Student Poster Session

October 10, 2015

BRB Lobby

Presentation times

Even numbers: 2-3pm

Odd numbers: 3-4pm

Contents

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~~Nicole Aiello~~ CANCELED

Cell & Molecular Biology (CAMB)
Developmental, Stem Cell and Regenerative Biology

Chemotherapy alters the natural history of metastatic progression

Nicole Aiello, David L. Bajor, Neha Bhagwat, Minh N. Pham, Toby C. Cornish, Christine A. Iacobuzio-Donahue, Robert H. Vonderheide, Ben Z. Stanger

Most cancer-associated deaths result from metastasis. Despite the importance of this process, it remains unknown whether the size, microenvironment, or other features of a metastatic lesion dictate the efficacy of chemotherapy in the adjuvant (micrometastatic) setting. Here, we delineate the natural history of metastasis in an autochthonous model of pancreatic ductal adenocarcinoma (PDAC), using lineage tracing to examine the evolution of disseminated cancer cells and their associated microenvironment during metastatic progression. With increasing size, lesions shifted from mesenchymal to epithelial histology, exhibited lower vessel density and accumulated a desmoplastic stroma, largely recapitulating the primary tumors from which they arose. Moreover, treatment with gemcitabine and nab-paclitaxel significantly reduced the overall number of metastases and shifted the size distribution toward small metastatic lesions. These results provide a window into the cellular dynamics of metastatic progression and suggest that adjuvant chemotherapy affords a survival benefit by directly targeting micrometastases.

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Alejandro Arroyo

Pharmacology (PGG)

Synthesis and characterization of fluorinated resazurin: a selective Cerenkov viability and redox probe

Alejandro Arroyo, Alexander Kachur, Elizabeth Browning, Eric Blankemeyer, Anatoliy V. Popov, Jim Delikatny

Resazurin(RA) is a common viability and redox dye. In reductive environments, RA is reduced to resorufin(RAred), a highly fluorescent molecule. Redox detection is a potential tool to non-invasively determine antioxidant capacity in cancer, inflammation and neurodegenerative diseases. Resazurin was fluorinated electrophilically under acidic conditions. Products were characterized using NMR, mass spectrometry and UV-vis spectra; chemical reduction and and cell activity was determined. RA fluorination identified five derivatives using preparative HPLC. Mass spectrometry confirmed synthesis of mono and difluorinated compounds. 1H and 19F-NMR confirmed fluorination sites. Absorption maxima and pKa of each fraction was determined. Chemical reduction experiment assessed the differential Cerenkov attenuation of FRAox and FRAred. DU145 cells were able to reduce MFRA and DFRA. Difference in photonic emissions between 620-660nm suggests these are the best wavelengths to distinguish FRA and FRAred for cell and in vivo applications. pKa results followed trend towards higher acidity with increased degree of fluorination. Ratio of emission between FRA and FRAred was 3.5(620nm). Fluorescent capability of FRAred seems to be the driving force of observed Cerenkov Radiation Energy Transfer(CRET). Eventual impact of this technique is the ability to translate into the clinic coupled with PET for dual imaging modality.

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Lauren Brady

Cell & Molecular Biology (CAMB)
Genetics and Gene Regulation

Investigating the impact of hypoxia-induced changes in isoform expression and splicing in the tumor microenvironment

Lauren K Brady, Vladimir M Popov, Mircea Ivan, Milan Radovich, Cameron Koch, Amit Maity and Constantinos Koumenis

Hypoxia is a cellular stress that influences both normal and tumor development. Tumor hypoxia shows a strong positive correlation with poor patient outcome for many types of cancer. To gain a deeper understanding of how the tumor microenvironment influences therapeutic response, our lab is investigating how transcription is differentially regulated in hypoxic tumors. To our knowledge, this is the first global sequencing analysis of hypoxic tumors to study transcriptional regulation at base-pair resolution. We optimized a method to isolate RNA from hypoxic regions of tumors and then carried out RNA-sequencing of normoxic and hypoxic tumor samples from a murine model of human head and neck cancer. The data revealed > 1,000 hypoxia-responsive isoforms; among these, we identified an overrepresentation of expression changes in last exons, introns and 3′ untranslated regions. Many of these splicing changes result in hypoxia-induced isoforms that code for substantially different protein isoforms than those expressed in normoxic conditions and represent pathways central to hypoxic adaptation, such as translation, glycolysis and cell cycle. Here we present evidence that hypoxia promotes intron retention in a major regulator of translation initiation, EIF2B5, to yield a previously-undescribed truncated isoform which may act to suppress translation in hypoxic cancer cells.

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Daniel Child

Cell & Molecular Biology (CAMB)
Genetics and Gene Regulation

Systemic analysis of wasting and heart atrophy in a murine model of Huntington's disease

Daniel Child

Huntington's disease (HD) is well known for its neurological phenotypes, but heart abnormalities have been observed in both patients and animal models. It is unclear, however, whether heart problems result from cardiac-specific pathophysiology or from systemic effects such as wasting. To explore this relationship, we performed indirect calorimetery on a well-characterized HD murine model at early-, mid-, and late-stage disease. Surprisingly, we found that HD males were hypometabolic during the light cycle. Total caloric intake was equal between HD and WT mice, but feeding patterns were disrupted, and HD mice were considerably more active than WT littermates. These results suggest that wasting may be associated with abnormalities in sleep-wake cycles. We next assessed heart involvement in wasting by measuring heart and body mass over the course of disease. We found that that HD hearts at late-stage disease were smaller than their WT counterpart, but the proportion of heart mass-to-body mass was greater in HD mice than in WT littermates. These findings suggest that heart atrophy occurs at a slower rate than other organs. Together, these results suggest a basis for the cardiac and systemic wasting observed in HD and provide a foundation for future studies into peripheral HD pathologies.

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Natalie Daurio

Pharmacology (PGG)

Tamoxifen induced bioenergetics stress uncovers novel estrogen receptor-independent therapeutic potential

Natalie Daurio, Stephen Tuttle and Constantinos Koumenis

Tamoxifen, a selective estrogen receptor modulator (SERM) is clinically used for adjuvant treatment of estrogen receptor (ER) positive breast cancer (BC). Our lab has shown that tamoxifen inhibits oxygen consumption at high, yet clinically relevant, doses. We hypothesize that this occurs through a non-ER dependent, direct effect on the mitochondria, and causes metabolic and signaling changes that can be exploited for therapeutic benefit. Tamoxifen inhibited oxygen consumption to a similar degree in ER+ MCF7 and ER- MDA-MB-231 cells. Moreover, pharmacological and genetic manipulation of ER expression did not affect the ability of tamoxifen to decrease oxygen consumption. In response to tamoxifen inhibition of oxygen consumption, tumor cells increased dependence on glycolysis. Glucose deprivation or treatment with glycolytic inhibitors, 2-deoxy glucose (2DG) or 3-bromo-pyruvate significantly sensitized (BC) cells to tamoxifen. Tamoxifen inhibition of oxygen consumption increased the AMP:ATP ratio and activated the AMPK signaling pathway in vitro and in an mouse orthotopic model of triple negative BC. We have also demonstrated that tamoxifen cytotoxicity is modulated by AMPK signaling in an isoform specific manner. Our results indicate a new and unanticipated mechanism of action of tamoxifen in cancer and suggest novel ways of using this agent in the clinic.

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Scott Dooley

Cell & Molecular Biology (CAMB)
Gene Therapy and Vaccines

SMaRT approaches to the treatment of retinal diseases

Scott Dooley, Lloyd Mitchell, Jean Bennett

Spliceosome-mediated pre-mRNA trans-splicing (SMaRT) is an emerging technology that has been used to correct genetic cardiovascular disease at the transcript level. This technique is advantageous for treatment of diseases driven by genes too large to fit in viral vectors currently used in clinical trials. We are adapting SMaRT approaches for the treatment of the retinal diseases LCA10 and Stargardt Disease. We screened for successful binding domains and have identified one for the genes that drive both diseases (ABCA4 and CEP290). We are currently evaluating endogenous splicing and the potential for disease correction using these constructs.

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Salika Dunatunga

Genomics & Computational Biology (GCB)

Developing a method to assess sleep-wake states in mice using high resolution video systems

Salika Dunatunga, Ray Galante, James Shackleford, Allan Pack

Sleep-wake studies performed in mice give insight to the biological workings of similar disorders in humans. Mice have long been known to be social sleepers and their social sleep patterns are thought to affect biological reactions to sleep disorders. The gold standard for assessing sleep in mice has traditionally been through analysis of an electroencephalography (EEG) signal; however, EEG surgery is invasive and potentially modifies sleeping patterns of mice through stress from surgery, physical limitations, and the required isolation. In recent years, high resolution video systems have allowed tracking of flies, humans, and ants in social settings with minimal invasion. A basic video system designed to extract physical features of the mouse showed that video differentiates between REM sleep, non-REM sleep, and wake. We further investigate the usefulness of a higher resolution video system and automated scoring of the sleep-wake state of a mouse, validated by EEG signals.

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Shawn Foley

Cell & Molecular Biology (CAMB)
Genetics and Gene Regulation

Global analysis of the RNA-protein interaction and RNA secondary structure landscapes of the Arabidopsis nucleus

Shawn W. Foley, Sager J. Gosai, Dongxue Wang, Ian M. Silverman, Nur Selamoglu, Andrew D.L. Nelson, Mark A. Beilstein, Fevzi Daldal,
Roger B. Deal, and Brian D. Gregory

Post-transcriptional regulation in eukaryotes requires cis- and trans-acting features and factors including RNA secondary structure and RNA-binding proteins (RBPs). However, a comprehensive view of the structural and RBP interaction landscape of nuclear RNAs has yet to be compiled for any organism. Here, we use our ribonuclease-mediated structure and RBP binding site mapping approaches, to globally profile these features in Arabidopsis seedling nuclei, in vivo. We reveal anti-correlated patterns of secondary structure and RBP binding throughout nuclear messenger RNAs (mRNAs) that demarcate sites of alternative splicing and polyadenylation. We also uncover a collection of protein-bound sequence motifs, and identify their structural contexts, co-occurrences in transcripts encoding functionally related proteins, and interactions with putative RBPs. Finally, using these motifs, we find that the chloroplast RBP CP29A also interacts with nuclear mRNAs. In total, we provide the first simultaneous view of the RNA secondary structure and RBP interaction landscapes in a eukaryotic nucleus.

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Hayley Hanby

Cell & Molecular Biology (CAMB)
Cell Biology, Physiology, and Metabolism

Dense granules mature and differentiate from lysosomes at a post-megakaryocyte differentiation stage

Hayley Hanby, Jialing Bao, Jiyoon Noh, Danuta Jarocha, Ronghua Meng, Mitchell J Weiss, Mortimer Poncz, and Michael Marks

Dense granules (DGs) are lysosome related organelles (LROs) in platelets, which store molecules like calcium and ADP for secretion during clot formation. DG biogenesis is thought to occur during maturation of the platelet precursor, the megakaryocyte (MK). However, the precise timing of DG formation and maturation has not been definitively characterized. To better determine the relationship between dense granules and lysosomes and to begin to stage DG biogenesis, we sought to identify DG and lysosomal structures in platelets and MKs using dyes and fluorescently-conjugated biomolecules that label various compartments in the endosomal-lysosomal pathway. In mouse platelets, mepacrine—a fluorescent weak base that accumulates in DGs—does not colocalize with either Lysotracker or DQ-BSA. This indicates that DGs are distinct from lysosomes in mature platelets. However, in murine fetal liver-MKs or MKs differentiated from the engineered hematopoietic progenitor cell line, G1ME2, labeling by mepacrine and Lysotracker overlapped completely. Mepacrine labeling in GIME2-MKs was sensitive to bafilomycin A1, whereas labeling in platelets was not, suggesting that mepacrine in MKs might accumulate as a weak base in lysosomes. Finally, dense granule segregation from lysosomes occurs during and/or after proplatelet formation, as we observed heterogeneous staining of mepacrine and Lysotracker in proplatelets from G1ME2 cells.

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Stella Hur

Cell & Molecular Biology (CAMB)
Developmental, Stem Cell and Regenerative Biology

Speciation between mouse and human causes incomplete establishment of imprinting at the humanized H19/Igf2 ICR in mouse

Stella Hur, Andrea Freschi, Folami Ideraabdullah, Joanne Thorvaldsen, Lacey Luense, Angela Hines, Andrea Riccio, Marisa Bartolomei

Genomic imprinting is a phenomenon in which a subset of genes is expressed in a parent-of-origin-specific manner. Imprinted genes are regulated by imprinting control regions (ICRs), cis-regulatory elements that are differentially DNA methylated according to the parental allele. Genomic imprinting is conserved among mammalian species; however, the extent to which genetically evolved ICR elements across species are functionally compatible in different organisms, has not been well-described. This question is essential to address when modeling human mutations associated with imprinting disorders in vivo, such as in mouse models. In this study, we have generated a knock-in mouse model in which the endogenous H19/Igf2 ICR (mIC1) is replaced by the orthologous human ICR (hIC1) element. We show that maternal transmission of hIC1 can functionally substitute for mIC1, as demonstrated by proper parental allele-specific H19 and Igf2 expression and IC1 methylation. Surprisingly, imprinting is incompletely established at hIC1 in the mature sperm of knock-in mice when hIC1 is paternally transmitted. This is, in part, due to increased enrichment of activating histone marks at hIC1 compared to mIC1 during spermatogenesis. Overall, we show that hIC1 is not correctly reprogrammed in the male germ cells in mouse, inhibiting proper establishment of imprinting at hIC1.

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Brian Johnson

Cell & Molecular Biology (CAMB)
Genetics and Gene Regulation

Biotin tagging of MeCP2 reveals transcriptome changes among genetically defined neurons in vivo

Brian S. Johnson*, Yingtao Zhao*, Maria Fasolino*, Janine Lamonica, Yolanda Cui, Daniel Bu, Zhaolan Zhou

*These authors contributed equally to this work

Mutations in the MECP2 gene are responsible for Rett syndrome (RTT), a severe X-linked neurological disorder characterized by loss of developmental milestones, intellectual disability, and motor impairments. Previous studies have reported that MeCP2 binds broadly across the genome and subtly affects the expression of hundreds of genes, but how neuronal function is selectively disrupted and contributes to RTT remains poorly understood. One confounding factor has been the inherent heterogeneity of the mammalian brain, which comprises distinct anatomical regions and cell types that differ not only in form and function, but gene expression and epigenetic profiles. To overcome this challenge, we engineered a genetic system that allows us to assess cell type-specific profiles of gene expression (nuclear RNA-seq) across two mouse models bearing RTT-associated missense mutations. We found that MeCP2 affects the expression of different sets of genes within different cell types; furthermore, the number of differentially expressed genes (DEGs) and the degree to which DEGs are perturbed correlate with the severity of the RTT mutation and phenotype. Finally, our approach circumvents the genetic mosaicism associated with X-chromosome inactivation and identifies DEGs between neighboring wild-type and mutant neurons from heterozygous female mice, thereby establishing novel clinically relevant models for RTT.

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Julia Kieckhaefer

Cell & Molecular Biology (CAMB)
Genetics and Gene Regulation

The Foxa transcription factors sustain murine intestinal health by regulating Crohn's Disease risk loci

Julia Kieckhaefer

While the primary cause of intestinal inflammation in IBD is thought to be an inappropriate immune response to bacteria, genome-wide association studies (GWAS) have also implicated multiple genetic loci associated with genes expressed in the intestinal epithelium. Defects in the mucus layer, which is required for limiting the exposure of epithelial cells to pathogens, results in inflammation. It was previously shown that mucin expression is altered and Mucin2 (Muc2), the main component of intestinal mucus is downregulated by deletion of the paralogous transcription factors Foxa1 and Foxa2 in the mouse intestinal epithelium. Mice deficient in Muc2 develop intestinal inflammation and colorectal cancer, suggesting a potential role for the Foxa factors in the maintenance of an intact mucus layer and intestinal epithelial health. To investigate the requirement of Foxa1/a2 in the maintenance of intestinal barrier function, we analyzed immune cell infiltration into the epithelium of Foxa1/a2-deficient colon in mice treated with dextran sodium sulfate (DSS), as well as in mice advanced in age. Immunohistochemical staining showed that the Foxa1/a2-deficient colonic epithelium displays increased susceptibility to both experimental and spontaneous colitis. To analyze the mechanism of increased susceptibility to colitis in Foxa1/a2 mutants, we performed Foxa1 and Foxa2 chromatin immunoprecipitation assays followed by ultra-high throughput sequencing (ChIP-Seq) analysis on wild type mouse colon followed by RNA-Seq on Foxa1/a2-deficient and control aged colon to identify relevant Foxa targets. Significantly enriched Foxa1/a2 binding sites were identified in close proximity to the IBD risk genes Fut2 and Itln1, which were downregulated in mutant colon.

Additionally, a Foxa1/a2 binding site was found downstream of the gene for the secreted immune effector Relm-β. These findings suggest Foxa factors regulate IBD-associated genes and may contribute to IBD pathogenesis.

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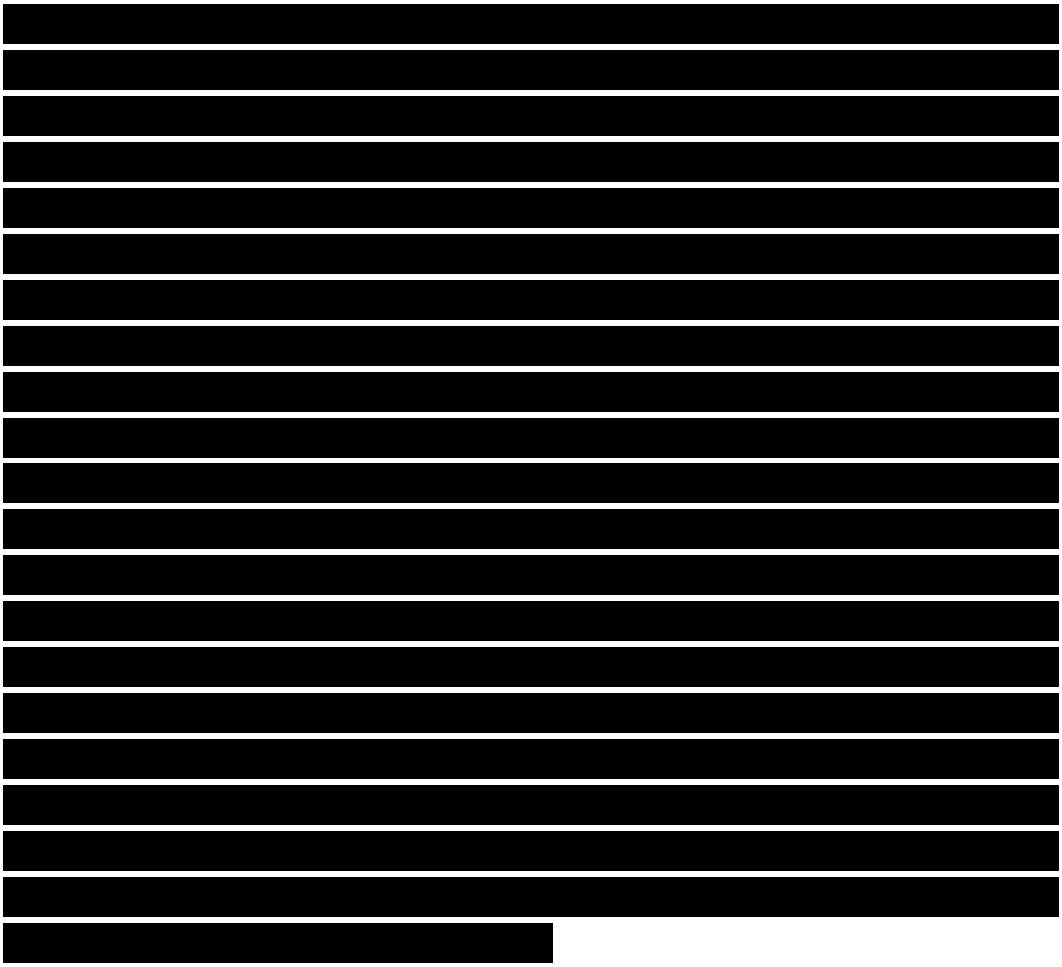
Michael Klichinsky

Pharmacology (PGG)

Chimeric antigen receptor macrophages as a novel cancer immunotherapy

Michael Klichinsky, Saad Kenderian, Marco Ruella, Olga Shestova,
Stephen Wallace, Decheng Song, John Scholler, Carl June, Saar Gill



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Mingen Liu

Cell & Molecular Biology (CAMB)
Cancer Biology

Determining CD47-independent regulation of macrophage phagocytosis in pancreatic carcinoma

Jason Mingen Liu and Gregory L. Beatty

Tumor-associated macrophages (TAMs) abound in solid tumors such as pancreatic ductal adenocarcinoma (PDAC), comprising up to half of all cells in the tumor microenvironment of PDAC. TAMs promote tumor growth and spread, but may adopt an anti-tumor role as well. The anti-tumor functions of TAMs include the ability to phagocytose tumor cells. The phagocytic capacity of TAMs is regulated by pro- and anti-phagocytic signals expressed by tumor cells. A major anti-phagocytic signal is CD47, which is highly expressed by multiple cancers, including PDAC. We have found that Panc-1 tumor cells were phagocytosed at low levels in comparison to BxPC-3 cells, though both lines robustly express similar levels of CD47. We hypothesized that additional signals can regulate the capacity of macrophages to engulf PDAC cells. To identify these signals, Toll-like receptors (TLR) agonists were screened in the presence or absence of CD47-blockade under co-culture conditions. Consistent with our hypothesis, stimulation of TLR1, TLR2 and TLR4 increased phagocytosis of tumor cells with concomitant CD47-blockade, but treatment with TLR1/2 agonist also increased phagocytosis in the absence of CD47-blockade. Together, these data suggest that additional signals regulate the capacity of macrophages to phagocytose PDAC cells.

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Elaine Liu

Neuroscience (NGG)

Loss of nuclear TDP43 leads to global RNA dysregulation in ALS/FTD

Elaine Liu, Jenny Russ and Edward Lee

Amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) are two fatal neurodegenerative diseases characterized by loss of nuclear TAR DNA binding protein 43 (TDP-43) into cytoplasmic TDP-43 aggregates. TDP-43 is a nucleo-cytoplasmic shuttling protein that binds to RNA and RNA binding proteins (RNABP). Neurons with cytoplasmic TDP-43 aggregates exhibit loss of normal nuclear TDP-43. Furthermore, a hexanucleotide repeat expansion in C9orf72 is the most common genetic cause of ALS/FTD. All C9orf72 carriers exhibit TDP-43 pathology. One of the proposed disease mechanisms is RNA foci formation that may sequester RNABPs, perturbing RNA processing. Loss of nuclear TDP-43 leads to global RNA dysregulation, leading to ALS/FTD. RNA-seq of nuclear RNA from sorted neurons with and without TDP-43 from post-mortem C9orf72 carriers and non-diseased post-mortem brain was done. There were 5576 differentially expressed genes (DEGs) due to loss of TDP-43 and 323 DEGs linked to the C9orf72 mutation. There were 118 common genes that were differentially expressed due to loss of TDP-43 and presence of C9orf72 mutation. These were enriched in signal transduction pathways. Interestingly, TDP-43 autoregulation was seen in the sorted nuclei. Future analyses will determine whether loss of nuclear TDP43 and the C9orf72 mutation exacerbate global RNA processing.

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Sarah McKee

Pharmacology (PGG)

The effect of early life methyl donor supplementation on obesity development

Sarah McKee

Excessive maternal weight gain during pregnancy contributes to an increased risk for obesity in the offspring. In a mouse model of excessive maternal weight gain, we find that offspring have increased preference for sucrose and fat, increased expression of genes that underlie reward-related behaviors, and both global and gene specific DNA hypomethylation. These changes in reward-related neural circuitry may contribute to the increased risk for the development of obesity in the offspring by altering the animal's response to highly palatable, energy dense foods. Methyl donor supplementation (MDS) during pregnancy can reverse some of these phenotypes, yet it is unknown whether postnatal MDS can reverse these phenotypes. To determine this, offspring from dams fed either a high fat diet (HFD) or control diet during gestation/lactation were fed a methyl donor supplemented diet during early life (age 3-6 weeks). We find that postnatal MDS significantly decreased body weight in both male and female adult HFD offspring, and does not alter body weights of control diet offspring. Further, postnatal MDS can normalize adult male fat preference and contributes to regional specific normalization of DNA hypomethylation.

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Minal Mehta

Cell & Molecular Biology (CAMB)
Gene Therapy and Vaccines

Hepatic-deficient PPP1R3B mice have impaired glucose homeostasis, and alterations in lipid metabolism

Minal Mehta, Salam Ibrahim, Nick Hand, Jeffrey T. Billheimer, Daniel J. Rader

Carbohydrate metabolism is a complex of critical physiological processes required to maintain plasma glucose homeostasis.The liver specifically plays an important role in these pathways. Insulin is essential for maintenance of creating a balance between carbohydrate and lipid metabolism.When insulin is stimulated to be released by pancreatic β cells, a large fraction of glucose is taken up by hepatocytes, which is converted into glycogen.The regulation of glycogen synthesis and breakdown is tightly regulated. Insulin promotes glycogen synthesis by promoting net dephosphorylation of glycogen synthase (GS) and phosphorylase (GP). This is achieved by inhibition of protein kinases and activation of protein phosphatases. Since these enzymes are ubiquitously expressed in all cellular compartments, it is now established that “targeting” subunits permit specific dephosphorylation of discrete pools of proteins within cells by regulating activity and compartmentalized expression of these enzymes where needed based on the physiological needs.These targeting subunits serve as scaffolding proteins by bringing enzymes to their substrates in macromolecular complex,and regulate PP1 activity, which has profound effects on glycogen metabolism. GL(PPP1R3B) is one such glycogen targeting subunit expressed in liver and we characterize a liver-specific knockout model and its role in regulation on glucose and lipid homeostasis.

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Jacquelyn Meisel

Genomics & Computational Biology (GCB)

Sequencing technique biases affect characterizations of the skin microbiome

Jacquelyn Meisel, Geoffrey Hannigan, Amanda Tyldsley, Adam SanMiguel, Brendan Hodkinson, Qi Zheng

Skin microbiome studies are increasingly common, due in part to affordable and accessible sequencing and analysis platforms. Compared to traditional culture-based techniques, DNA sequence-based methods, such as sequencing of the bacterial 16S ribosomal RNA (rRNA) gene, provide more precise characterizations of microbial communities. Although outcomes of microbiome studies heavily depend on protocols employed, optimal experimental approaches for the skin have not been established. Here we compare 16S rRNA gene to whole metagenomic shotgun (WMS) sequencing for deciphering skin microbiome community composition and functional genetic enrichment. We show that reported relative abundance of microbiota is highly dependent on the region of the 16S rRNA gene that is amplified and sequenced. Primers amplifying hypervariable region 4 (V4) are not optimal for skin microbiome studies, as they poorly recapitulate relative abundance of widespread commensals. Alternatively, sequencing of hypervariable regions 1-3 (V1V3) provides similar genus-level classifications of bacterial communities to WMS sequencing. A perceived advantage of WMS sequencing is that it provides evidence of a community's functional potential; however, metagenome predictions based on the 16S rRNA gene closely approximate WMS genetic functional profiles. This work highlights the importance of experimental design for skin microbiome studies while recommending optimal strategies depending on experimental objectives.

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Ian Mellis

Genomics & Computational Biology (GCB)

Development of a compressed sensing framework for approaching the distributed encoding of quantitative traits in the genome

Ian Mellis

A core goal of systems biology is understanding—at a quantitative level—how genotype influences phenotype. Of particular interest is how gene network organization informs this central mapping. Many high-throughput experiments demonstrate widespread and often small effects on gene expression from essentially any perturbation of a genetic network. Currently, many in the molecular biology community take a reductionist view of gene networks and attribute most of these small effects to systemic “noise”; that they have little or no intrinsic functional consequence. We plan to explore an alternative explanation for this phenomenon: that the observed system of weak genetic interactions encodes a high-dimensional genotype-phenotype mapping compressively and isometrically. Recent advances in random matrix theory reveal that some high-dimensional datasets with appropriate properties of “sparseness” can be represented in much lower dimension by compressive and isometric mapping. In this project, we will develop a biologically grounded theory of compressive and isometric mapping between genotype and phenotype, wherein information is encoded in weak interactions between genes, to explain heritability of quantitative traits. This work could aid significantly in developing a useful framework for approaching the interpretation of gene networks, and could help answer long-standing questions about the heritability of quantitative traits.

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Sarah Middleton

Genomics & Computational Biology (GCB)

Finding motifs that direct RNA localization in neurons

Sarah Middleton, Junhyong Kim

Neurons regulate protein expression in space and time by transporting mRNAs to the dendrites and translating them locally at synapses. This enables quicker synaptic remodeling in response to stimulation and allows remodeling to be restricted to a single synapse or synaptic neighborhood, which is required for many forms of long-term memory formation. It is thought that dendritic localization is primarily mediated by the recognition of motifs within the RNA sequence, but only a few motifs have so far been found. In this study, we extend and improve on previous searches for localization motifs using a combination of experimental and computational techniques. First, using sub-single cell RNA-seq, we directly compare the dendritic and somatic compartments of individual neurons to detect RNAs that are enriched in the dendrites. Using this improved catalog of potentially localized RNAs as input, we then apply a novel motif searching algorithm that can detect both linear and structural RNA motifs. Preliminary results indicate that several genes show differential expression of 3'UTR isoforms between the dendrites and cell body, suggesting preferential localization of certain isoforms to the dendrites. Motif searches within these dendritic isoforms identified 42 significantly enriched motifs, many of which are structural. Future work will evaluate these candidate motifs for localization function in vivo.

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Katelyn Miller

Cell & Molecular Biology (CAMB)
Cell Biology, Physiology, and Metabolism

Persistent measles virus in the brain after resolution of acute infection

Katelyn D Miller, Kevin J O'Regan, Christine Solomos, Glenn F Rall

Viral infections of the central nervous system (CNS) can lead to debilitating diseases such as encephalitis and often cause death. Viral persistence and latency within the CNS is generally not considered for potentially neurotropic RNA viruses such as measles virus (MV), which is thought to be sterilely cleared following acute infection. It has been shown that MV RNA persists in the CNS of elderly individuals who died of natural causes, implicating retention of replication-competent virus decades after primary exposure. Our laboratory has shown that immunocompetent mice, engineered to express a measles virus receptor in CNS neurons, functionally resolve MV from the CNS with no lasting neurological defects. However, viral RNA and mRNA persist in the brains of these mice for greater than 90 days after challenge. To understand how this virus evades sterile clearance from neuronal populations, we depleted the adaptive immune response in these infected mice. After depletion, the levels of MV RNA, mRNA, and protein increased within the CNS, implicating the adaptive immune response in restricting active viral replication. A better understanding of the host factors that control neurotropic virus replication, persistence and reactivation may be especially relevant to inflammatory CNS diseases.

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Andrew Moore

Neuroscience (NGG)

Regulation of optineurin-dependent mitophagy by TANK binding kinase 1

Andrew Moore

The ALS-associated protein optineurin was recently identified as an autophagy receptor for parkin-mediated mitophagy. However, the regulation of optineurin in this pathway is not well understood. Here, we investigated the role of optineurin phosphorylation in the autophagic clearance of damaged mitochondria. We used live-cell microscopy in HeLa cells to examine the recruitment of GFP-LC3 to depolarized mitochondria in the presence of wildtype, phosphodeficient (S177A), or phosphomimetic (S177E) optineurin. We found that after 90m of treatment with the depolarizing agent CCCP, all three optineurin constructs were robustly recruited to depolarized mitochondria. However, cells expressing S177A optineurin showed five-fold lower levels of LC3-positive mitochondria than cells transfected with wildtype or S177E-optineurin. We next examined the regulation of optineurin by TANK Binding Kinase 1 (TBK1), a serine/threonine kinase previously shown to phosphorylate optineurin at S177 and recently demonstrated to be a risk factor for ALS. Knockdown of TBK1 by siRNA significantly attenuated LC3 recruitment to mitochondria at 90m post-CCCP. This mitophagy defect was partially rescued through the overexpression of S177E-optineurin. These observations indicate that two ALS linked alleles, optineurin and TBK1, operate in the same mitophagy pathway, suggesting that ineffective clearance of damaged mitochondria may be a key factor underlying ALS pathogenesis.

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Danielle Mor

Neuroscience (NGG)

Dopamine-mediated neurotoxicity of α-synuclein in vivo

Danielle Mor, Elpida Tsika, Joseph Mazzulli, Jennifer Grossman, John Wolfe and Harry Ischiropoulos

Parkinson's disease (PD) is defined by loss of dopamine (DA) producing neurons in the substantia nigra (SN) and an abundance of Lewy body inclusions comprised of aggregated α-synuclein (α-syn) protein. Mutations and duplications/triplications of the α-syn gene cause dominantly inherited PD, highlighting a central role for α-syn in PD pathogenesis. However, a mechanistic link is lacking between α-syn aggregation and DA neuron biology that explains the selective loss of this cell population. In vitro, DA itself is a modifier of α-syn aggregation, resulting in potentially neurotoxic oligomers. We used a novel lentiviral approach and an established mouse model of α-synucleinopathy to investigate the interaction of DA and α-syn in vivo. DA levels in SN neurons were increased by targeted expression of mutant R37E/R38E tyrosine hydroxylase (TH-RREE), which is insensitive to feedback inhibition by DA. In mice expressing A53T mutant human α-syn, TH-RREE expression induced a previously undescribed phenotype of nigrostriatal degeneration and locomotor deficit. Biochemical analysis revealed conformationally distinct oligomers. Moreover, recombinant DA-stabilized oligomers were toxic to primary neurons. We report here the first in vivo account of DA and α-syn acting synergistically to drive neurodegeneration, and propose a novel mechanism whereby neurons may be selectively vulnerable in PD.

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Pierce Nathanson

Cell & Molecular Biology (CAMB)
Microbiology, Virology and Parasitology

The genome-wide DNA binding properties of the ALS-associated protein TDP-43

Pierce Nathanson, Xiang Yu, Travis Unger, Qi Zheng, Shawn Foley, Jordan Mak, Brian Gregory, Alice S. Chen-Plotkin

Amyotrophic Lateral Sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are fatal adult-onset neurodegenerative diseases characterized by premature neuronal loss. In the majority of cases of ALS and ~50% of the cases of FTLD, pathology is characterized by mislocalization and accumulation of TAR DNA Binding Protein of 43kD (TDP-43) in neurons and glia. Whether neurodegeneration is predominantly due to a loss of nuclear TDP-43 or toxic gain of function is highly debated. It is clear, however, that mutations of TARDBP are sufficient to cause both of these genetically heterogeneous diseases.

TDP-43 is an evolutionarily conserved, ubiquitously expressed nucleic acid binding protein. Originally described as a protein capable of binding the HIV transactivation response element DNA, research has been dominated by investigation of TDP-43's role in RNA processing, thereby neglecting a fundamental property of TDP-43 biology. Given that neurodegeneration in ALS and FTLD-TDP is likely due, at least in part, to the loss of TDP-43 nuclear function, the elucidation of TDP-43's DNA binding properties is warranted. Here we have characterized the genome-wide DNA-binding properties of TDP-43 in HEK293 cells, verified key TDP-43 binding sites, and performed functional assays suggesting that TDP-43 has distinct effects on expression of a subset of genes.

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Kristy Ou

Pharmacology (PGG)

Interrogating enhancer function and causal variants at the ADAMTS7 coronary artery disease GWAS locus

Kristy Ou

No abstract provided.

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M. Kazim Panjwani

Immunology (IGG)

Establishment of feasibility for the treatment of canine B cell malignancies with cCD20 CAR-bearing T cells

M. Kazim Panjwani*, Jenessa Smith*, Keith Schutsky, Josephine Gnanandarajah, Sondra Calhoun, Colleen O'Connor, Nicola Mason, Daniel J. Powell Jr.

*equal contribution

Chimeric antigen receptor (CAR) T cell therapy has shown great promise in treating human hematological malignancies, but preclinical murine models limit prediction of safety and efficacy in humans. Here, we have established a treatment model for CAR T cell therapy in canines with spontaneous lymphoma. We first established a methodology that yields robust (50-120x) expansion of canine T cells from normal or lymphoma-diseased dogs. We then utilized mRNA electroporation to express a CAR targeting the B cell antigen CD20 in canine T cells. T cells efficiently and transiently expressed the cCD20-Z CAR and exhibited specific lysis and IFN-γ secretion against cCD20+ target cells. We administered autologous cCD20-Z RNA CAR+ T cells to a canine patient with twice-relapsed B cell lymphoma. Treatment was well-tolerated and we observed no evidence of serious adverse events. The anti-tumor effect, however, was transient, suggesting that modification to attain durable anti-tumor effects in vivo is necessary. Our study establishes the methodologies and feasibility of anti-cCD20 CAR therapy in canines with spontaneous B cell lymphoma. This work lays the foundation for study of a large, outbred animal model that better resembles spontaneous human malignancies, enabling improved assessment for potential toxicity risks prior to translation into human studies.

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Isaac Perron

Neuroscience (NGG)

Diet/energy balance affect sleep and wakefulness independent of body weight

Isaac Perron, Allan Pack

Obesity is strongly associated with excessive daytime sleepiness (EDS), even in individuals without sleep apnea. Remarkably, obese patients who undergo weight loss therapy report dramatic improvements in EDS before significant weight loss occurs, suggesting that excess weight alone cannot fully explain EDS. We hypothesize that energy balance (i.e., weight gain/loss) has independent effects on EDS from body weight. We used rodent models of obesity, which spend more time asleep and cannot maintain long sleep/wake bouts compared to lean controls, to disentangle diet/energy balance from body weight on sleep/wake behavior. We fed adult mice either regular chow (RC) or high fat diet (HFD) for eight weeks. Then subsets of mice from each group were fed the opposite diet (a.k.a. diet switch), causing newly-fed HFD mice to gain weight and RC-fed mice to lose weight; one week later, the two diet switch groups had similar body weight ($p>0.05$). We found that sleep/wake behavior was dissimilar between these groups, with weight-loss mice exhibiting significantly increased wake time ($p<0.05$) and consolidated sleep/wake bouts ($p<0.05$) compared to mice gaining weight. Therefore, acute changes to diet and/or weight can affect sleep and wakefulness separately from body weight.

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David Reiner

Neuroscience (NGG)

Novel neural circuits mediating amylin's energy balance effects

David Reiner, Diana Olivos, Lauren McGrath, Derek Zimmer,
Elizabeth Mietlicki-Baase, Matthew Hayes

Amylin is a pancreatic-derived hormone that acts centrally to reduce food intake and its effects have predominantly been attributed to the area postrema. Recently, the ventral tegmental area (VTA), was highlighted as a physiologically relevant site of action for amylin-mediated control of energy balance, encouraging a broader assessment of the CNS circuitry mediating amylin's effects on food intake. Given that the lateral dorsal tegmental area (LDTg): [1] sends cholinergic/glutamatergic input to the VTA to modulate motivated behavior, [2] binds amylin, and [3] receives input from other feeding-relevant centers, we tested the hypothesis that amylin receptor signaling in the LDTg controls food intake in rats. First, qPCR analyses and immunohistochemical data show that the amylin receptor complex are expressed in the LDTg. Unilateral LDTg injection of the amylin receptor agonist salmon calcitonin (sCT) dose dependently reduced chow intake and body weight, which is driven by a suppression of meal size. Knockdown of amylin receptors in the LDTg increases body weight and food intake, suggesting that amylin receptor signaling in the LDTg is required for the normal control of energy balance. Together, these data identify the LDTg as a novel nucleus mediating the energy balance effects of amylin receptor signaling.

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Seth Rhoades

Pharmacology (PGG)

3D time-dependent fluxomics: probing metabolic flux across the circadian cycle using computational and experimental approaches

Seth Rhoades

Recent studies suggest that not only what we eat, but when we eat, play a role in the development of metabolic disorders. Systems-level research on circadian rhythms has focused on finding oscillatory patterns in ‘omics data in both altered feeding paradigms and genetic clock disruption. While this data can provide snapshots of metabolism, the dynamic nature of these rhythms cannot be proven with static measurements. We hypothesize metabolic abnormalities in disrupted rhythms and altered feeding paradigms can be found by delineating metabolic flux. Here we incorporate computational and analytical tools to assess flux across the circadian cycle. We employ constraint-based modeling (CBM) to generate untargeted predictions of metabolic flux across the circadian cycle using publicly available circadian expression datasets. Our objective is to compare these predictions across multiple metabolic tissues in different feeding paradigms and in the presence of a dysfunctional clock. Additionally, these predictions of diurnal metabolic changes can guide experimental flux experiments. We have also developed untargeted metabolomics methods using liquid chromatography mass spectrometry (LC-MS) to analyze polar metabolites. These tools, coupled with recent advancements in metabolomics data processing, we can utilize differential labeling analysis to identify pathways which utilize isotopically labeled nutrients in vitro.

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Alex Rohacek

Cell & Molecular Biology (CAMB)
Developmental, Stem Cell and Regenerative Biology

Loss of Esrp1 leads to defects in inner ear development associated with congenital hearing loss

Alex Rohacek, Thomas W. Bebee, Ricky Tilton, John Germiller, Ian Krantz, Russ P. Carstens, Douglas J. Epstein

Hearing loss is the most common form of congenital birth defect affecting an estimated 35 million children worldwide. To date, nearly 100 genes have been identified which contribute to a deafness phenotype in humans. The functions of these genes span the breadth of inner ear development to include transcription factors, signaling pathways and components of the sensory hair cells. Little is known, however, about how alternative splicing contributes to inner ear development and function. We recently identified mutations in Epithelial Splicing Regulatory Protein 1 (ESRP1), a critical regulator of alternative splicing, in a family with deafness. Here we show that loss of Esrp1 in mouse embryos leads to gross morphological defects of the inner ear. Esrp1 mutants further display defects in the timing of the auditory hair cell differentiation program. We performed RNA-seq experiments comparing control and mutant cochlea and observed a significant downregulation of many deafness genes. Of note, we found severely compromised expression of genes within the stria vascularis, a tissue required for ion homeostasis, which may be largely attributed to altered splicing of FgfR2. These results implicate Esrp1 as a critical regulator of inner ear development and as a novel locus for hearing loss in humans.

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Samantha Russell

Cell & Molecular Biology (CAMB)
Developmental, Stem Cell and Regenerative Biology

Identifying core components of the non-canonical Fra signaling pathway

Samantha Russell, Alexandra Neuhaus-Follini

Neural circuits form in early development and require axons to be guided accurately by conserved pathways. At the Drosophila ventral midline, ligands including Slit, which signal repulsion through Roundabout (Robo) receptors, and Netrin, which signal attraction through the Frazzled (Fra) receptor, signal locally through the cytoskeleton. Predominantly, research on axon guidance has been focused on understanding how ligand-receptor interactions affect local cytoskeletal rearrangements. However, we have found that Fra also acts independently of Netrin, as a transcription factor that activates expression of commissureless (comm), which encodes a protein that antagonizes Slit-Robo signaling. This allows commissural axons to avoid premature response to Slit and cross the midline.

We intend to identify genes that interact with Fra and are necessary for Fra to regulate transcription. The Fra intracellular domain (ICD) is cleaved and released by gamma-secretase, and contains an activation domain. However, it is unknown what proteins Fra interacts with that Fra requires to regulate transcription. We conducted a yeast two hybrid screen to identify proteins that interact with the Fra ICD. Initial screening has identified both proteins that are consistent with what is known about canonical Fra signaling, as well as novel interactors that have roles in transcriptional regulation.

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Brenda Salantes

Cell & Molecular Biology (CAMB)
Gene Therapy and Vaccines

Potency and kinetics of the autologous HIV-1 neutralizing antibody response during analytical treatment interruption

Brenda Salantes, Ben Scheinfeld, Katharine J. Bar

Background: Studies using a rhesus macaque model of HIV-1 infection have suggested that infusion of HIV-specific broadly neutralizing antibodies (bNAbs) into viremic animals leads to delayed virus rebound and potential boosting of the autologous adaptive immune response. Penn is currently conducting a Phase I clinical trial in which the bNAb VRC01 is infused into virally suppressed HIV-infected individuals who then undergo an analytic treatment interruption (ATI). To assess the baseline kinetics of rebound viremia and the humoral response, we analyzed a cohort of subjects who underwent ATI without bNAb immunotherapy.

Methods: We sequenced gp160 env by single genome sequencing (SGS) of plasma RNA from 11 subjects upon first detectable viremia post-ATI through three months post-ATI. Approximately 25 sequences per subject were analyzed phylogenetically by Maximum Likelihood analysis. Env sequences representing the consensus of low-diversity lineages were then cloned and assayed for autologous plasma neutralization using the TZM-bl assay.

Results: We generated a mean of 21 envs (range 13-29) from plasma taken at the first timepoint after detectable viral recrudescence. Phylogenetic analysis of post-ATI sequences revealed that rebounding viruses formed multiple, low diversity lineages (median 4, range 2 to >10) indicating multiple genetically distinct viruses arise from latency in the first weeks after ATI. Analysis of circulating viruses over time revealed shifts in the relative predominance of neutralization-sensitive lineages, with the more lineages with relatively greater

resistance to autologous antibodies persisting over time. These results suggest that the autologous antibodies can potently select against certain viruses and are able to drive of the composition of the viral quasispecies.

Conclusions: The study reported here suggests that HIV-infected individuals undergoing ATI exhibit rebound viremia from multiple reactivated cells within the first weeks after ATI. In this setting, the autologous neutralizing antibody response continuously exerts pressure on plasma virus after viral recrudescence. These findings serve as an essential first step in understanding the kinetics of viral rebound from latency and evaluating the potential adjuvant effects of bNAb infusion in humans.

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Mengge Shan

Genomics & Computational Biology (GCB)

Dynamics of changes in global protein-RNA interaction in Physcomitrella patens transcriptome

Mengge Shan and Matthew Willmann

RNA binding proteins (RBPs) mediate the regulation of eukaryotic RNAs at every point in the RNA 'life cycle' including stabilization, transcription, translation, degradation and other processes. Despite the frequency of RBP-RNA interactions, there was no established method for characterization of the entire set of RBP-RNA interaction sites in a eukaryotic organism. To address this gap we developed a ribonuclease-mediated protein footprinting approach called protein interaction profile sequencing (PIP-seq). Using data from this method, we uncovered previously uncharacterized signatures of RBP-RNA interaction sites in the model plant Physcomitrella patens (P. patens). Furthermore, we revealed that treatment of P. patens with the phytohormone abscisic acid (ABA), a key hormone involved in development and stress response, results in large-scale changes of its transcriptome. Finally, we found that gene expression changes are often a result of specific changes in the interactions between RBPs and their target RNAs. Based on these initial results, we hypothesize that many of the changes are the consequence of reordering of RNA secondary structure, a well known feature required for RBP-RNA interactions, and its subsequent effects on these binding events.

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Nishita Shastri

Pharmacology (PGG)

Defining the signature of genomic instability arising from replication stress

Nishita Shastri and Yu-Chen Tsai

Previous studies have defined genomic sequences that are difficult to replicate and are vulnerable to replication-associated breaks. However, many of these sequences have been identified through indirect and biased approaches. To identify genomic sequences that contribute to replication-associated breakpoints, we have performed genome-wide screens to determine the location, sequence, and frequency of replication perturbations within the mammalian genome upon replication stress. Ataxia telangiectasia and Rad3-related protein (ATR) is a checkpoint kinase that is a key upstream regulator of the response pathway to replication fork stalling during replication stress. Through inhibition of this response pathway, our aim is to determine the genomic regions that most frequently lead to double-strand breaks. A novel Break-Seq assay has been developed to isolate high frequency break sites throughout the genome that become sensitized to collapse into double-strand breaks. Since replication protein A (RPA) binds to single-stranded DNA that becomes exposed when replication forks stall, RPA ChIP-Seq has also been used to map sites of frequently stalled replication forks, however not necessarily ones that collapse into breaks. With these complementary approaches to map replication stress-sensitive regions, we can better understand features of the identified genomic regions that make it prone to fork stalling and/or collapse.

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Ewa Stypulkowski

Cell & Molecular Biology (CAMB)
Developmental, Stem Cell and Regenerative Biology

Understanding the role of protein palmitoylation during asymmetric cell division

Ewa Stypulkowski

Generating cellular heterogeneity is fundamental for the development of multicellular organisms and tissue homeostasis. A mechanism of generating cellular diversity is through asymmetric cell divisions, which generate morphologically and functionally distinct daughter cells through the unequal segregation of cellular components, such as proteins, mRNAs, and polarized cytoskeletal structures. The resulting daughter cells activate unique transcriptional programs specifying their identity. What remains unclear is how signaling molecules are targeted and anchored to the plasma membrane to establish cell polarity. Protein palmitoylation is a reversible lipid modification that promotes shuttling of proteins between the membrane and the cytosol. Thus, palmitoylation may be a novel mechanism for establishing cell polarity in dividing cells. We hypothesize the depalmitoylating enzyme, acyl- protein thioesterase 1 (APT1), regulates polarized cell divisions by asymmetrically targeting proteins to the plasma membrane during mitosis. Using cancer cell lines to model polarized cell divisions, we find that APT1 and signaling molecules, β-catenin and Numb, are asymmetrically localized and this is dependent on APT1 activity. Furthermore, we find that APT1 activity is required for regulating asymmetric gene expression using reporter cell lines. Taken together, our findings provide a novel role for palmitoylation during asymmetric cell division and cell-fate decisions.

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Camille Syrett

Cell & Molecular Biology (CAMB)
Developmental, Stem Cell and Regenerative Biology

Female mouse lymphocytes have unusual features of X-Chromosome Inactivation: can reinstating X-silencing correct overexpressed X-linked genes in lupus?

Camille Syrett, Jianle Wang, Ian Penkala, Montserrat C Anguera

Systemic lupus erythematosus (SLE) is a female-biased autoimmune disorder whereby over 90% of those affected are women. Interestingly, overexpression of X-linked autoimmune genes has been uniquely observed in lymphocytes of female SLE patients. Normally, the contribution of genes from the second X chromosome is silenced through the process of X chromosome inactivation (XCI), which is mediated by the long noncoding RNA XIST. We examined the epigenetic properties of the inactive X in naïve and activated murine lymphocytes and made the remarkable discovery that Xist RNA is abundantly expressed yet abnormally dispersed thought nuclei in naïve T cells compared to their stimulated counterparts. Furthermore, we observed a reduction in heterochromatin modifications that typically localize to the inactive X chromosome. Using murine models of lupus we are testing whether Xist deficiency exacerbates SLE-like disease development and severity. Additionally, we seek to genetically correct abnormal expression of immunity-related X-linked genes using the XCI machinery, by inserting an inducible human XIST transgene into the endogenous X chromosome or an inducible promoter upstream of XIST with CRISPR technology. In sum, these studies aim to provide a novel link between Xist-mediated XCI and female autoimmunity.

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Miklos Szantai-Kis

Biochemistry & Molecular Biophysics (BMB)

Broadening the utility scope of thioamides

D. Miklos Szantai-Kis, J. Yanxin Wang, Eileen M. Hoang, E. James Petersson

Thioamides are a single-atom substitution in canonical amide bonds. Through their altered physical properties, thioamides are able to quench fluorescence via FRET and PET mechanism. Thus far thioamides have only been incorporated into the backbone of full length proteins via solid phase peptide synthesis followed by native chemical ligation. Our current studies aim to facilitate thioamide incorporation into full length proteins: Our first approach is to optimize conditions, so we can selectively desulfurize beta-thiol groups in the presence of thioamides. This would allow the use of beta-thiol derivatives of amino acids for native chemical ligation and would greatly increase the number of potential ligation sites. Our second approach is to put the thioamide motive into the side-chain of amino acids, specifically of Asn and Gln. These probes would potentially be able to be incorporated into proteins by the ribosome. Altogether these two approaches will broaden the utility scope of thioamides.

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Benjamin Tajer

Cell & Molecular Biology (CAMB)
Developmental, Stem Cell and Regenerative Biology

Distinct signaling roles for type I receptors Bmpr1 and Acvr1l, and the type II receptors Bmpr2 and Acvr2 within the BMP receptor complex

Benjamin Tajer and Mary Mullins

The Bone Morphogenetic Protein (BMP) pathway patterns dorsal-ventral (DV) tissues during gastrulation. A dimeric BMP ligand assembles a receptor complex composed of two type-I and two type-II receptors. Type-II receptors phosphorylate and activate type-I receptors, which then phosphorylate Smad proteins, which regulate gene expression. This, however, is overly simplistic as there are two conserved classes of type-I receptor, Bmpr1 and Acvr1l, and two conserved classes of type-II receptor, Bmpr2 and Acvr2, all of which are necessary for vertebrate development. In the zebrafish embryo, Bmp2/7 heterodimers are the only ligands that signal in DV patterning, due to the heterodimer's unique ability to integrate both type-I receptors into the BMP receptor complex, as Bmpr1 preferentially binds the Bmp2 ligand, and Acvr1l exclusively binds Bmp7. I hypothesize that Bmpr1 and Acvr1l have distinct functional roles. I am performing a series of domain swap experiments to determine the components required for each receptor's specific function. We do not currently know the contribution of the two BMP type-II receptor classes, Bmpr2 and Acvr2, to the signaling complex. I am creating zebrafish mutants null for each type-II receptor class using CRISPR technology, to determine whether both classes have independent, necessary signaling functions in DV patterning.

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Feven Tameire

Cell & Molecular Biology (CAMB)
Cancer Biology

The stress response transcription factor ATF4 mediates cytoprotective pathways in c-Myc overexpressing cells

Feven Tameire

The proto-oncogene c-Myc is often deregulated in human tumors and its overexpression associates with poor prognosis in patients. While c-Myc is a potent activator of pro-tumorigenic pathways, it can also induce antitumorgenic pathways such as apoptosis. However, in c-Myc driven cancers the anti-tumorigenic state is evaded via a yet poorly understood pro-survival mechanism. We previously reported that activation of the PERK arm of the Unfolded Protein Response (UPR) is one such pro-survival mechanism induced by c-Myc to bypass c-Myc induced apoptosis. ATF4, one of the main downstream effectors of PERK, regulates amino acid metabolism, antioxidant response and autophagy. Here, we show ATF4 expression is induced in Myc overexpressing cells. Activation of c-Myc in ATF4-deficient cells resulted in enhanced apoptosis and decreased clonogenic survival. We observe increased oxidative stress in ATF4 deficient cells during c-Myc activation that can be rescued with addition of Trolox, a potent antioxidant. Accordingly, we also observe enhanced protein levels of ATF4 in lymphocytes isolated from a c-Myc driven mouse model of lymphoma (Eμ-Myc) compared to lymphocytes from WT control mice. We further explore regulation of ATF4 expression during c-Myc activation and downstream pathways that ATF4 regulates.

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Matthew Thompson

Biochemistry & Molecular Biophysics (BMB)

Influenza A M RNA presents a novel example of host proteins hnRNP K and NS1-BP regulating alternative splicing

Matthew Thompson, Ke Zhang, Beatriz M. A. Fontoura, Kristen W. Lynch

The regulation of eukaryotic and viral RNA alternative splicing is known to be highly important to cellular and viral function. Splicing regulation is primarily achieved by the association of RNA binding proteins with specific splicing elements on an RNA, impacting the recruitment of spliceosome machinery, and resulting in the skipping or inclusion of an exon. We have previously identified that host cell RNA-associated proteins hnRNP K and NS1-BP regulate the alternative splicing of influenza A M RNA. The influenza A genome consists of 8 single-stranded RNA segments, with two segments, M and NS, being spliced. The M segment RNA has two major isoforms, the unspliced M1 RNA and the spliced M2 RNA. Each of these isoforms encodes for proteins required for optimal viral infection and replication. We have shown that hnRNP K and NS1-BP are required for the production of M2 RNA splice products. This is the first known example of hnRNP K and NS1-BP cooperatively regulating a splicing event in either viral or host cell contexts. Thus, we have sought to study M RNA splicing at a detailed level to better understand the roles of hnRNP K and NS1-BP as splicing regulators.

To understand how hnRNP K and NS1-BP regulate M RNA splicing we first looked at how the proteins interact with the transcript. Previous results showed that hnRNP K directly binds M RNA while NS1-BP indirectly associates through a protein-protein interaction. We now show that the binding of hnRNP K to M RNA occurs within the first 106 nucleotides (M 1-106) of the 1004 nt transcript. This binding event is key to the assembly of protein complexes on M RNA, as shown by a loss of nuclear protein binding to M RNA when hnRNP K binding is

perturbed. Additional to a hnRNP K binding site, M 1-106 contains the M2 5' splice site. In cell-based assays where M 1-106 is fused to a heterologous intron-exon pair, the M2 splice site is utilized in a manner comparable to wildtype M RNA. These data suggest that M 1-106 is a key region for regulation of M RNA splicing. Future experiments will address if hnRNP K and NS1-BP regulate splicing by utilizing elements within M 1-106. We will then characterize the specific regulatory sequences within M 1-106 to define a clear mechanism for how hnRNP K and NS1-BP are influencing splicing. The data gained from these experiments will provide valuable information about how hnRNP K and NS1-BP regulate splicing in viral and host cell RNAs.

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James Townsend

Biochemistry & Molecular Biophysics (BMB)

Organized water: addressing the paradoxes of acellular bodies in ctenophores

James Townsend, Blanca Aguilar, Felix Barber, Guillermina Ramirez-San Juan

In the genomic era, we know that the earliest-branching animal phyla include ctenophores, sponges, and cnidarians. Intriguingly, these animals all share bodies that are more amorphous, acellular materials by volume than they are living cells. Ctenophores—perhaps the first-branching extant metazoan taxon—are primarily composed of a voluminous hydrogel called the "mesoglea" that is derived from the ECM of the thin epithelium that surrounds it. We report that this gel is far from simple, but has sophisticated material properties. First, it behaves as a non-Newtonian fluid that is also self-healing. It is also molecularly crowded, with a viscous fluid phase and very slow diffusion of sub-micron beads. Paradoxically, while the gel appears "full" to diffusing particles, it looks "empty" to light, with a refractive index almost identical to seawater and vanishingly low concentrations of proteins or sugars. How can a structure be "empty" to light, yet otherwise exhibit these sophisticated properties? Further, what do these material properties teach us about the bodies of the earliest animals? We will present results on the biochemical constituents of ctenophore mesoglea, their organization within the gel, and on the viscoelastic, shear-hardening properties of this enigmatic tissue in the context of the animals' development and ecophysiology.

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Michael Werner

Immunology (IGG)

Using chimeric BET proteins to study how they bind chromatin and contribute to transcription

Michael Werner, Sarah Hsu, Aaron Stonestrom, Gerd Blobel

Bromodomain and extra-terminal motif (BET) proteins BRD2, BRD3, and BRD4 bind chromatin and "read" acetylated lysines to cue transcription. Inhibition of the acetyl-binding BET bromodomains has proven effective in treating inflammation, cardiac remodeling, and many cancers. Despite excitement in this new field of therapy, a comprehensive understanding of how BET proteins bind to a given locus and contribute to transcription is lacking. Using a gain-of-function approach in a BRD2-/- cell line, we observed that BRD3 but not the short or long isoforms of BRD4 (BRD4s and BRD4l) can rescue transcription, suggesting that functional overlap exists between BRD2 and BRD3 but not BRD4. Given that these two proteins are structurally similar to BRD4s, we designed chimeric BRD2 and BRD4s constructs to determine in an unbiased manner which domains underlie BRD2 function. Intriguingly, a chimera containing the bromodomains of BRD4 and the carboxy-terminal domains of BRD2 was sufficient to rescue BRD2-/- cells. Future experiments will refine our domain mapping of BET proteins to understand how individual domains contribute to localization on the genome, recruitment of transcriptional cofactors, and subsequent transcriptional control.

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Frances Xin

Cell & Molecular Biology (CAMB)
Cancer Biology

Assessing the transmission of an altered epigenotype and phenotype following exposure to endocrine disrupting compounds

Frances Xin, Martha Susiarjo, Amita Bansal, Martha Stefaniak, Changhong Li, Rebecca Simmons, Marisa Bartolomei

Fetal exposure to endocrine disrupting compounds (EDCs) results in aberrant developmental outcomes and increased disease susceptibility in adult life. These effects can also be transmitted across multiple generations. Although the precise mechanisms by which these compounds act remain to be elucidated, it has been proposed that epigenetic pathways mediate their effects. Exposure to the EDC bisphenol A (BPA) has been shown to alter DNA methylation, an epigenetic regulatory mechanism critical for proper development. DNA methylation is also a well-established mechanism of imprinted gene regulation. In our mouse model, fetal exposure to BPA results in aberrant regulation of imprinted genes in a gene- and tissue-specific manner, which corresponds with altered DNA methylation at regulatory elements of imprinted genes. In adulthood, BPA-exposed male offspring exhibit altered metabolism. Interestingly, these phenotypic changes persist to the next (F2) generation. Misregulation of Igf2, a growth-promoting imprinted gene, is associated with the observed metabolic phenotype. Because humans are rarely exposed to a single EDC at once, we are assessing the effects of combinatorial exposures. Identifying the detrimental effects of early-life EDC exposure on fetal and postnatal development across multiple generations and determining their mode of action will ultimately improve human health risk assessments of these compounds.

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Bihui Xu

Cell & Molecular Biology (CAMB)
Cancer Biology

Stromal regulation of breast cancer treatment resistance by STAT1 and NOTCH3

Bihui Xu, Tony J. Wu, Barzin Y. Nabet, Mirjam C. Boelens, Dianna Azzam, Joyce Slingerland, Andy J. Minn

Besides cell intrinsic mechanisms that control resistance to cancer therapy, cellular interactions with the microenvironment can also confer protection from treatment. Here, we show that tumor-stroma interaction protect breast cancer cells from radiation through expansion of a population of therapy resistant cells with tumor initiating properties. Interferon-stimulated genes (ISGs) are induced in breast cancer after interaction with stroma in a STAT1-dependnent manner. Concomitantly, cell-cell contact results in expression and activation of breast cancer NOTCH3 by JAG1. The activation of NOTCH and STAT1 pathways synergizes to enhance transcription of NOTCH target genes through increased STAT1 binding at promoter regions of NOTCH target genes. Treatment of mice with a gamma secretase inhibitor counteracted the protective effects of stroma and led to long term survival. In total, these results highlight the cooperatively of STAT1 and NOTCH pathways in mediating therapy resistance.

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Ying Zhang

Cell & Molecular Biology (CAMB)
Gene Therapy and Vaccines

Metabolic stress contributes to exhaustion of tumor infiltrating CD8+ T lymphocytes

Ying Zhang, Ling Liu, Raj Kurupati, Xiangyang Zhou, Hudaihed A, Filisio F, Wynetta Giles-Davis, Joshua Rabinowitz, Hildegund C.J. Ertl

The efficacy of immunotherapy of solid tumors is dampened by T cell exhaustion, which is generally attributed to continuous antigenic stimulation. On the contrary, we observed in a mouse melanoma model that bystander CD8+ tumor-infiltrating lymphocytes (TILs) became exhausted, suggesting alternative mechanisms. CD8+TILs increasingly experienced metabolic stress during tumor progression. Our data show that metabolic challenges profoundly affected differentiation and functions of TILs. As shown with vaccine-induced CD8+TILs and in vitro activated polyclonal CD8+T cells, hypoxia directly increased LAG-3 expression through hypoxia-induced factor (HIF)-1α, while restricting Glu supply enhanced PD-1 expression. Both conditions reduced CD8+T cell functions and polyfunctionality. Further studies showed that T cells in late stage tumors depended on FAs uptake, triacylglycerol (TG) synthesis and lipolysis to fuel FA ß-oxidization (FAO). Increased energy production through FAO when Glu and O2 were limited in turn increased PD-1 expression but preserved effector functions of activated CD8+T cells. Our findings highlight that metabolic stress plays a critical role in driving exhaustion of CD8+TILs, suggesting metabolic interventions as an added strategy to improve the efficacy of cancer immunotherapy.

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Yuxiang Zhang

Pharmacology (PGG)

Discrete functions of nuclear receptor Rev-erbα couple metabolism to the clock

Yuxiang Zhang

No abstract provided.

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Atrish Bagchi

Biochemistry & Molecular Biophysics (BMB)

Extracellular dysregulation of the epidermal growth factor receptor

Atrish Bagchi, Nicholas Bessman, Mark Lemmon, Kathryn Ferguson

A subset of oncogenically activating epidermal growth factor receptor variants are located in its extracellular region, including EGFR variant III (EGFRvIII), found in ~50% of glioblastoma multiforme (GBM) patients. EGFRvIII is an important clinical target in glioblastoma multiforme. It is not fully understood how EGFRvIII leads to transactivation of EGFR and Met. Additional extracellular single amino acid substitutions in EGFR are sufficient to transform NIH3T3 cells and cause tumor growth in murine xenograft models. The activating point mutations are concentrated in critical regions around domain II, leading us to hypothesize that these mutations may cause ligand independent dimerization of the EGFR extracellular region (ECR); however, sedimentation equilibrium analytical ultracentrifugation analysis suggests that R84K, A265D and A265V do not affect dimerization of the EGFR ECR in the absence or presence of ligand. Surface plasmon resonance and isothermal titration calorimetry analysis indicate a significant increase in the ligand binding affinities for these EGFR ECR variants. Together these data suggest that these mutations alter the thermodynamic linkage of ligand binding and dimerization. We will present our progress towards undertsanding the mechanistic basis of activation of these extracellular EGFR variants based on biophysical techniques, including X-ray crystallography, using a nanobody/VHH domain.

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Kellie Woll

Pharmacology (PGG)

A novel bifunctional propofol analogue reveals selective GABAA receptor subunit binding in native neuronal tissue

Kellie Woll

Propofol, an intravenous anesthetic, is a positive modulator of the GABAA receptor, but the mechanistic details, including the relevant binding sites, remain disputed. Here we designed and synthesized a photoactive and clickable propofol analogue, ortho-alkynyl-meta-azipropofol (AziPm-click 1), for the unbiased identification of propofol-binding proteins in their native state within mouse synaptosomes. After confirmation of retained in vivo and GABAergic anesthetic character, this affinity-chemoproteomic strategy captured ~4% of the synaptosomal proteome, including five α or β GABAA receptor subunits. Lack of γ2 subunit capture was not due to low abundance. Molecular dynamics simulations revealed that higher affinity interactions for propofol at αβ relative to γ-containing interfaces were due to differential hydrogen-bond probability. This investigation provides the first evidence for direct propofol interaction of specific GABAA receptor subunits within native neuronal tissue. Finally, the complex alkylphenol-binding proteome provides a rich source of targets that might underlie the many effects of propofol.

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