ERs, suggesting ER activity may influence actin remodeling by EVL transcription. Recently, we identified a novel actin structure, mediated by EVL, that suppresses membrane protrusions and 3D migration through the generation of Suppressive Cortical Actin Bundles (SCABs). In preliminary experiments, ER+ human breast cancer cell lines treated with estrogen generate SCABs, show reduced membrane protrusions and suppressed invasive potential in a 3D collagen matrix. Conversely, cells treated with ER antagonists show decreased SCAB formation, display abundant membrane protrusions and enhanced 3D migration. From these data, we propose a model in which ER activity suppresses cancer cell invasion through the EVL-mediated SCAB formation, generating an actin architecture that is unfavorable for invasion. If successful, our proposed model will characterize a mechanism by which ER activity plays a protective role against cancer cell invasion and influence future clinical studies focused on treatments for patients afflicted with ER+ breast cancer.

Andrew Paek

MCB

New Faculty!

“Live cell imaging of the chemotherapy response in single cells reveals mechanisms of resistance”

Many chemotherapeutic drugs kill only a fraction of cancer cells, limiting their efficacy. For many cancer types, the p53 transcription factor is a key player in the decision of whether a cell lives or dies in response to chemotherapy. We use live-cell imaging to investigate the role of p53 dynamics in the fractional response to chemotherapy. We have found that the probability that a cell will die in response to treatment depends on both the time and amount of p53 induction. Cells must reach a threshold level of p53 to execute apoptosis and this threshold increases with time after treatment. The increase in p53 apoptotic threshold is due to drug-dependent induction of anti-apoptotic genes in the inhibitors of apoptosis (IAP) family. In addition, we find that both p53 dynamics and cell fate are correlated in sister cells that divided before chemotherapy treatment. This suggests that non-genetic differences between cells might predispose cells to a particular cell fate.

Julieann Puleo

CMM

Gus Mouneimne

“EVL: A New Regulator of Focal Adhesion Maturation and Durotaxis”

Tumors exist within a complex microenvironment that includes an extracellular matrix composed of different biochemical and mechanical stimuli. These diverse environmental cues serve to actively regulate cellular behavior including tumor cell invasion and migration, processes that are required for cancer metastasis. Tumor cells have the ability to sense and respond to a multitude of stimuli present in the microenvironment including migration towards increasing chemical gradients (Chemotaxis) and increasing matrix stiffness (Durotaxis). While the specific signaling cascades responsible for sensing a chemical gradient have been well delineated, only recently have researchers begun to investigate the mechanisms responsible for sensing of the mechanical environment – specifically, the sensing of stiffness gradients. Due to the gaps in knowledge regarding the mechanisms responsible for Durotaxis, there exists an even greater deficiency in understanding what mechanisms regulate cellular navigation through a microenvironment consisting of both chemical and mechanical stimuli. This navigation is crucial to ultimately understanding tumor cell migration and the metastatic process. Resolving this larger question however, first demands a mechanistic understanding of the Durotactic process. Current literature in the field suggests that focal adhesions (adhesive plaques which connect the cell to the extracellular environment) are the sensory location responsible for Durotaxis. Preliminary data from our lab now presents EVL (an actin elongation factor that localizes to focal adhesions) as a unique and necessary regulator of focal adhesion dynamics, and of Durotaxis. Our overall hypothesis is that through actin polymerization at focal adhesions, EVL is able to mature focal adhesions to an extent necessary to withstand the mechanical demands of Durotactic migration.

John Purdy

IMB

New Faculty!

“Human Cytomegalovirus Remodeling of Host Metabolism “

Viral infection is a result of a multitude of interactions between viral and host factors. Host metabolic pathways play an essential role during replication of viruses, including human cytomegalovirus (CMV) since metabolism provides all of the energy and building materials required to construct an infectious virus particle. CMV is a β-herpes virus that is wide-spread, infecting a majority of humans. Infection is usually asymptomatic; however, pre-natal infection or infection of immunocompromised individuals may cause a wide-range of clinical illness including birth defects, hearing loss (in the case of pre-natal infection), and complications post organ transplantation. Previous systems-level examination of metabolites and their fluxes found that CMV notably increased the flow of carbon from glucose to fatty acid metabolism. Using high resolution mass spectrometry we established CMV dramatically increased synthesis of lipids with very-long chain fatty acid tails (VLCFAs) that are used to build the viral envelope. Recently, we found that metabolic remodeling by CMV may alter other cellular processes, specifically cell stress. Our data suggest that infection requires a carefully balanced metabolic hijacking program. Overall our findings demonstrate that CMV institutes a specific metabolism program to generate a unique cellular metabolite environment for the production of infectious viral progeny.

Casey Romanoski

CMM

New Faculty!

“Systems Genetics of Human Aortic Endothelial Cells For Atherosclerosis”

The buildup of plaque in arteries, or *atherosclerosis*, is the leading cause of heart attack and stroke in the Western World. Atherosclerosis is an inflammatory disease of the arterial wall that is caused by complex environmental and genetic risk factors. Endothelial cells, which form a single cell layer lining blood vessels, are important mediators of inflammation in atherosclerosis.

Genome-wide association studies have demonstrated that most disease variants for cardiovascular disease (and almost all complex diseases) are located outside of gene coding regions of DNA, thus demonstrating that regulatory function is a major disease mechanism. However, altered gene regulatory function in disease is challenging to predict from DNA sequence alone largely because it is highly cell type and context-specific.

This talk will outline how recent advances in systems genetics are being applied in my laboratory to identify the dynamic regulatory landscape perturbed in primary human aortic endothelial cells in inflammatory environments. By combining genome-wide molecular phenotyping with genetic variation, this project is aimed to better understand the regulatory mechanisms of human atherosclerosis.

Kun Xiong

MCB

Joanna Masel

“How does the complex structure of transcription factor networks emerge?”

Transcription factor networks (TFNs) display some interesting internal structures, such as power-law distributions of the node degrees, and enrichment for certain network motifs. These structures can perform special functions, leading to the suggestion that these observed structures are adaptations for these functions. An alternative view emphasizes the possibility that the same internal structures of TFNs could also arise non-adaptively, i.e. in the absence of selection for these particular functions. Here, we propose a model of the evolution of TFNs that includes the realistic simulation of mutations to the *cis*-regulatory binding sequences controlling the expression of TFs. We demonstrate how to use the model to test whether the over-representation of coherent type-1 feed-forward loops (C1-FFL) is an adaptation for resistance to external noise.

Sebastian Zeltzer

CMM

Felicia Goodrum

“Human Cytomegalovirus: All Roads Lead to the Early Endosome”

Endocytic trafficking is a critical mechanism by which cells sense and respond to their environment. Plasma membrane proteins encompassing mitogenic receptors, immune receptors, and nutrient transporters are all subject to endocytosis and subsequent trafficking. The ultimate fate of these cargos — recycled or degraded—influences both the immediate and long term survival of the cell. Human Cytomegalovirus (HCMV), a betaherpesvirus that infects the majority of the world, results in a significant remodeling of the host endocytic and secretory pathway for the production of virus. While numerous studies indicate how altered trafficking contributes to the assembly and envelopment of virus particles, little is known regarding how infection alters the trafficking and fate of host receptors. Here we report that infection with HCMV results in a significant alteration to the Clathrin Independent Endocytosis (CIE) pathway, targeting and retaining CIE receptors in an enlarged structure consistent with the early endosome. Further, we demonstrate that CIE receptors that ordinarily traffic independently of the early endosome are also rerouted and retained within the same structure. These findings represent a global change in the fate of numerous critical host proteins including the immune molecule Major Histocompatibility Complex I. Together these findings offer a novel means of HCMV induced immune regulation, as well as regulation of numerous CIE regulated signaling and growth pathways. Lastly, these results highlight the significance of altered receptor trafficking for survival of the host cell.

Nasiha Ahmed

MCB

Jacob Schwartz

“Mapping EWS-Fli1 Structure/Function to Transcriptional Reprogramming in Ewing’s Sarcoma”

Ewing’s sarcoma is the second most common pediatric bone cancer, driven by the novel oncogenic fusion protein EWS-Fli1. EWS-Fli1 is capable of modulating transcription of target genes to reprogram cells toward malignant transformation. However, the mechanism behind EWS-Fli1 activity to induce such global changes is poorly understood. What is known is that EWS-Fli1 has unique structural properties and cofactor interactions that contribute to its activity, but have yet to be mapped to its role in transcriptional regulation or tumorigenesis. We hypothesize EWS-Fli1 acts as a scaffold between transcriptional activators and repressors, such as LSD1, CBP, and p300, to initiate chromatin remodeling and transcriptional reprogramming in Ewing’s sarcoma pathology. Furthermore, as Ewing’s sarcoma is a prototypical model of transcriptional dysregulation in cancer, insight into the mechanism of how a single oncogenic factor is capable of rewiring cells toward tumorigenesis will illuminate transcriptional regulation pathways in overall tumor development.

Gaius Augustus

CBIO

Nathan Ellis

“The spectrum of somatic mutations in African American colorectal cancers”

Objective. African Americans have higher incidence and mortality rates for colorectal cancer (CRC) compared to other US populations, and they present with more right-sided, microsatellite stable disease and are diagnosed at earlier ages. To gain insight into these trends, we conducted whole exome sequencing and copy number analysis of African American colorectal cancer from the Chicago Colorectal Cancer Consortium.

Results. The frequency of APC mutations (27/43 = 63%) was lower than expected compared to TCGA NHW data (p=0.05). The group of CRCs without APC mutations (N=16) was associated with an earlier onset of CRC (51 vs. 62, p=0.01) and with a previous cancer (25%, p=0.06). The frequencies of mutations in the some driver genes identified by the TCGA (ACVR1B, EDNRB, FAM123B, GPC6, KIAA1804, NRAS, SMAD2, SOX9, CTNNB1, and TCF7L2) were much lower than expected in African American CRCs, which implies that other driver genes or other mutational mechanisms are driving African American CRC. By copy number analysis, we found that the gains and losses were broadly similar between African American and NHW CRCs, indicating that copy number variation does not explain the under-representation of known driver genes in African American CRCs. Examination of other possible cancer driver genes showed that somatic mutations in AXIN2, CHD5, FLT3, GLI2, SMO, and TOP1 were significantly more mutated as a group in African Americans compared to NHW CRCs (p=1.8 x 10-7).

Conclusion. Our results indicate that a novel group of cancer driver genes are operating in the development of CRCs in African Americans. Further studies are needed to investigate the biological bases for the differences in tumorigenesis between African Americans and NHWs.

Matthew Bienick

CBC

Indraneel Ghosh

“Design of Modular Switches for Allosteric Control over Protein Kinases and Protein Phosphatases”

Selectively modulating the activity of a desired enzyme in vivo is a major goal in protein design and can aid in the development of methods for understanding and rewiring signaling pathways. Protein kinases and phosphatases are complementary enzymes that catalyze the addition and removal of phosphate groups upon substrate proteins, respectively. Kinases and phosphatases are implicated in many signaling pathway and their deregulation is implicated in many diseases such as cancer. The high structural homology of these enzymes presents a challenge in designing selective inhibitors for understanding their cellular roles. Though powerful genetic knockdown or knockout tools exist, they are susceptible to compensatory cellular mechanisms and do not allow for titratable activity. We have addressed this problem by designing a potentially general allosteric approach for gating kinase and phosphatase activity. We have utilized the well-studied protein-protein interactions between Bcl-2 and BH3-only peptides and their small molecule inhibitors. We have designed a system where specific BH3-only peptides, 20 to 25-residues, are inserted into an enzyme, at predetermined non-homologous positions. BH3-only peptides, such as Bad, are unstructured but adopt a rigid, α-helical conformation upon the addition of a protein binding partner, such as Bcl-xL. Thus in our system, Bcl-xL acts as a poison and allosterically inhibits the function of the Bad-inserted-enzyme. Subsequently, the addition of a small molecule inhibitor, ABT-737, binds to and displaces Bcl-xL, acting as an antidote, thus restoring enzymatic activity. We have shown this method allows for controlling the activity of kinases and phosphatases with a small molecule in a dose dependent fashion both in vitro and in cellulo. We are currently optimizing these systems in order to study cell signaling and redesign pathways.

Matthew Bronnimann

IMB

Sam Campos

“Translocation of the Papillomavirus L2/vDNA Complex Across the Limiting Membrane Requires the Onset of Mitosis”

The human papillomavirus type 16 (HPV16) L2 protein acts as a chaperone to ensure that the viral genome (vDNA) traffics from endosomes to the trans-Golgi network (TGN) and eventually the nucleus, where HPV replication occurs. En route to the nucleus, the L2/vDNA complex must translocate across limiting intracellular membranes. The details of this critical process remain poorly characterized. We have developed a system based on subcellular compartmentalization of the enzyme BirA and its cognate substrate to detect membrane translocation of L2-BirA from incoming virions. We find that L2/vDNA translocation requires transport to the TGN and is strictly dependent on entry into mitosis. Cell cycle arrest causes retention of L2/vDNA in the TGN lumen; only release and progression past G2/M enables translocation and subsequent infection. Microscopy of EdU-labeled vDNA reveals a rapid and dramatic shift in vDNA localization during early mitosis. At late G2/early prophase, while the TGN is undergoing mitotic fragmentation, vDNA egresses from the TGN to a pericentriolar location, accumulating there through prometaphase where it begins to associate with condensed chromosomes. By metaphase and throughout anaphase the vDNA is seen bound to the mitotic chromosomes, ensuring distribution into both daughter nuclei. Mutations in a newly defined chromatin binding region of L2 potently blocked translocation, suggesting that translocation is dependent on chromatin binding during prometaphase. This represents the first time a virus has been shown to functionally couple the penetration of limiting membranes to cellular mitosis, explaining in part the tropism of HPV for mitotic basal keratinocytes.

Nico Contreras

IMB

Janko Nikolich-Zugich

“The Immunological Consequences of mCMV Infection on Adipose Tissue”

Cytomegalovirus (CMV) infection results in a lifelong and persistent infection that is characterized by stochastic bouts of replication. Furthermore, the primary and definitive location of viral replicative senescence has yet to be identified. As CMV has a broad tissue and cellular tropism the identification of a ‘viral reservoir’ has been difficult. The objective of this study was to investigate the potential involvement of adipose tissue in acute and chronic immune responses during CMV infection. Adipose tissue is a highly heterogeneous tissue containing the adipocytes and stromal vascular fraction (SVF). The SVF consists of numerous immune cells and specifically CD8a T cells, which are crucially important for the control of CMV infection. Inflammation within adipose tissue has been increasingly investigated in the context of obesity, but whether CMV infection adipose tissue and the downstream consequences of such an infection have not been reported. Here we demonstrate, using the mouse model of CMV infection (mCMV) that mCMV is capable of infecting the cellular constituents of adipose tissue and this results in a significant CD8a+ T cell response that is maintained in both the acute and lifelong infection. These results have far reaching implications for metabolic health, increase our knowledge of mCMV tropism, and identify a neglected reservoir for viral replication and persistence.

Avery DeVries

CMM

Donata Vercelli

“Neonatal Smad3 Promoter Hypermethylation Predicts Asthma In Children Of Asthmatic Mothers From Three Birth Cohorts”

Asthma is the most common chronic disease of childhood, but the timing and mechanisms of disease inception remain imprecisely defined. In this respect, epigenetic mechanisms are worth investigating because of their likely contribution to the developmental and environmental components of asthma pathogenesis. However, little is known about asthma epigenetics. Our recent epigenome-wide analysis of DNA methylation in the Infant Immune Study (IIS), an unselected birth cohort followed to age 9 for asthma diagnosis and active symptoms, identified 589 regions in cord blood mononuclear cells (CBMCs) that were differentially methylated (DMRs) in subjects who did and did not develop asthma by age 9. Subsequent analyses focused on the DMR in the promoter of SMAD3, an immunoregulatory transcription factor that emerged as the most connected hub in the network of asthma-associated DMRs. Our goal was to further characterize the association between neonatal SMAD3 methylation and asthma during childhood and most importantly, to assess whether the association detected in IIS could be confirmed in comparable birth cohorts.

Brittany Forte

CBIO

Sam Campos

“Activation of Cytosolic DNA Sensors Depends on Golgi Integrity”

Oncogenic human papillomaviruses (HPVs) cause nearly 5% of all cancers worldwide, and essentially all cervical cancers. During infection, HPV traffics its viral genome (vDNA) to the nucleus; minor capsid protein L2 facilitates trafficking of the L2-vDNA complex to the Golgi, where it resides during interphase. During mitosis, the L2-vDNA complex leaves the Golgi lumen and translocates to the nucleus, initiating viral infection. Innate cytosolic DNA sensors (CDS) recognize cytosolic DNA as a pathogen-associated molecular pattern. Activation of CDS results in the stimulation of the type-I interferon response via translocation of ER-resident Stimulator of Interferon Genes (STING) to the Golgi, where it recruits TANK binding kinase 1 to phosphorylate and activate interferon regulatory factor 3 (IRF3). CDS pathways are predicted to be inactive during open mitosis to avoid detection of self-DNA, however this has never formally been tested. We hypothesize STING-dependent CDS pathways are inactivated during mitosis due to the natural dispersal of the mitotic Golgi. Further, we hypothesize HPV has specifically evolved to traffic to and translocate from the mitotic Golgi as an immunoevasive tactic to avoid detection by STING-dependent CDS pathways. To test our hypothesis, HaCaTs, human keratinocytes, were transfected with foreign nucleic acids and analyzed for CDS activation. Nucleic acid transfection resulted in phosphorylation and nuclear translocation of IRF3 as well as translocation of STING to the Golgi, indicating CDS pathway activation. Strikingly, chemical disruption of the Golgi ribbon decreased the phosphorylation and nuclear translocation of IRF3 in response to foreign nucleic acids. Thus, CDS pathways may be inactivated by Golgi dispersal during mitosis, allowing vDNA to evade CDS detection during translocation from the safety of the Golgi to the cytosol/nucleus during infection.

Kotaro Fujimaki

MCB

Guang Yao

“Autophagy plays a critical role in maintaining quiescence depth”

Cellular quiescence is a phenomenon in which cells are maintaining its proliferative ability while ceasing in cellular proliferation. In other words, quiescent cells are those that are not proliferating but do proliferate when the right stimulus comes. Cellular quiescence is prevalent in many biological processes such as tissue regeneration or cancer dormancy. Thus it is imperative to dissect the mechanism of quiescence if we were to understand these processes. However much fewer researches have been conducted on cellular quiescence compared to proliferation. One of the major interests of our lab is the molecular mechanism of quiescence heterogeneity: some quiescent cells require more stimulus to start proliferation (termed “deeper”), and some require less (termed “shallower”). Whether the cells are in deeper or shallower quiescence can greatly alter the outcome of the aforementioned processes, and thus imperative to understand.

We have conducted RNA-Seq experiment on rat fibroblast cells that were induced to different quiescence depth by varying durations of serum deprivation. By conducting bioinformatics analysis on the RNA-Seq data, autophagy has emerged as a candidate function that is increased while cells move deeper into quiescence. By inhibiting lysosomal function and thus autophagy, we have observed that cells that are inhibited in autophagy require more stimulation to re-enter the cell cycle compared to the control. Furthermore, we have observed that the influence of autophagy inhibition is greater on deeper quiescent cells compared to shallower quiescent cells. Our experimental results and analysis suggest that autophagy plays a critical role in maintaining cellular homeostasis to allow the cells to respond to growth stimulus properly, especially in deeper quiescent cells.

Xuezhen Ge

MCB

Tricia Serio

“The Effects of Environmental Stresses on Prion Propagation in Yeast”

Prions are proteins that can adopt multiple self-replicating conformations, which can confer transmissible epigenetic traits including infectious neurodegenerative diseases in mammals and heritable phenotypes in yeast. Unlike stress-induced aggregates of misfolded proteins, which can be resolved by the cellular quality control (proteostasis) network, the ordered amyloid aggregates formed by prion proteins are resistant to clearance under normal growth conditions. However, recent work in our lab showed heat shock unexpectedly disassembles prion aggregates by altering the accumulation of a quality control factor, the chaperone Hsp104, through asymmetric retention in cells that experience the stress. To gain insight into the components of the network that promote prion clearance, we have been investigating the impact of a range of other stress conditions (i.e. H2O2, ethanol, DTT and KCl) on prion propagation and prion aggregate clearance. Although all treatments alter the steady-state size of prion aggregates and by inference protein misfolding/resolution dynamics, only ethanol treatment leads to prion loss. As expected, factors known to be important for prion propagation, such as Hsp104, Ssa1 and Sis1, are efficiently induced upon ethanol treatment, and inhibition of Hsp104, by either treatment with a specific inhibitor GdnHCl or heterozygous disruption, blocks ethanol-induced prion curing, indicating that this process is Hsp104 dependent. In addition, the asymmetric retention of Hsp104 is also required for prion curing upon ethanol treatment, as alterations to the yeast bud neck, which are known to increase chaperone transmission, also block curing. Thus, heat shock and ethanol appear to alter cellular proteostasis through a similar pathway to evoke prion curing.

Jeffrey Grover

MCB

Rebecca Mosher

“AGO4 Nuclear Import and Export Are Necessary For DNA Methylation and Transposon Silencing By RdDM”

RNA-directed DNA Methylation (RdDM) is an epigenetic pathway that results in the establishment of DNA methylation at cytosines in all sequence contexts and transcriptional gene silencing (TGS) of transposable elements, genes, and virus-derived sequence. RdDM requires 24 nucleotide short interfering RNAs (siRNAs) produced by sequential action of a plant-specific RNA polymerase (Pol IV), an RNA-dependent RNA polymerase (RDR2), and dicer (DCL3) to be bound by an Argonaute protein (AGO4) in order to establish DNA methylation at genomic loci through interaction with a scaffold transcript produced by Pol V, and recruitment of the methyltransferase DRM2. Motif prediction software identifies three putative nuclear localization sequences (NLSs) and two nuclear export sequences (NESs) within the Arabidopsis thaliana AGO4 protein sequence. We have investigated AGO4 localization through targeted mutagenesis of each NLS or NES on AGO4. Disrupting NLSs or NESs results in reduced or increased nuclear AGO4 by microscopy, respectively, impaired RdDM by methylation-sensitive qPCR at RdDM target loci, and defective transposon silencing. Additionally, transient expression of heterologous protein with ectopic NLSs from A. thaliana AGO4 demonstrate they are sufficient to cause nuclear import. This expands upon previous work in the field by demonstrating an important role for nuclear export of AGO4 and multiple potential routes of nuclear entry. Such behavior may indicate nuclear-cytoplasmic cycling, and function for a cytoplasmic pool, of AGO4 in RdDM.

Alex Hamby

MCB

Charles Wolgemuth

“Swimming bacteria power microspin cycles”

Dense suspensions of swimming bacteria are living fluids, an archetype of active matter. For example, *Bacillus subtilis* confined within a disk-shaped region forms a stable vortex that counter-rotates at the periphery. Here we examined *Escherichia coli* under similar confinement and found that these bacteria, instead, form microspin cycles: a single vortex that periodically reverses direction on timescales of seconds. Using experimental perturbations of the confinement geometry, medium viscosity, bacterial length, density and chemotaxis pathway, we show that morphological alterations transition a stable vortex into a periodically reversing one. We develop a mathematical model based on single cell features that quantitatively recreates the dynamics of these vortices and predicts that density gradients power the reversals. Our results define how microbial physics drives the active behavior of dense bacterial suspensions and may allow one to engineer low Reynolds number micromixers for use in applications such as drug discovery.

Christy Harrison

IMB

Pawel Kiela

“Interactions between sodium transport and the microbiome in the development of colitis”

The microbiome is an increasingly appreciated aspect of our health, and has been found to be altered in numerous chronic disease states, including inflammatory bowel disease (IBD). Sodium exchange in the colon is mediated largely by the sodium hydrogen exchanger NHE3, which is disrupted during active IBD. Our lab has previously demonstrated that an endogenous defect in sodium transport through loss of NHE3 is sufficient to drive a spontaneous colitis in mice that is ameliorated by the administration of broad-spectrum antibiotics, suggesting a required role of the microbiome in this process. We asked whether the microbiome alone was sufficient to transfer this susceptibility to healthy mice. To address this, we transplanted fecal microbiome from NHE3 -/- mice into NHE3-wildtype mice in two different models of colitis: Il-10 -/- and CD4+CD45RBHI T cell transfer into Rag -/- recipients. In both Il-10 -/- colitis and T cell transfer colitis, and regardless of whether recipients were cleared with antibiotics or germ-free to begin with, mice who had received fecal transplant from NHE3 -/- donors developed a more pronounced disease phenotype than those that had received fecal transplant from NHE3 +/+ controls. However this susceptibility was not as drastic as in mice that endogenously lacked NHE3, indicating that the microbiome alone could not recapitulate full colitic susceptibility. This suggests that some colitogenic capacity exists in the microbiome from NHE3 -/- mice, but also that the ongoing dysfunction of NHE3 itself in IBD may be an important and necessary factor driving colitogenic dysbiosis and disease. Additionally, it subtly highlights not just the influence of the microbiome on the host, but the reciprocal influence of the host’s genetics and microenvironment on the microbiome and its functional outcome in disease.

Vic Keschrumrus

BME

Henk Granzier

“Drug Screening and Therapy Development Using Engineered Heart Tissues”

Engineered heart tissues (EHTs) are three-dimensional, fibrin-based heart muscle constructs generated for a 24-well format. They are capable of generating advanced in vitro disease models for studying biological cues involved in cardiovascular disease. These constructs allow for high throughput screening of drugs and therapies in a fully enclosed and automated environment. They can be made from a variety of cell sources including primary cell lines and human induced pluripotent stem cells. When combined with clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) technology, EHTs have the capacity to reproduce known pathological mutations and validate patient-specific therapies. Isoprenaline, a β- adrenoreceptor agonist, was administered to EHTs to observe their response over time at increasing concentrations. Using a custom-built analysis system, we collected data on beats per minute, contraction velocity, relaxation velocity, average force produced, contraction time, and relaxation time. The results prove that EHTs have the capacity for preclinical applications and can potentially reduce the labor and costs associated with animal models and drug development.

Balazs Kiss

CMM

Henk Granzier

“Nebulin Modulates The Left-Handed Helical Pitch And Cross-Bridge Behavior Of Thin Filaments”

Nebulin, the giant sarcomeric protein, interacts and colocalizes with thin filaments in skeletal muscle. Although nebulin is thought to play a fundamental role in thin filament length regulation, little is presently known about the nanoscale basis of nebulin’s influence on the thin filament structure in passive and contracting skeletal muscle. In the present work we investigated the ultrastructure of soleus muscle dissected from conditional nebulin-knockout (cKO) and control mice by using small-angle X-ray diffraction.
Soleus samples were dissected and mounted vertically in a custom-made, temperature controlled experimental cell filled with oxygenated Ringer solution. Each muscle was stretched to L0 followed by tetanic activation. X-ray diffraction images were recorded in both the passive state and during tetanic contraction using a high-flux 12 keV X-ray beam. In order to characterize the helical ultrastructure of the thin filaments we studied the 5.1 nm and 5.9 nm meridional reflections. To assess the mass distribution between thick and thin filaments the 1,0 and 1,1 equatorial spacings and intensities were analyzed.
While the passive 5.1 nm spacing does not differ significantly between cKO and the control group, the 5.9 nm spacing was found to be 5.914±0.002 nm in control and 5.888±0.002 nm in cKO, respectively ($p<0.0001$). The significantly shorter left-handed thin filament helical pitch is maintained during contraction in cKO muscles. Through the equatorials we found significantly less mass attached to the thin filaments in passive cKO muscles and inefficient myosin mass transfer during their contraction. The myofilament lattice spacing tends to increase with tension in cKO but decreases in control samples. These findings suggest that nebulin plays a role in thin filament structure and cross-bridge behavior.

Josh Kochanowsky MCB

Anita Koshy

“CRISPR/Cas9 mediated gene disruption of Toxoplasma gondii”

Toxoplasma gondii is a model for studying the phylum Apicomplexa, in part due to the availability of various genetic tools and its ability to be readily cultured. Genetic tools exist for widely utilized laboratory strains. However, these techniques are not easily implemented in non-laboratory strains. Here we utilized a CRISPR/CAS9-system to disrupt genes in *T. gondii*. Our CRISPR/CAS9 system provided an efficient system for targeted gene disruption.

Luke Kosinski

MCB

Joanna Masel

“Translational errors purge structural order from potential polypeptides, facilitating future innovation”

De novo genes are genes that originated from ancestrally non-coding DNA and are a source of genetic and phenotypic novelty, but how they emerge is unclear. Non-coding sequences may be translated in error. Their polypeptide products may interfere with existing cellular machinery, making them deleterious, or may have a minimal impact, making them benign. The magnitude of their impact is expected to depend on the level of their expression, with highly translated polypeptides having greater effects. We hypothesized that selection acts on non-coding sequences when error rates are high, purging deleterious polypeptides and increasing the pool of benign variants capable of being coopted as a de novo gene.

To test our hypothesis, we examined genes in Saccharomyces cerevisiae for an association between readthrough frequency, measured by ribosome profiling, and whether the readthrough product is benign. High intrinsic disorder (ISD), measured here using IUPred, is likely to indicate a benign polypeptide because high ISD polypeptides are hydrophilic and, as a result, tend to avoid dangerous amyloid aggregation. This is supported by the fact that the average ISD of 3′ untranslated regions (3′ UTRs), which are non-coding and may be deleterious if expressed, is much lower on average than the ISD of coding regions, which are regularly expressed and cannot be deleterious. We then tested whether the ISD of readthrough products increases with readthrough frequency and found a five-fold increase in readthrough from low to high ISD. This indicates that selection acts on 3′ UTRs in S. cerevisiae to purge low ISD variants in favor of high ISD variants as error rates increase.

Sarah Kwon

MCB

Guang Yao

“Controlling Depth of Cellular Quiescence by an Rb-E2F Network Switch”

Quiescence is a reversible non-proliferative cellular state that plays a critical role in the health of higher organisms. Reactivating quiescent cells to proliferate is fundamental to tissue repair and regeneration. Often described as a “G0 phase”, quiescence is in fact not a single homogeneous state. Previous studies have shown that as cells remain quiescent for longer durations, they move progressively “deeper” into quiescence and exhibit a prolonged standby period before reinitiating DNA replication upon growth stimulation. Yet, control mechanisms underlying deep vs. shallow quiescence remain elusive. Here by coupling modeling and single-cell measurements, we show that the depth of cellular quiescence is controlled by the activation threshold of an Retinoblastoma (Rb)-E2F network switch. Particularly, deeper quiescent cells feature a higher Rb-E2F activation threshold, require a stronger growth stimulation to exit quiescence, and exhibit a delayed traverse through the restriction point. We further show that different Rb-E2F pathway components have varying efficacies in regulating Rb-E2F activation threshold and quiescence depth. The ability to manipulate cellular quiescence depth may provide a future strategy against hypo- and hyper-proliferative diseases.

Rachel Langston

MCB

Ted Weinert

“Errors in telomeric DNA replication lead to chromosome instability within a single cell cycle”

Errors in DNA replication can trigger cycles of chromosome instability that then lead to gross chromosomal rearrangements (GCRs). Here we report how defects in Cdc13, a telomere specific protein, can cause chromosome instability that originates in the telomere of Saccharomyces cerevisiae. We find that cdc13-induced unstable chromosomes arise in a single cell cycle, require passage through S phase, and synergize with defects in DNA replication. We propose that after replication error, sister chromatids fuse by a process that likely requires ssDNA but not canonical double strand break repair pathways. This system, using a conditional Cdc13 mutation, promises a more complete ontogeny of chromosome instability: in this case from the initial telomere replication error to formation of unstable chromosomes and their subsequent resolution.

Frank Li

CMM

Henk Granzier

“Nebulin’s C-terminus is Necessary for Sarcomere Structure and Function”

Nebulin is a massive structural protein that is bound to most of the sarcomeric thin filament, yet despite the strides to fully characterize this protein, its functions within the sarcomere have only been inferred through complete knockout models. In order to avoid the compounding effects of a loss of multiple protein interactions, it is necessary to study the functions of specific regions of the protein instead. We created a mouse lacking only the unique C-terminal domains of nebulin in order to study their function within the Z-disc as well as their importance to the sarcomere. We found that such a small truncation was able to induce a myopathy phenotype in mice. Most limb muscles showed signs of underdevelopment, contributing to an overall lighter and smaller mouse. Investigation into protein expression and interactions found that structural proteins like T-mod and α-actinin were up-regulated. These findings correlated with structural abnormalities observed in electron microscopy studies. And while nebulin protein levels varied, near normal levels of nebulin did not correlate to a healthier, more functional muscle. In fact, in the soleus, where there was a larger portion of normal-looking sarcomeres and normal levels of nebulin, there still remained a significant force deficit, suggesting that the C-terminus plays a role in force production as well. Studies into growth pathways are ongoing but seem to suggest that, while growth-promoting phosphorylation of GSK-3β is up-regulated, the expression of GSK-3β undergoes a greater up-regulation, leading to a loss in muscle mass. From this study, we have determined that nebulin’s C-terminus is vital to structure and function, and its loss alone is sufficient to result in myopathy phenotypes.

Andres Morera

MCB

Jacob Schwartz

“Transcriptional regulation by TDP-43”

Amyotrophic Lateral Sclerosis (ALS) is an age-related neurodegenerative disease of motor neurons, leading to gradual paralysis and death within 2 - 5 years of symptom onset. The RNA-binding protein TDP-43 is commonly observed in aggregates within motor neurons and surrounding glia in patients, and is mutated in a subset of cases. TDP-43 can also associate with chromatin and regulate transcription. This current work is focused on characterizing transcriptional targets of TDP-43, and testing the structure/function relationship between TDP-43 and transcriptional output. In support of at least one model of transcriptional regulation by TDP-43, we find that TDP-43 may bind RNA Pol II.

Fen Pei

MCB

Tricia Serio

“Determining the Mechanism(s) of Dominant-Negative Inhibition of Different Prion Strains”

The prion mechanism underlies several previously inexplicable phenomena, including transmissible neurodegenerative disease in mammals and the non-Mendelian inheritance of unique traits in fungi. Despite the efficient and autocatalytic pathway of prion protein misfolding, dominant-negative prion mutants are able to interfere with this pathway for wild-type prion proteins. However, controversies persist about the actual mechanism of dominant-negative inhibition. For example, in the yeast S. cerevisiae, the inhibitory mechanism of G58D, a dominant-negative mutant of the prion protein Sup35, was linked to either an enhancement of the fragmentation reaction or to a failure to transmit existing aggregates to daughter cells upon division. These studies used different strains of the prion protein, raising the possibility that the mutant could impact prion propagation through distinct pathways depending upon Sup35 conformation. To resolve this controversy, we have compared the mechanism of prion curing in the [PSI+]Strong, [PSI+]Sc4 and [PSI+]Weak Sup35 strains. Our studies indicate that the G58D mutant inhibits propagation of all Sup35 conformations in a dose-dependent manner, leading to a decrease in aggregate thermodynamic stability. We show that incorporation of G58D into wildtype prion aggregates composed of any of these conformations promotes aggregate disassembly in vivo, leading to a change in the number of transmissible aggregates. Heterozygous disruption of Hsp104, the cellular factor mediating prion aggregate fragmentation, partially reversed the dominant-negative effects of G58D expression. Despite these mechanistic commonalities, the ratio of mutant to wildtype protein required for dominant-negative inhibition correlates with differences in their basal thermodynamic stabilities. Thus, the conformational variation does not modulate the mechanism by which the inhibition occurs but rather the sensitivity of these strains to prion loss.

Kelvin Pond

CMM

Nathan Ellis

“Regulation of Homologous Recombination by NSMCE2”

Background: DNA damage generated during replication is a major source of mutations and failure to repair this damage can cause genomic instability. Homologous Recombination (HR) is a high-fidelity DNA repair pathway and numerous HR proteins are regulated by sumoylation. The mechanisms that control sumoylation and their roles in HR are poorly understood. Cells deficient in the SUMO E3 ligase NSMCE2 are sensitive to DNA damaging agents and have defects in HR. Our preliminary data indicated that BLM sumoylation is dependent on NSMCE2. Because BLM sumolyation is required to recruit RAD51 to stalled forks, we hypothesized that NSMCE2-deficient cells are deficient in HR due to a defect in BLM-dependent RAD51 recruitment.

Results: To test this hypothesis, we transfected HeLa cells with siRNAs specific to NSMCE2 and tested whether cells could recruit RAD51 to replication forks stalled with hydroxyurea (HU). Contrary to our hypothesis, we found that the amount of RAD51 protein that accumulated at stalled forks was greater in HU-treated NSMCE2-deficient cells compared to HU-treated control cells. The amount of single-stranded DNA binding protein RPA was diminished by half in HU-treated NSMCE2-deficient cells and DNA damage signaling was similarly diminished as evidenced by the lower levels of γ-H2AX. The double-strand breaks (DSBs) that normally form after extended treatment with HU were also greatly diminished in NSMCE2-deficient cells. These data indicated that despite the over-accumulation of RAD51 to sites of stalled replication forks, cells are unable to perform HR repair efficiently, indicating that RAD51 is unable to complete its function there.

Conclusions: The hyper-accumulation of RAD51 at stalled forks we observed in NSMCE2-deficient cells suggests the sumoylation of one or more substrates by NSMCE2 is required for the remodeling of stalled forks that normally leads to unloading of RAD51 at the fork, strand breakage, and repair by HR.

John Ryniawec

CMM

Gregory Rogers

“How do cells control centriole length?”

Centrosomes are small, intricate organelles whose precise architecture is necessary to perform their diverse functions in cellular signaling, cytoskeletal organization, and during cell division. Two centrioles sit at the core of each centrosome and ensure proper duplication and assembly of the organelle. Each centriole is composed of triplet microtubules arranged in a radially symmetrical, nine-fold, barrel-like structure of a defined width and length. Although we understand how proper width of the barrel is achieved, there is little insight into mechanisms that impose precise control of the length. To elucidate mechanisms of centriole length regulation, we are focusing our studies on the Distal Tip Complex (DTC) of proteins, which caps each centriole and is required to maintain proper length. We hypothesize that the hierarchical recruitment of DTC components, followed by their cell cycle-coordinated modification, controls distinct phases of centriole growth. By understanding mechanisms that regulate centriole length, we will open new paths to investigate the functional consequences of architectural changes to the centriole seen throughout development and in disease.

Rebecca Slater

BME

Henk Granzier

“Exercise Mitigates Increased Diastolic Stiffness In The Titin IA KO Mouse”

The giant sarcomeric protein titin spans the length of the half sarcomere and consists of an I-band region that functions as a molecular spring and an A-band region. Here we use a mouse model in which a portion of titin near the IA junction has been removed (IA KO) resulting in diastolic dysfunction and increased total and titin-based passive tension. We test the hypothesis that exercise ameliorates increased titin-based stiffness. In this study, 3 month old male IA KO and WT mice were allowed free access to a running wheel for 28 days and distance, speed, and duration were recorded each night. After 28 days mice were sacrificed and the LV was used for skinned muscle mechanics, analysis gels, and western blots. WT and IA KO mice ran for the same duration but IA KO mice ran slower and therefore less distance than their WT counterparts (WT mice ran ~6 km per night while IA KOs ran ~4.5 km). Stiffness in both WT and IA KO mice was reduced after exercise; titin-based tension at a sarcomere length of 2.3 μm was reduced by ~16% and ~20% respectively. No changes in titin isoform ratios were observed but exercised mice showed hypo-phosphorylation of S26, a PKC site in titin’s PEVK region. S26 phosphorylation is known to increase titin’s stiffness so hypo-phosphorylation is consistent with the reduction in stiffness observed in exercised mice. We conclude that exercise improves diastolic dysfunction though modulation of titin phosphorylation.

Edgar Tapia

CBIO

Justine McEvoy

“A role for epigenetic deregulation of long non-coding RNAs in rhabdomyosarcoma”

Most pediatric solid tumors arise from perturbations during development, however the mechanisms that drive these malignancies remain poorly understood. Next generation sequencing of pediatric cancers has revealed that in a subset of tumors, genomic instability is not a driving mechanism for tumor progression but that epigenetics may be a major contributor. To test this idea, we are focusing on rhabdomyosarcoma (RMS), a developing muscle tumor with two major histological subtypes, embryonal (ERMS) characterized by a high mutation rate and alveolar (ARMS) characterized by one recurrent chromosomal translocation and a stable genome. From our preliminary epigenetic analysis, we discovered a group of novel long-non coding RNAs (lncRNAs) that are epigenetically deregulated and aberrantly expressed in either one or both of the RMS subtypes. In addition, we identified a well-characterized lncRNA called HOTAIR, which is aberrantly expressed in several adult malignancies and promotes invasion and metastasis. Previous studies have demonstrated that lncRNAs are important for development but may also function as tumor suppressors and oncogenes, suggesting a potential mechanism for tumorigenesis in RMS. We hypothesize that lncRNAs transcriptionally control cancer consensus genes and developmental genes that are essential for RMS development through epigenetic mechanisms. Our preliminary data demonstrates that loss of HOTAIR inhibits cell proliferation in rhabdomyosarcoma cell lines. In addition, knockdown of the novel lncRNA 19_31 results in increased cell death. Determining the contribution of lncRNAs to RMS tumorigenesis will allow us to shed a light on the molecular drivers of this disease.

Joshua Trujillo

MCB

Rebecca Mosher

“The Argonaute-binding platform of NRPE1 evolves through modulation of intrinsically disordered repeats”

Argonaute proteins are important effectors in RNA silencing pathways, but they must interact with other machinery to trigger silencing. Ago hooks have emerged as a conserved motif responsible for interaction with Argonaute proteins, but little is know about the sequence surrounding Ago hooks that must restrict or enable interaction with specific Argonautes. Here we investigated the evolutionary dynamics of an Argonaute-binding platform in NRPE1, the largest subunit of RNA Polymerase V. We compared NRPE1 sequences from more than 50 species, including dense sampling of two plant lineages. This study demonstrates that the Argonaute-binding platform of NRPE1 retains Ago-hooks, intrinsic disorder, and repetitive character while being highly labile at the sequence level. We reveal that loss of sequence conservation is due to relaxed selection and frequent expansions and contractions of tandem repeat arrays. These factors allow a complete restructuring of the Ago-binding platform over 50-60 million years. The presence of labile repeat arrays in all analyzed NRPE1 Ago-binding platforms indicates that selection maintains repetitive character, potentially to retain the ability to rapidly restructure the Ago-binding platform.

Robbert van der Pijl

CMM

Coen Ottenheijm

“Diaphragm hypertrophy following passive stretch: a role for titin-based mechanosensing?”

Background: Titin has been proposed to play a key role in mechanosensing and striated muscle trophicity: spanning half-sarcomeres, single titin molecules bind hypertrophy signaling proteins in its I-bands extensible region. However, there is no conclusive data to support this proposition in skeletal muscle, mainly due to the lack of appropriate tools.
Hypothesis:Titin-based passive tension modulates skeletal muscle hypertrophy.
Methods & results: We used unilateral diaphragm denervation (UDD) in mice, a model that induces a transient hypertrophy in the denervated hemidiaphragm. Using ultrasound imaging, we reveal that the denervated hemidiaphragm undergoes cyclic passive stretch (26±2% muscle lengthening), followed by an increase in wet weight of ~48±3% after six days of UDD. Mass increase resulted from both an increase in cross-sectional area of, and an overall switch to, 2a fibers.
Next, to test whether titin-based passive tension plays a role in the hypertrophy response, we used two mouse models: one with decreased (RBM20ΔRRM; RBM20) and one with increased (TtnΔIAjxn; IA) titin-based passive tension. In RBM20 mice the denervated hemidiaphragm showed a blunted response (20±6% less hypertrophy), whereas the IA mice showed an exaggerated response (18±8% more hypertrophy) relative to wt mice.
Titin-binding proteins implicated in muscle trophicity were induced after UDD, in particular Ankrd1 & 2, FHL1, and MuRF1. Interestingly, Ankrd1 was differentially induced, with a higher induction in the RBM20 mice and a blunted induction in the IA mice. Ankrd2 and FHL1 showed blunted induction in both models, and MuRF1 was similarly induced in both models.
Conclusion: In skeletal muscle, titin modulates hypertrophic remodeling in a passive tension dependent manner. Ankrd1 might play an important role in titin-based hypertrophy signaling.

Ryan Wallace

MCB

Andrew Capaldi

“Body Building: Controlling Yeast Quiescence Through Functional Protein Aggregation”

Efficient control over cellular growth requires fine-tuned regulation of numerous metabolic processes and is essential for cell survival in a naturally fluctuating environment. This metabolic coordination is achieved through the Target of Rapamycin Complex 1 (TORC1), which controls metabolism and energy homeostasis by phosphorylating and regulating a large network of proteins involved in lipid, nucleotide, and protein synthesis. While the mechanisms underlying regulation of TORC1 in nitrogen and amino acid deficiency are well-understood, there is evidence that alternate, unknown pathways control TORC1 in other stress and starvation conditions. Thus, TORC1 both controls growth and fine-tunes metabolic flux based on the condition of the cell but it remains unclear how most signals are transmitted through the complex to ensure optimized metabolic output.

In *Saccharomyces cerevisiae* the mechanism of nitrogen and amino acid regulation of TORC1 is well-understood and highly manipulatable through molecular biological techniques. This regulation happens through modulation of the GTPase activity of the EGO complex (EGOC) which binds TORC1 and tethers it to the vacuole through a lipid modification on one of its constituents. In contrast, glucose regulation of TORC1 does not depend on the GTPase activity of EGOC. Instead, my laboratory has shown that glucose starvation controls TORC1 activity by triggering the dissociation of the complex and movement of the essential regulatory subunit, Kog1, to a single punctate structure on the vacuolar membrane (Kog1-bodies). As Kog1-bodies, like the native TORC1, remain tethered to the vacuole, EGOC likely maintains its association with Kog1 in glucose starvation conditions. These recent discoveries led me to hypothesize that EGOC plays a novel role in TORC1 regulation by driving the clustering of Kog1 and altering TORC1 substrate specificity.

Yuanzhang Yang

CMM

Samantha Harris

“Ca^{2+}-dependent interactions between cMyBP-C and calmodulin and impact on cardiac muscle contraction.”

Cardiac myosin binding protein-C (cMyBP-C) is an essential regulator of actomyosin cycling and muscle contraction and its dysfunction is one of the biggest factors accounting for hypertrophic cardiomyopathy (HCM). Understanding its physiological significance is the long-term goal of this research, including understanding the mechanisms by which cMyBP-C regulates cardiac muscle contraction, how cMyBP-C is affected by its regulators, and the mechanisms by which mutations on cMyBP-C cause.

Calmodulin (CaM) is expressed in all eukaryotic cells as an intracellular target of the secondary messenger Ca2+, and Ca2+/calmodulin participates in many crucial processes in all the muscle types, such as Ca2+ control of various kinases to regulate muscle contraction and regulation of Ca2+ pump and release channel on sarcoplasmic reticulum. Recently an interaction between Ca2+/calmodulin and the M domain of cMyBP-C, the major regulatory domain that modulates actin and myosin interactions to influence muscle contraction, was partly described. However, the features of this interaction (eg. binding affnitiy and binding condition) are not fully understood and its functional significance is unknown. The objective of the proposed project is to determine how interactions between cMyBP-C and Ca2+/calmodulin are relevant to the physiological role of cMyBP-C. Here we hypothesize that CaM interacts with cMyBP-C at multiple specific sites to regulate cMyBP-C function in a calcium-dependent manner. We further propose that interactions of cMyBP-C with Ca2+/calmodulin are influenced by HCM-related mutations on cMyBP-C, thus providing insights into how mutant cMyBP-C causes HCM. The proposed research with a combination of multiple in vitro and ex vivo approaches is crucial for evaluating the physiological relevance of interactions between Ca2+/calmodulin and cMyBP-C to muscle contraction and cardiac functioning.

Patricia Zagallo

MCB

Molly Bolger

“Investigating how students create and use models to interpret and reason about authentic biological data”

A primary goal in biology instruction reform is integration of scientific reasoning skills and content knowledge. We developed a curriculum designed to foster the development of such skills in a large-enrollment, upper-division biology content course called Teaching undergraduates Real data Interpretation using Models (TRIM). We provide students with models that convey key course topics to aid in their interpretation of published data figures and to provide practice with model-based reasoning, a primary disciplinary skill. In this study, we ask 1) Can TRIM students draw their own productive models to interpret authentic data? And 2) How do students coordinate models (provided or self-generated) with data interpretation? How do the two conditions compare? To answer these questions, we recruited TRIM students (n=30) to interpret data figures in clinical think-aloud interviews. Participants were randomly assigned to a provided-model condition or asked to draw their own model from the data. Coding analysis results revealed no statistical difference in the interpretation quality between provided-model and draw-model groups (p=0.20, Student’s t-Test), suggesting that student-derived models may be equally productive as instructor-provided models for fostering students’ interpretations of novel data. We found the more abstract the drawing, the higher the data interpretation score for biology-specific points, r=.69; p=.009, suggesting models stimulate students to consider biological implications in the data figures. Likewise, students were more likely to make key biological connections during the model task than during the data interpretation task, suggesting that simply asking students to interpret data does not necessarily lead to all desirable biological connections. Our work aims to inform instructional design decisions for integrating disciplinary practices such as modeling and data interpretation in undergraduate biology classrooms.

Jacob Zbesko

IMB

Kristian Doyle

“Characterization of Inflammation in Acute and Non-Acute Ischemic Infarcts in Human and Mouse Brain Tissue”

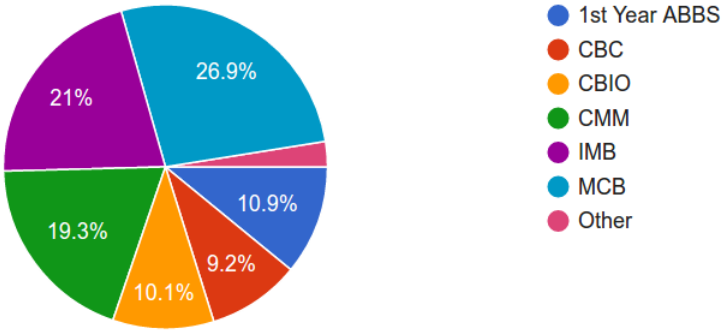
Approximately one third of stroke sufferers develop delayed dementia following a stroke. We hypothesize that one of the mechanisms that leads to this delayed dementia is a chronic inflammatory response that persists at the site of the lesion and causes bystander damage to surrounding tissue. To address this hypothesis we present a characterization of the cytokine response to stroke in the human brain at different stages of wound healing. Our data indicate that inflammation following stroke may not be as efficiently self-limiting as it is in other tissues. In addition, due to most preclinical research being conducted in rodent models of stroke, we also present a comparison of the cytokine response to stroke in the human and mouse brain at two different stages of wound healing, using both C57BL/6 and BALB/c mice to control for mouse strain related differences in the immune response. Our data indicate that the acute inflammatory response to stroke is not significantly different in different strains of mice, or between mice and humans. However, in the weeks after stroke, during the stage of wound healing termed liquefactive necrosis, the chronic inflammatory response to stroke diverges in different mouse strains and in humans.

For Fun

Most likely to . . .
. . . not answer emails - Jeff
. . . send emails to Ryan Gutenkunst, not Wallace - Julie
. . . stay on top of everything - Jess
. . . skip committee meetings - Brett
. . . have a large rope handy – Emily
. . . steer the heaviest cart at Costco – Brittany

Length of Costco receipt – 36 inches
Texts sent between student committee – 217
Emails sent between student committee - 97
Number of tears shed – 6
Beers consumed during planning - ???

Attendance



Presentations