CHARACTERIZING IMMUNE RESPONSES TO AAV9 IN A MOUSE MODEL OF DUCHENNE MUSCULAR DYSTROPHY

MICHAEL EMAMI (MBIDP/ CBB, Spencer lab)

Duchenne muscular dystrophy (DMD) is an X-linked disease caused by out-of-frame mutations in the DMD gene. Gene replacement strategies use AAV to deliver a truncated DMD gene and are accompanied by a series of severe autoinflammatory and pre-existing T cell responses against dystrophin. It is unclear why certain patients develop AAV-specific responses and the specific immune responses that arise following AAV gene therapy. In order to comprehensively and unbiasedly characterize AAV-induced immune responses in vivo, we dosed a dystrophic mouse model with AAV serotype 9 carrying a vector encoding CRISPRCas9 and mCherry reporter and performed 10x Genomics single cell RNA-sequencing (scRNA-seq). We injected AAV9-mCherry and PBMCs were isolated and ana- lyzed prior to AAV administration and then 2 weeks post- AAV administration. AAV9-mCherry single injection of AAV-mCherry, phenotypic shifts in monocytes, NK cells, B cells, and T cells were observed. We also tested the effect of two AAV- CRISPR mCherry injections. Upon second exposure to AAV- mCherry, the mCherry expression was not detected, suggest- ing CRISPR/Cas9 and mCherry reporter and performed 10x Genomics single cell RNA-sequencing (scRNA-seq). We injected AAV9-mCherry and PBMCs were isolated and ana- lyzed prior to AAV administration and then 2 weeks post- AAV administration. AAV9-mCherry single injection of AAV-mCherry, phenotypic shifts in monocytes, NK cells, B cells, and T cells were observed. We also tested the effect of two AAV- CRISPR mCherry injections. Upon second exposure to AAV- mCherry, the mCherry expression was not detected, suggest- ing CRISPR/Cas9 and mCherry reporter and performed 10x Genomics single cell RNA-sequencing (scRNA-seq). We injected AAV9-mCherry and PBMCs were isolated and ana- lyzed prior to AAV administration and then 2 weeks post- AAV administration. AAV9-mCherry single injection of AAV- mCherry, phenotypic shifts in monocytes, NK cells, B cells, and T cells were observed. We also tested the effect of two AAV- CRISPR mCherry injections. Upon second exposure to AAV- mCherry, the mCherry expression was not detected, suggest- ing CRISPR/Cas9 and mCherry reporter and performed 10x Genomics single cell RNA-sequencing (scRNA-seq). We injected AAV9-mCherry and PBMCs were isolated and ana- lyzed prior to AAV administration and then 2 weeks post- AAV administration. AAV9-mCherry single injection of AAV- mCherry, phenotypic shifts in monocytes, NK cells, B cells, and T cells were observed. We also tested the effect of two AAV- CRISPR mCherry injections. Upon second exposure to AAV- mCherry, the mCherry expression was not detected, suggest- ing CRISPR/Cas9 and mCherry reporter and performed 10x Genomics single cell RNA-sequencing (scRNA-seq). We injected AAV9-mCherry and PBMCs were isolated and ana- lyzed prior to AAV administration and then 2 weeks post- AAV administration. AAV9-mCherry single injection of AAV- mCherry, phenotypic shifts in monocytes, NK cells, B cells, and T cells were observed. We also tested the effect of two AAV- CRISPR mCherry injections. Upon second exposure to AAV- mCherry, the mCherry expression was not detected, suggest-
to GCSF as a proof-of-concept model protein. Therefore, a
new type of interferon (IFN) response defends against viral infection by stimulating expression of antiviral genes known as IFN-stimulated genes (ISGs). One such ISG is zinc fin-
ger antiviral protein (ZAP), which inhibits viral mRNA translation. However, the mechanism by which ZAP recognizes viral proteins and triggers translation inhibition is not fully understood. In this study, we propose a model where ZAP interacts with viral proteins in a manner that leads to their degradation.

ELUCIDATING ALPHAVIRAL DETERMINANTS OF SENSITIVITY TO ZINC FINGER ANTIVIRAL PROTEIN (ZAP) LEAN NGUYEN (Mailman lab)

The type I interferon (IFN) response is a critical innate immune response that provides broad antiviral protection. In this study, we investigated the mechanism by which ZAP (also known as MDA5) responds to alphaviruses, a family of viruses that cause a wide range of diseases in human and non-human primates.

NupJ is a nuclear pore protein that plays a crucial role in cellular functions such as nucleocytoplasmic transport and signal transduction. In this study, we investigated the role of NupJ in response to cellular stress, particularly in the context of interferon-stimulated gene expression.

CUL3 SUBSTRATE ADAPTOR SPOP REGULATES THE NUCLEAR POLE PROTEIN NUPJ

JOSEPH ONG (Chemi & Biochem/BBS, Torres lab)

Cell processes like growth and division are tightly regulated. The nuclear envelope, which separates the nucleus from the cytoplasm, is a vital organelle for maintaining these processes. In this study, we explored the role of the nuclear envelope protein NupJ in response to cellular stress.

REGULATION OF THE CHROMATIN REMODELING SNF2 IN S. CEREVISIAE

LAUREN THURLOW (MBDIP/BBS, Johnson lab)

Eukaryotic cells have evolved complex gene regulation networks to coordinate responses to external conditions and stimuli. One such network involves the chromatin remodeling complex SNF2, which plays a crucial role in regulating gene expression.

DROPLETS INTO CELLS AND ORGANISMS. TO MONITOR THE LOCALIZATION OF DYES AND LABELS, bioluminescence imaging (BLI) and fluorescence imaging (FLI) have been widely used. However, these methods are limited in their ability to resolve small structures and to monitor the localization of single molecules within the cell. To overcome these limitations, researchers have developed new methods that allow for the localization of dyes and labels with high accuracy and resolution.

In this study, we investigated the potential of using bioluminescent probes in single molecule localization (SML) experiments to study the localization of dyes and labels within cells and organisms. We found that bioluminescent probes can be used to accurately localize dyes with high resolution, even within the sub-cellular scale.

The bioorthogonal nature of perfluorocarbons provides a unique platform for introducing dyes and labels into cells and organisms. To monitor the localization of dyes and labels, bioluminescence imaging (BLI) and fluorescence imaging (FLI) have been widely used. However, these methods are limited in their ability to resolve small structures and to monitor the localization of single molecules within the cell. To overcome these limitations, researchers have developed new methods that allow for the localization of dyes and labels with high accuracy and resolution.

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mammals) appears to play a role in carrying out the down-regulation of Snf2 protein levels, a clue that will be further examined. Investigating the dependence of cancer cells on their mtDNA will provide fresh insight into tumor heterogeneity and evolution in the conditions of severe mitochondrial dysfunction. Understanding content over time in vivo, which has been shown to occur in these cells in both in vitro and in vivo conditions, we will gain further insights into how mtDNA affects cancer growth and metastasis independently of the nuclear genome. Additionally, single cell RNAseq data afford characterization of heterogeneity within cultures such that nuanced statements can be made about the relationship between the sources of heterogeneity, and their interactions with QTL. To this end, we have generated single cell RNA seq data for haploid parents, diploid parents, and segregants of a cross between strains BY and RM. First, we recapitulated parent qEQL results from bulk RNA seq using scRNA data. Next we used the haploid parent data to develop penalized logistic regression classifiers of cell cycle phase (evaluated in diploid and haploid segregants), along with other annotations of the sources of heterogeneity within the datasets. We then used these annotations to look for interactions with qEQL.

**ASSESSING THE EXISTENCE OF NER1 ISOFORMS IN THE DEVELOPING SPINAL CORD**

**SANDY ALVAREZ** (MBIDP/ CDB, Butler lab)

The function of the nervous system is dependent on the correct assembly and development of neural circuits. Circuits in the spinal cord are generated when growth cones at the tips of axons use molecular cues in the environment to guide axon extension. Netrin 1 is a long-range, diffusible, chemoattractant that emanates from the floor plate (FP). However, recent work by the Butler lab and other groups, has suggested this model is incorrect. In the mouse spinal cord, DCC is expressed in both FP cells and neural progenitor cells (NPCs) in the ventricular zone (VZ). In the absence of either netrin1 or its receptor, DCC, axons innervate the VZ and commissural axons either stall or defasciculate. These phenotypes are only observed on the dorsal surface of the spinal cord. In the visual system, netrin1 can stall or defasciculate. These phenotypes are only observed in diploid and haploid segregants. Additionally, single cell RNAseq data afford characterization of heterogeneity within cultures such that nuanced statements can be made about the relationship between the sources of heterogeneity, and their interactions with QTL. To this end, we have generated single cell RNA seq data for haploid parents, diploid parents, and segregants of a cross between strains BY and RM. First, we recapitulated parent qEQL results from bulk RNA seq using scRNA data. Next we used the haploid parent data to develop penalized logistic regression classifiers of cell cycle phase (evaluated in diploid and haploid segregants), along with other annotations of the sources of heterogeneity within the datasets. We then used these annotations to look for interactions with qEQL.

**EPIGENETIC MODIFICATION IN NK CELL ANTI-TUMOR RESPONSES**

**MANDY CHENG** (MBIDP/ IMMP, Su lab)

Natural Killer (NK) cells play a crucial role in cancer immuno-surveillance. However, multiple cell surface receptors that recognize stress-induced ligands on malignant cells. Engagement of NK cell surface receptors elicits an intricate signaling network that results in the release of cytokotic granules and cytotoxic granules. The frequency of NK cells in human peripheral blood correlates with increased cancer incidence, indicating importance of NK cells in tumor immunity. However, the factors that regulate NK cell maturation remain unclear. Accumulating evidence suggests a critical role for epigenetic regulation in immune cell differentiation. Interestingly, our preliminary data indicates that 3′UTR RNASEQ results suggest that UTX controls NK cell maturation and responses. Our preliminary data demonstrates NK cell-specific deletion of UTX results in reduced expression of eQTL in some NK cell subtypes suggesting that UTX controls NK cell maturation. Interestingly, a recent study shows UTx demethylate activity is 

**DETERMINING SOURCES AND TARGET CELLS OF SPP1 IN DYSTROPHIC MUSCLE NICHES USING SINGLE-CELL RNASEQ**

**RAQUEL ARAGON** (MBIDP/ CDB, Spencer lab)

In DMD, chronic cycles of degeneration and regeneration lead to intramuscular inflammation and accumulation of fibrosis. Our previous work showed that Spp1 exacerbates progression of muscular dystrophy. We showed that Spp1 promotes muscle fibrosis and shifts macrophages to a pro-inflammatory phenotype. However, the cellular source of Spp1 and its target cells in dystrophic muscle are unknown. It is likely that different cellular sources of Spp1 could have disparate effects on muscle regeneration. In this study, we used mass cytometry to cross-link muscle stem cell (MUSC)-specific and myofibroblast-specific Cre drivers to parse how Spp1 derived from these cell types promotes disease, and to dissect the impact of local sources of Spp1 on the dystrophic cellular milieu. To dissect the source of Spp1, we conducted functional testing and assessed how Spp1 ablation affected muscle performance. To characterize the effect of cell specific sources of Spp1 on cellular targets, we carried out scRNAseq. Functional testing showed a mild improvement of Musc-specific (Pax7-Cre) Spp1 conditional knockout. Bulk RNAseq of sort-purified Pax7(+) cells revealed Spp1 ablation had an auto-crine effect. Pax7-Cre induced Spp1 expression is pro-mitogenic genes and inhibition of inflammatory and fibrotic genes. scRNAseq analysis revealed that Pax7 Cko shifted macrophage phenotype. Myofibroblast-specific (Lys2) Spp1 Cko showed a significant improvement in muscle function. scRNAseq revealed that macrophage-specific Spp1 Cko led to ablation of a specific population of Pdgfra(+) stromal cells with upregulated ApoD expression. These findings suggest that different cellular sources of Spp1 exert diverse downstream effects on cells in dystrophic muscular dystrophy.
TRIM28 IS ESSENTIAL FOR MOUSE GERMLINE DEVELOPMENT

JONATHAN DIRUSO (MBIDP/ CDB, Clark lab)

The germline is responsible for transmitting the genome between parent and offspring, therefore the maintenance of germline integrity is critical for offspring health. During the initial stages of mammalian germline development, retrotransposons are repressed despite global loss of DNA methylation from the germline. While most retrotransposons in the soma have degraded and are no longer capable of active transposition, some young retrotransposons retain the ability to mobilize, presenting a potential threat to germline genome integrity and, consequently, fertility if aberrantly expressed. We have shown that the tripartite-motif containing-28 (Trim28) plays a crucial role in repressing retrotransposons, particularly piwi family retrotransposons, in the mouse germline. We have demonstrated that Trim28 inactivation results in derepression of several piwi family retrotransposons and amplification of a large number of long interspersed nuclear elements (LINEs). These findings demonstrate an essential role for Trim28 in maintaining repression of LINEs, revealing the potential role of Trim28 in maintaining retrotransposon repression in the mouse germline.

SYNTHESIS OF FLUORINE-18 LABELED SMALL MOLECULES FOR USE IN PET IMAGING

BALKWIN LIWANAG (Chem & Biochem, Murphy lab)

Recent advances in cancer therapy have highlighted the need for improved diagnostic tools and targeted therapies for improving patient outcomes. One promising area is the use of positron emission tomography (PET) imaging with fluorine-18 labeled small molecules (FSMMs) to assess tumor metabolism, response to therapy, and potential biomarkers of disease progression. The synthesis of FSMMs is critical to enhance the efficacy of both endogenous NK cell responses and potential NK cell therapies.

ENGINEERING A YEAST-BASED PLATFORM FOR PRODUCTION OF NOVEL MONOTERPENE INDOLE ALKALOIDS

JOSHUA MISA (Chem & Biomolecular Engineering, Tang lab)

Monoterpane indole alkaloids (MIAs) are an expansive class of plant natural products, many of which have been named on the World Health Organization’s List of Essential Medicines. Their biological activities include anti-cancer, anti-malarial, anti-addiction, and more. However, MIAs are some of the costliest small-molecule drugs due to low production of natural hosts. Thus, a more sustainable and reliable source of these drugs is critical to meet global demand. The model eukaryote, Saccharomyces cerevisiae (Baker’s yeast), has proven to be an effective host for production of numerous challenging compounds. In our work, we report the development of a yeast-based platform for high-throughput production of the universal MIA precursor, strictosidine, using a combination of synthetic biology and metabolic engineering techniques. Our fed-batch platform produces 5 mg/L stricostidine starting from the inexpensive commercially available, geranium, and is the highest titer reported thus far. Additionally, our robust platform can produce stricostidine analogues through feeding modified substrates to our host. Our platform will enable future reconstitution of downstream biosynthetic pathways towards production of more elaborate MA analogues, which are critical for drug discovery.

SYNTHESIS OF FLUORINE-18 LABELED SMALL MOLECULES FOR USE IN PET IMAGING

MATTHEW LOWE (MBIDP/ CDB, Clark lab)

Primary Germ Cells (PGCs) are the source of the entire adult germline and their proper differentiation is essential for the maintenance of fertility. Our group has shown that the DNA methyltransferase Setdb1 to establish repressive marks. We have demonstrated differential depletion of DNMT1 which maintains DNA methylation and represses genome-wide demethylation in PGCs, and show that these promoters are also used in the adult germline and their proper differentiation is essential for the maintenance of fertility. We have demonstrated that while EED and H3K27me3 are not necessary for the maintenance of fertility, their loss leads to defects in dendritic branching, and neurophysiological abnormalities. Our group’s primary focus is the synthesis of Fluorine-18 labeled compounds for PET imaging.

TOWARDS A MECHANISTIC UNDERSTANDING OF A TYPE III POLYKETIDE SYNTHASE INVOLVED IN CANNA-BINOID BIOSYNTHESIS

KRISTOFER GONZALEZ-DEWHITT (MBIY/ BSBB, Abramson lab)

Plant-specific type III polyketide synthases catalyze the iterative condensation of acetyl-CoA with malonyl-CoA to produce a variety of polyketide scaffolds. In Cannabis sativa, a polyketide synthase generates a linear poly-β-keto tetraketide, which downstream enzymes cyclize to form tetracycloannorobinal or cannabinol. In mouse models of anxiety, the cannabinoid, reveal the protein’s catalytic cysteine undergoes irreversible oxidation to yield an inactive enzyme, making biochemical and biophysical characterization impossible. Current efforts are focused on identifying and preventing this inhibitory reaction in order to fully characterize the enzyme. The overarching goal of the project is to develop a biosynthetic approach that improves cannabinoid production for pharmaceutical purposes.

SYNTHESIS OF FLUORINE-18 LABELED SMALL MOLECULES FOR USE IN PET IMAGING

BALKWIN LIWANAG (Chem & Biochem, Murphy lab)

Retrotransposon reactions are a major source of genetic and potential applications of these reactions include in situ drug delivery, uncaging reactions, and bioimaging. Highly selective retrotransposon reactions have been used to image relevant biochemical processes, such as cancer metabolism, disease progression, and biomolecule localization. One area of research we are interested in exploring is immune cell imaging; imaging of immune cells allows for non-invasive visualization of immune cell response and its localization. Our research goal is to develop new bioorthogonal reactions with favorable kinetics for use in positive emission tomograpy (PET) imaging of immune cells.

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**Determining whether RNAi core components are involved in mammalian pericentromeric regulation**

**RAFAEL SANDOVAL** (MBIDP/ BSSB, Zamudio lab)

RNA interference (RNAi) is a highly conserved gene regulatory process that utilizes small RNA to target messenger RNA (mRNA) for transcriptional repression. Although the role RNAi plays in posttranscriptional regulation has been well-characterized in a myriad of eukaryotic systems, studies show RNAi factors Argonaute (Ago) and Dicer also serve essential roles in establishing and maintaining the heterochromatic state of the pericentromere therefore regulating its transcriptional output and higher order structure. Although the intersection between RNAi and heterochromatin assembly is well resolved in most eukaryotic systems, it remains elusive in mammals. To characterize the Ago-mediated regulation at the mammalian pericentromere, we utilize a mouse embryonic stem cell (mESC) system that has an Ago1-4 null background and a doxycycline inducible Ago2. Here, we confirm the existence of multiple pericentromeric ncRNA species that vary in size. Furthermore, with the loss of Ago proteins, we observe a change in the size heterogeneity and an increased abundance of pericentromeric ncRNAs. Using live-cell imaging and histone H2B-GFP cell lines, we observe colcemid treated Ago null cells bypass the mitotic spindle checkpoint at higher frequency compared to cells expressing Ago2.

**Halting and visualizing heme acquisition by pathogenic Staphylococcus aureus using a photocaged Hb receptor**

**JESS SOULE** (Chem & Biochem, Rodriguez & Clubb labs)

The Center for Disease Control has identified methicillin-resistant *Staphylococcus aureus* (MRSA) as a serious threat, which annually in the United States is responsible for over 300,000 hospitalizations and 10,000 deaths. *S. aureus* acquires the essential nutrient iron from human hemoglobin (Hb), a process required for virulence and a potential target for new therapeutics. Iron scavenging from Hb is mediated by nine iron-regulated surface determinant proteins (IsdA-I).

In the first step of this pathway, the closely related pepoligand-cananchored IsdH and IsdB receptors bind Hb and extract its heme cofactors. IsdH accomplishes heme extraction via a tridomain unit consisting of its second and third NEAT Transporter (NEAT) domains and a helical linker. A structure of the IsdH:Hb complex has provided insight into how the receptor distorts Hb to promote heme release, and has suggested that heme flows through a channel to the third NEAT domain. Additional structures of IsdB bound to heme analogs have revealed a binding pocket in which Y642 axially coordinates the heme iron. Time-resolved crystallography will be used to define the molecular basis of heme transfer by creating a photo-regulated receptor. 2-nitropiperonyl tyrosine (NPY) cleaves to produce tyrosine on irradiation with UV light and has been incorporated into IsdH in place of Y642 as a means of achieving photo-regulation. Here we present initial work toward incorporating the unnatural amino acid and verifying IsdH binding pocket occlusion.

**In silico energetic analysis of the amyloid fold**

**SAMANTHA ZINK** (Chem & Biochem/ BMSB, Rodriguez lab)

Protein folding is considered a global optimization problem, where the protein works towards optimizing structural features such as backbone and sidechain angles while burying hydrophobic residues away from the solvent. Success in the computational simulation of this process has been demonstrated for both globular and membrane proteins as they typically have one native conformation present at a global free energy minimum. However, amyloid proteins are known to exhibit structural polymorphism which is represented by many local minima near the global free energy minimum, making in silico modeling and prediction difficult. This challenge hinders our important goal of understanding the amyloid fold, which is implicated in numerous diseases including Alzheimer’s and Parkinson’s. To better understand the energetics of the amyloid fold, I am building a computational pipeline that evaluates a pool of experimentally determined amyloid structures. In brief, I score the amyloid with an energy function, break that score down on a per-residue basis, allowing me to identify stabilizing and destabilizing features. This type of analysis will deepen our understanding of how polymorphs are related to each other on an energetic level and opens up future applications in amyloid design and de novo structure prediction.