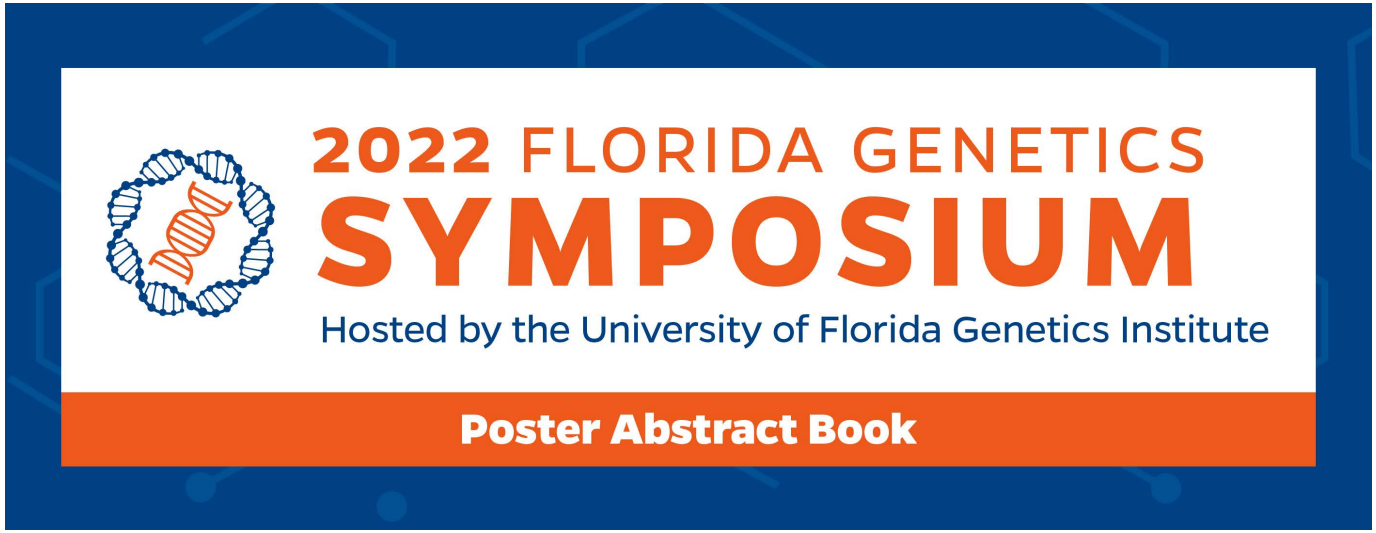

Poster Abstracts for the 2022 Florida Genetics Symposium

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Network analysis of multi-omics microbiome data to identify keystone unknown taxa

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Microorganisms have shaped Earth's biochemical and physical landscapes by inhabiting diverse metabolic niches. However, most microbial species remain unknown and are referred to as "microbial dark matter", highlighting a gap in our understanding of structured complex ecosystems. There are different omics methodologies to study microbial communities and combining these omics can provide a better look into the distinct biological layers in the ecosystem. However, there are no standard methodologies for meta-omics integration of microbiome data. We evaluate the role of 'microbial dark matter' in structured communities, using microbialites as a model ecosystem, and different types of data: metagenomics, metatranscriptomics and amplicon sequencing. Metagenomics (n=56) and metatranscriptomics (n=34) samples and were input into SortMeRNA to extract 16S reads. The output, as well as amplicon sequencing samples (n=14), were processed through QIIME2 for taxonomy analysis. Afterwards, R package MDMnets was utilized to build co-occurrence networks. Most hubs presented unknown classifications, sometimes up to the phyla level. A lack of classification at higher taxonomy levels suggests unknown microbes represent poorly characterized microbial lines. Future comparison of the highest scoring hubs of each data type using sequence similarity networks can yield the most similar important hubs occurring across the different data types, allowing the identification of the most relevant hubs. This work highlights the importance of unknown taxa in community structure and proposes microbiome data-integration and ecosystem network construction to identify significant unknown taxa for further characterization.

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Characterization of sleep phenotypes and CNS transcriptome in Myotonic Dystrophy Type 1 CNS mouse models

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Myotonic Dystrophy type 1 (DM1) is a multisystemic disease caused by an CTG trinucleotide repeat expansion in the 3' untranslated (UTR) region of the *Dystrophia Myotonica Protein Kinase* (DMPK) gene. Although DM1 presents with a large variety of symptoms spanning different body systems, excessive daytime sleepiness and other CNS symptoms are largely understudied. To assess the hypothesis that Muscleblind-like (MBNL) 2 protein depletion contributes to CNS symptoms in DM1 in general and sleep phenotypes specifically through the splicing mis regulation of the Gamma subunit of the $GABA_A$ receptor, we use the Muscleblind-like (MBNL) 2 knockout mouse. Specifically, we characterize sleep phenotypes in the MBNL2 KO mouse using the PiezoSleep mouse behavioral tracking system.

AAV-barcoding for High-throughput Screening of Vector Transduction Efficiency in the CNS of Cynomolgus Macaques Compared to C57BL/6 Mice

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Adeno-associated virus (AAV) vectors remain a popular choice for both research applications and investigational therapeutics. Tissue-specific transduction efficiency between serotypes has led to many novel engineered vector capsids. This has left an excess of AAV capsid variants to test for specific use-case scenarios and a relative paucity of methods to efficiently differentiate multiple capsids simultaneously. This is further complicated when considering the multitude of methods to administer AAV therapies to the CNS. To demonstrate transcription-level ranking of 19 AAV capsid serotypes among various CNS tissues, we injected a pooled vector mix of barcoded AAV vectors into Cynomolgus Macaques as well as C57BL/6 mice. Each species was divided equally into two different CNS injection methods. Tissue from each animal was processed for DNA and RNA extraction, and AAV barcode regions were subsequently amplified using metabarcoding primers to indicate each amplicon's tissue of origin, animal of origin, and whether it was extracted as DNA or RNA. These samples were pooled, processed, and sequenced by Illumina NextSeq®. Barcode sequence calls for the 19 AAV serotypes were analyzed with anatomical resolution. This was later compared between species by Spearman's rank correlation analysis. To our surprise, despite there being some obvious differences between primates and mice for some serotypes, there was a very strong correlation between macaques and mice across the CNS when considering bulk CNS transcription ($R > 0.75$; $p <<< 0.01$). This is important to consider when designing studies for a clinical translational pipeline, as mouse studies can be far more efficiently powered compared to primates.

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Single Nuclei RNA-seq data analysis of *Medicago truncatula* rhizobial infection and nodulation

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Chemical nitrogen fertilizer production uses around 5% of the global natural gas produced each year. Some plants, like the model legume *Medicago truncatula,* benefit from a symbiotic relationship with nitrogen-fixing bacteria known as rhizobia. In this relationship, the plant recognizes the presence of rhizobia and develops a modified root structure, a root nodule, to house the bacteria. The genetic basis of root nodule symbiosis (RNS) has been extensively studied for over a century, but identifying the genes that regulate the development of this modified root organ has remained elusive. A deeper understanding of the transcriptional program underpinning the establishment of this symbiotic relationship could uncover the elements necessary to engineer RNS into non-nodulating plant species. Engineering nodulation into agricultural crops and bioenergy feedstocks could reduce nitrogen fertilizer application, in turn preventing water pollution and decreasing dependency on fossil fuels. To investigate the initial transcriptomic changes regulating root nodule symbiosis, we collected root samples of wildtype *M. truncatula* at 0, 24, 48, and 96 hours after inoculation with *Sinorhizobium meliloti*. Using 10X Genomics Single Cell RNAseq protocols on each set of samples, we produced transcriptomic profiles of individual cell types that reveal the trajectory of cell lineages, as they differentiate to form the various components of the nodule. The single-cell RNA sequencing data is now being explored to identify specific regulators that control the developmental transitions between different cell types in a lineage.

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Lipid and Hormone Indicators of Reproductive Status in Florida Manatees (*Trichechus manatus latirostris*)

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Abstract

Research focused on reproductive physiology is essential to conservation and management of threatened wildlife populations. Florida manatees (*Trichechus manatus latirostris*) are protected as a threatened species by federal and state laws. However, there are limited data for Florida manatee reproductive physiology. This study aimed to (1) quantify plasma steroid hormones in Florida manatees at different gestational stages and to (2) determine the relationship between plasma progesterone concentrations and lipid biochemistry to develop a diagnostic lipid panel for pregnant manatees. Ultra-high performance liquid chromatography-tandem mass spectrometric analysis was used to measure plasma steroid hormones and lipid concentrations. Pregnant female manatees were morphometrically distinct from male and non-pregnant female manatees, characterized by larger body weight and maximal girth. Progesterone concentrations in manatees were also elevated during early gestation versus late gestation. Cholesterol, a lipid precursor for reproductive steroids, was not different between groups. However, plasma concentrations of a sphingolipid, ceramide non-hydroxy fatty acid-sphingosine and several glycerophospholipids, including lysophosphatidylcholine, phosphatidylethanolamines, plasmenyl-phosphatidylserines and monomethyl phosphatidylethanolamines, were associated with pregnancy status in the Florida manatee. This research contributes to the field of manatee reproductive physiology by providing critical data on plasma steroid hormones relative to reproductive status and generates a novel dataset of plasma lipids in healthy Florida manatees. These data are expected to advance understanding of manatee physiology to inform prospective population management.

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Juvenile Idiopathic Epilepsy in Arabian Horses is not a Single Gene Disorder

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Valued for their temperament, beauty, athletic ability, and exhibition in the show ring, Arabian horses are an important component of the horse industry. Juvenile Idiopathic Epilepsy (JIE), a seizure disorder, specifically impacts Arabian foals from birth to six months of age. Affected foals exhibit tonic-clone seizures lasting as long as five minutes and risking secondary complications like blindness and disorientation. Some foals outgrow this condition, while others die or suffer lifelong complications if not treated.

Previous work suggested a strong genetic component to JIE and proposing JIE to be a single-gene trait. In this work we conducted a GWAS in 62 cases of JIE and 118 genetically matched controls, identifying several loci suggesting JIE is not caused by a single locus. Coat color (chestnut, grey) phenotypes were used as positive control traits to assess the efficacy of GWAs in this population. The identification of polymorphisms contributing to JIE could provide a basis for genetic testing for the disease, a tool that may illuminate new treatment options, and help to eliminate production of affected foals.

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Lysine acetylation and its role in maintaining redox homeostasis: insight through study of an extremophile

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Oxidative stress adaptation strategies are a central topic in cell biology due to being linked to cardiac, neurodegenerative disease and cancer. Representatives of the domain *Archaea* have been used as model organisms based on their close evolutionary relationship with eukaryotes and extremophilic properties. Study of the halophilic archaeon *Haloferax volcanii* reveals lysine acetylation to have an apparent role in oxidative stress response. The strong oxidant hypochlorite: i) stimulates an increase in the abundance of Pat2:Pat1 lysine acetyltransferases and ii) selects for lysine deacetylase *sir2* mutants. In this study, we report that the lysine acetylome increases in abundance and shifts in profile when glycerol-grown *H. volcanii* is exposed to hypochlorite. Mass spectrometry analysis reveals ~20% of the theoretical proteome to be lysine acetylated with the number of acetylated proteins 3.4-fold higher during hypochlorite stress compared to non-stress conditions. Of note, four proteins related to electron transfer and central metabolism, 2Fe-2S ferredoxin (HVO_2995), two acetyl-CoA synthetases (HVO_1917 and HVO_1374) and glycerol kinase (HVO_1541). These alterations in the proteome may help maintain NADH:NAD+ ratios for redox balance. Understanding how lysine acetylation alters the activity of these proteins may give us new answers into mechanisms of stress response in halophilic archaea. Expansion of this knowledge can provide a balanced evolutionary perspective of post translational modifications in living organisms.

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Evolutionary consequences of a Caribbean anole recolonizing secondary forest

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Over half of tropical forests worldwide are regenerating forests, which eventually may provide suitable habitat for recolonization by locally extinct fauna. Locally extinct animals recolonize secondary forests in well-known community-level patterns, but less understood are the population-level processes underlying them. Current understanding of these eco-evolutionary processes is rooted on predictions from range expansion theory: first, expanding populations undergo a genetic bottleneck caused by the low number of founders; second, they experience different selection pressures than the source population. However, support for these predictions comes from other types of expansions—almost exclusively biological invasions. Recolonization may differ due to factors like the proximity of the source population or a shared evolutionary history between native fauna and secondary forest. To advance our understanding of these processes, we asked two questions: 1) do recolonizing populations have low genetic diversity and high genetic variation relative to the source population? and 2) do recolonizing populations experience different selection pressures (i.e., have distinct allele frequencies) than the source population?

To answer them, we conducted a comparative genetics study among *Anolis gundlachi* lizard populations along a chronosequence of forest succession in Puerto Rico. Our results show similar heterozygosity levels among populations from different-aged forests. Similarly, pairwise FST values between populations are low and do not support the occurrence of genetic differentiation. The preliminary results of our genetic clustering analysis show no clear evidence of genetic structure. Therefore, contrary to our predictions, these results suggest high connectivity between young forest and nearby remnants of old forest.

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Characterizing the functional and metabolic role of a candidate gene, *PHGDH*, in idiopathic small fiber peripheral polyneuropathy

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Millions of Americans are impacted by peripheral neuropathy, a heterogenous class of neurological diseases characterized by debilitating neuropathic pain and sensory loss. One-third of peripheral neuropathies are idiopathic lacking etiological understanding and effective treatments. Here, we describe the characterization of a candidate gene for small fiber peripheral polyneuropathy, *Phosphoglycerate dehydrogenase* (*PHGDH*), identified by whole genome sequencing from patient derived blood samples. We identified two allelic mutations in *PHGDH*; 1468G>A and 792+6T>G. The former reduces the activity of PHGDH while the effect of the latter is unknown. Mini-gene splicing assays revealed c.792+6T>G results in exon 7 skipping by weakening the 5' splice site. RNA and western analysis of patient fibroblasts detected a reduction in *PHGDH* transcript and protein levels. As PHGDH plays an important role in central, one carbon, amino acid and lipid metabolism, we performed metabolic flux analyses on patient fibroblasts to determine whether reduction in PHGDH function disrupted metabolism. Patient derived fibroblasts displayed perturbations in central carbon, amino acid, and glycolytic metabolism. To develop an antisense-oligonucleotide (ASO) based strategy that blocks *PHGDH* exon 7 skipping and restores optimal levels of this enzyme, we carried out a high-throughput point mutagenesis screen of *PHGDH* exon 7 and flanking introns. This approach has identified potential exon 7 regulatory splice sites serving as ASO targets. Our work is the first of its kind to identify defective cell metabolism as a basis for peripheral neuropathy and sets the stage for the development of ASO based therapeutics for peripheral neuropathies and inborn errors of metabolism.

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Deep learning enabled clinical translation of extracellular vesicle precision gene delivery

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Abstract

Intercellular genetic transmission and regulatory control within tissues will unlock advances in clinical therapy. We are now understanding that extracellular vesicles released from cells contain mRNA/miRNA content that influence the fate of a recipient cell. Specifically, small extracellular vesicles, known as exosomes, are universally secreted from all living cells as 30-300 nm nanoparticles that are emerging as markers into understanding epigenetic regulations. However, clinical translation of exosomes remains challenged by their secreted and tropic heterogeneity, subsequently limiting our understanding of precise molecular regulation initiating communication, alteration, and therapeutic conditioning of target tissues. To address this clinical challenge, we have used artificial intelligence to design precision targeting modality onto exosome isolates using a combination of generative modelling and molecular engineering (ExoGAN). For demonstration, we use ExoGAN to systematically understand naïve CD8+ T cell activation through exosome presentation of antigens bound to MHC-I, showing downstream cytokine secretion of activated naïve CD8+ T cells. We highlight how ExoGAN's latent understanding of receptor-ligand interfaces projects strong ligand affinity when compared to existing antigens using selected computational and experimental approaches. We expect that ExoGAN will serve as a central framework to establish a universal receptor targeting strategy, allowing researchers to unravel exosome-mediated genetic regulatory mechanisms in a systematic manner. Broadly, ExoGAN will facilitate clinical translation as an end-to-end method benefiting clinician, researcher, and patient alike.

The target-directed microRNA degradation interactome in cancer

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Binding of microRNAs (miRNAs) to mRNAs normally results in post-transcriptional repression of gene expression. However, extensive base-pairing between miRNAs and target RNAs can trigger miRNA degradation, a phenomenon called target RNA-directed miRNA degradation (TDMD). As TDMDs can rapidly ablate mature miRNAs that are often dysregulated in cancer, the dynamics between miRNAs and their TDMD targets (triggers) in cancer initiation and progression remains to be determined. To probe this question, we utilize the AGO-CLASH (Argonaute cross-linking, ligation, and sequencing of miRNA-target RNA hybrids) method, which we have recently adapted to reveal widespread TDMD triggers in the human and mouse transcriptome. Identification of TDMD triggers was facilitated by focusing on their ability to induce non-templated nucleotide addition at the miRNA 3' end. From AGO-CLASH data sets obtained from multiple cancer cells, we identified eight triggers that can induce degradation of their corresponding miRNAs when exogenously expressed in various cell lines. We found that the TDMD base-pairing and surrounding sequences were essential for TDMD, and that CRISPR knockout of endogenous trigger or ZSWIM8 (a ubiquitin ligase essential for TDMD) reduced miRNA degradation. Interestingly, we uncovered the degradation of miR-221/222 by a trigger in BCL2L11, which encodes a pro-apoptotic protein, enhances apoptosis. This represents the first example where a TDMD trigger within an mRNA can functionally cooperate with the encoded protein. We are currently attempting AGO-CLASH in clinically-derived colorectal tumor samples. By analyzing healthy and tumor tissue using AGO-CLASH, RNA-seq, and small RNA-seq, we probe the dynamic changes in miRNA targeting and TDMD interactome in colorectal cancer.

Antisense-to-Latency Transcript Long Noncoding RNA in Kaposi's Sarcoma-associated Herpesvirus Perturbs Host Alternative Splicing Regulation

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiologic agent for Kaposi's sarcoma, Primary effusion lymphoma and Multicentric Castleman's disease. Recent transcriptome studies have uncovered multiple non-coding RNAs in KSHV, most of which are expressed during the lytic stage. The Antisense-to-latency transcript (ALT) is a lytic nuclear transcript of approximately 10,000-nucleotides. It is transcribed from the antisense to the KSHV latency-associated region including LANA, vFlip, vCyclin, and most of the microRNA genes.

To study potential functions of ALT, we performed RNA antisense purification from lytically induced BC3 cells and identified 51 host proteins and three KSHV proteins, ORF11, ORF59 and ORF51, as direct ALT binding partners. Interestingly, 41 of the identified host proteins are involved in splicing regulation including core-splicing factors U2AF2 and SRSF1; strong suppressors of cryptic exon inclusion, PTBP1 and PTBP2, as well as HNRPLL, HNRNPDL and HNRNPA2B1, suggesting that ALT may perturb splicing via a sponging mechanism. Target validation was performed using RNAscope and IFA co-localization.

Transient knockdown and over-expression of ALT revealed alternative splicing (AS) changes when ALT expression is altered. RNAseq-based splicing analysis identified AS events associated with nonsense-mediated mRNA decay (NMD) by mistaken exon skipping and nonconserved cryptic exon inclusion.

In summary, our findings indicate that the KSHV lncRNA ALT perturbs host mRNA splicing by association with many splicing factors, leading to AS events during lytic replication. Since most KSHV genes, especially the lytic transcripts, are not spliced, ALT-dependent sponging during lytic reactivation may provide a viral-favored environment and function as a novel host shut-off mechanism.

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Shifts in the coral microbiome in response to *in situ* experimental deoxygenation

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Abstract:
Global climate change impacts ocean communities through rising surface temperatures, ocean acidification, and deoxygenation. While the response of the coral holobiont to the first two effects has been relatively well studied and documented, little is known about the response of the coral microbiome to deoxygenation. In this study, we investigated the response of the microbiome to hypoxia in two coral species that differ in their relative tolerance to hypoxia. We conducted *in situ* oxygen manipulations on a coral reef on the Caribbean coast of Panama, which has previously experienced episodes of low dissolved oxygen concentrations. Naïve coral fragments (previously unexposed to hypoxia) of massive starlet coral (*Siderastrea siderea*) and whitestar sheet coral (*Agaricia lamarcki*) were transplanted to a reef and either enclosed in chambers that created hypoxic conditions or left at natural oxygen levels. We collected surface samples of mucus and tissue after 48 hours of exposure to these conditions and characterized the microbiome by sequencing 16S rRNA genes. We found that the microbiomes of the two coral species were distinct from one another and remained so after exhibiting a shift of similar magnitude in response to hypoxia. Additionally, there was an overall increase in anaerobic microbes after hypoxic exposure, and fourteen families that changed significantly in abundances, including Desulfovibrionaceae, Midichloriaceae, Nitrincolaceae, and Clostridia Family XII. We also identified potential coral pathogens, including stony coral tissue loss disease. Our findings provide a basis for further investigation into understanding how microbial shifts may mediate coral resilience in response to ocean deoxygenation.

Poster Number: 14 Day 1

Title: Comparing the Transcriptomes of T cells and B cells in Type 1 Diabetes Cases and Controls using Long Reads

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Type 1 diabetes (T1D) is an autoimmune disease that arises from the action of both genetic and environmental factors. Previous linkage and genome-wide association studies (GWAS) have identified more than 100 chromosomal regions that contribute significantly to T1D risk. Short read RNASeq studies of immune cells (CD19+ B cells, CD4+ and CD8+ T cells) have shown that alternative splicing events in genes associated with T1D risk are cell type specific. Typical short read RNASeq data (e.g. 100-300 nt) can be used to identify novel junctions and proportion of exons spliced in (PSI). However, short read data cannot reconstruct entire transcripts, nor can they identify novel isoforms. In this study, we are using long-read (PacBio) sequencing to overcome these limitations in our characterization of the transcriptomes of immune cells in T1D cases and controls. We are constructing long read transcriptomes of CD19+ B cells, CD4+ and CD8+ T cells from T1D cases and controls. These long read transcriptomes together with RNASeq data, are used to i) quantify transcripts across immune cell types, ii) identify alternative splicing and iii) differential transcript usage between immune cell types and T1D cases and controls.

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Optimization of Sorghum Transformation and Regeneration Efficiency

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Sorghum is a versatile cereal crop grown worldwide and used for food, animal feed, biofuels, and bio-based chemicals. Genetic improvement of sorghum using traditional breeding methods is limited by a finite pool of genetic variation. Genome editing offers the prospect of targeted genetic changes, but relies on transformation, which is slow and inefficient in sorghum. We recently developed a biolistic transformation protocol using leaf whorl explants, which makes the process faster compared to the use of immature embryo explants, but transformation and regeneration rates need to improve to enable genome editing. Tissue culture conditions, including the choice and concentrations of phytohormones, were evaluated for improved regeneration rates. Culturing the calli on a hormone-free medium in between callus induction and shoot regeneration produced more shoots faster than the original protocol. The effect of microparticle size and speed (a function of the helium pressure) on biolistic transformation efficiency in leaf whorl and immature embryo explants was evaluated using a *GFP* reporter gene. The combination of 0.8-µm particles and helium pressure of 1350psi produced the most transient fluorescent foci 7-12 days after bombardment. Several transgenic plants were regenerated from the bombarded calli and confirmed with PCR. Using the smaller particles and higher pressure relative to our initial experiments is expected to improve transformation rates in future experiments.

Containerized CUT&RUN Analysis Allows Characterization of Histone H3.3 Deposition in Kaposi's Sarcoma-Associated Herpesvirus (KSHV) Infection

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The Cleavage Under Targets and Release Using Nuclease (CUT&RUN) experimental technique has recently been developed as an alternative to Chromatin Immunoprecipitation (ChIP) for studying the epigenetic landscape of the genome, providing a significantly increased signal-to-noise ratio by reducing off-target sequence capture and background noise. Analysis of CUT&RUN experiments has increased complexity compared to traditional ChIP-Seq, and there is a lack of robust methodology available for analyzing CUT&RUN data in non-model organisms. Here, we present a novel analysis pipeline developed in the Nextflow bioinformatics programming language to facilitate flexible, modular CUT&RUN data analysis. This method includes fully containerized execution with publicly available programs, allowing for top-tier flexibility and reproducibility of results across diverse computational systems. We applied this pipeline to analyze CUT&RUN data from a Kaposi's Sarcoma-Associated Herpesvirus (KSHV)-infected cell line to characterize the role of HIRA and DAXX in KSHV infection and establishment of latency.

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Choroid plexus spliceopathy in myotonic dystrophy type 1

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Dmpk CTG^{exp} knockin (KI) mouse models for myotonic dystrophy type 1 (DM1) demonstrated the choroid plexus (ChP) is particularly susceptible to DM1 spliceopathy. The ChP is important for neurodevelopment, brain homeostasis, circadian rhythms, and sleep via its production and regulation of cerebrospinal fluid (CSF). To clarify how the ChP is affected in DM1, we investigated transcriptomic changes by RNAseq. As a first step, DM1 is characterized by impairments in developmental pre-mRNA splicing transitions regulated by the MBNL family of RNA binding proteins (RBP). Thus, we performed RNAseq on wild-type mouse ChP from late embryogenesis to adults and correlated ChP developmental splicing transitions with the corresponding changes in regulatory RBP expression. Next, we determined that both mouse *Dmpk* CTG^{exp} KI and human DM1 ChP shows a spliceopathy that primarily reverts splicing patterns to an earlier developmental pattern, and *Mbnl2* KO and *Dmpk* CTG^{480} KI mice, as well as human DM1, choroid plexi show mis-splicing of important ion channels and secreted proteins. Based on these and additional findings, we propose that ChP mis-splicing leads to an alteration in CSF composition resulting in pathological features of the DM1 CNS.

Predicting Transcription Factors Biological Roles in P. vulgaris

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Phaseolus vulgaris or common bean is a model organism for studying the evolution of crops due to the existence of two gene pools developing independently for more than 165,000 years. However, little is known about the regulation of genes involved in evolutionary and agriculturally important traits, which limits our understanding of regulatory processes leading domestication. Studying the differences in regulation of traits such as starch accumulation or oligosaccharides biosynthesis between wild and domesticated accessions is important to build effective breeding programs.
Here, we combined comparative genomics, affinity motif scanning and functional profiling to infer a functionally informed Transcription Factor regulatory network in common bean. We screened homologous promoters of orthologous genes using an adaptation of a conservation test which is anticipated to select evolutionary conserved transcription factor binding sites. Thus, we were able to identify evolutionarily conserved motifs between *P. vulgaris* and phylogenetically close species such as *G. max*, *V. angularis*, and *V. radiata*. Additionally, we used an affinity-based motif scanning approach to identify transcription factor binding sites that could have been missed by the conservation test. Biological roles of transcription factors families were assigned based on enriched functional annotations within genes with conserved binding motifs to a specific TF family and a regulatory network was constructed based on shared functional roles. We validated our predictions using available knowledge about the starch biosynthesis pathway in plants.

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Translational Regulation of Target-Directed miRNA Degradation

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MicroRNAs (miRNAs) are a class of non-coding RNAs that interact with the target mRNAs to induce mRNA degradation and translational repression. Generally, miRNAs are stable when associating with Argonaute (AGO). However, miRNA can turnover rapidly when binding to a target RNA with extensive complementarity, a phenomenon called target-directed miRNA degradation (TDMD). So far, the most known “TDMD trigger” sequences that can induce miRNA degradation are located either in the 3' untranslated region (UTR) of mRNAs or in noncoding RNAs. To understand if non-coding region is the preferred location for TDMD triggers, we tested the efficacy of an engineered TDMD trigger against miR-16 when placed either in the coding sequence (CDS) or the 3' UTR of a superfolderGFP reporter. 3'UTR TDMD trigger induced more significant miRNA degradation than the CDS trigger. Presumably, the translating ribosomes regulate miRNA levels through TDMD inhibition. Indeed, blocking translation of the GFP reporter by morpholino oligos or various translation inhibitors increased miR-16 degradation by the CDS trigger to a similar level as the 3' UTR trigger. Because translation repression has been linked to neurodegenerative diseases, we are currently performing AGO-CLASH in mouse striatal cells modeling a brain disorder, with the goal to identify disease-associated CDS TDMD triggers. Our study explains the predominant localization of effective TDMD triggers in the non-coding transcripts, and globally identifies potential triggers within the protein-coding sequences after translation inhibition. Future research along this line will bring a better understanding of the molecular mechanisms driving TDMD and provide potential therapeutic strategies for neurodegeneration.

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Transcriptomic Analysis of MRSA Biofilms treated with Nitroxoline and Halogenated Quinoline

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Abstract

Bacteria exist in two different forms: free-floating bacteria or dormant biofilms. Bacterial biofilms are surface-attached communities of slow- or non-replicating cells embedded within a protective extracellular matrix. Unlike their planktonic counterparts, biofilms are innately tolerant to conventional antibiotics. Nitroxoline, as a broad-spectrum biofilm-eradicating agent, was used to probe biofilm viability. Transcript profiling (RNA-seq) showed that 452 of 2594 transcripts in methicillin-resistant *S. aureus* (MRSA) biofilms were differentially expressed following a 2 h treatment of nitroxoline. WoPPER cluster analysis revealed that iron uptake clusters (*sbn*, *isd*, MW2101, MW0695, *fhu*, and *feo*) were significantly upregulated upon nitroxoline treatment. RT-qPCR further demonstrated that nitroxoline upregulates iron uptake genes in established *Staphylococcus epidermidis* and *Acinetobacter baumannii* biofilms. In addition, the ability of halogenated quinoline RA-HQ-12 to induce rapid iron starvation in MRSA biofilms was also evaluated via RT-qPCR. Time-course study testing select genes in MRSA biofilms was performed to understand first/second responses induced by RA-HQ-12.

Single-cell RNA sequencing reveals cell-type localization of the trehalose-6-phosphate pathway in maize leaves

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Trehalose-6-phosphate (Tre6P) has been proposed to act as a sucrose availability signaling metabolite by informing the metabolic status of the plant. It has an essential role in developmental processes by regulating sink-source tissue relationships. The Tre6p synthesis pathway is composed of two enzymes, trehalose-6-phosphate synthase (TPS), which synthesizes Tre6P, and trehalose-6-phosphate phosphatase (TPP), which dephosphorylates Tre6P to produce trehalose. These enzymes are encoded by large multigene families that present spatial and temporal specificity. Here, we used a single-nuclei RNA sequencing approach to determine cell-specific expression patterns of TPS and TPP genes in maize leaf tissue. We found that *ZmTPS1*, which encodes a catalytically active enzyme, is expressed in the vascular bundle, specifically in phloem parenchyma, xylem parenchyma, and companion cells/sieve elements complexes. *ZmTPS12* shares this expression pattern but was also found to be expressed in mesophyll cells. *ZmTPS1* is expressed at a highly strategic site for phloem loading and sucrose export to sinks. We also found that three TPP genes, are expressed in mesophyll and bundle sheath cells. These results improve our understanding of the possible role of Tre6P as a signaling molecule in maize and provide insights into the compartmentalization of the Tre6P enzymes in maize source tissue. We show the first evidence of cellular localization of the Tre6P pathway in a crop with strong sink tissues that remobilize sugar. Further studies will be undertaken to translate this novel discovery into crop improvement for abiotic stress tolerance.

Diminished vasculogenesis under inflammatory conditions is mediated by Activin A.

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Clinically many ischemia-associated diseases coincide with substantial systemic elevation of Activin A. We have previously shown that Activin A plays a key role in vasculogenesis; its activity is essential for vessel stabilization. However, its expression in the early phase of vessel remodelling has an angiostatic effect. We hypothesized that inflammatory cells, known to accumulate in ischemic tissue, cause upregulation of Activin A in the cells of the vessel wall, which in turn limits endogenous vessel remodelling. To test this, cocultures of human vasculogenic cells, endothelial (EC) and adipose pre-pericyte/stromal cells (ASC), were exposed to LPS-activated human peripheral blood mononuclear cells (aPBMC) and analysed for EC tubulogenesis. While EC organized into dense vascular networks in intact EC-ASC cocultures, the presence of aPBMC had a detrimental effect, decreasing the vessel density by 60%. aPBMC also upregulated Activin A expression in EC and induced its expression de novo in ASC. These effects were mediated by TNFα in EC and by IL1β in both cell types. Blocking Activin A activity in EC-ASC-aPBMC cocultures rescued EC tubulogenesis. Additionally, extended exposure of ASC to aPBMC or IL1β upregulated expression of markers of smooth muscle cell/myofibroblasts in ASC; which again was mediated by Activin A, acting in an autocrine fashion. Thereby, this study indicates that inflammatory stimuli upregulate Activin A in vascular cells which in turn prevents effective vessel remodelling and generates contractile smooth muscle cells/myofibroblasts. Hence, this study also highlights Activin A as a potential therapeutic target to limit the detrimental effects of inflammation on vasculo/angiogenesis.

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An Epigenetic Signature Predicts Outcome in Pediatric AML Patients

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250 word maximum

Acute Myeloid Leukemia (AML) is a devastating disease associated with high morbidity and mortality. Though the development of precision medicine efforts such as small molecule inhibitors and immunotherapy have proved successful in improving survivorship, results have been underwhelming, with 5-year survival rates for young patients below 70%. Recently, a 6-gene transcriptomic score named pLSC6 highlighted the importance of DNA methyltransferase 3 B in pediatric AML prognosis, which invites a deeper investigation of leukemic stem cells through the scope of their DNA methylome. Here, we describe a machine learning pipeline in a cohort of pediatric AML patients from the multi-center Children's Oncology Group's (COG) clinical trials AAML1031 and AAML0531. Methylation array data from pre-treatment, diagnostic biopsies were processed from raw files, and the discovery cohort selected after clinical exclusion criteria consisted of 1028 patients and 308,968 CpGs. We first used supervised UMAP for dimensionality reduction, then deployed an optimized distributed gradient boosting (XGBoost) classifier to predict event-free survival (EFS). The validation cohort included separately processed methylation data from 164 diagnostic, bone marrow samples from the multi-site St. Jude AML02 clinical trial. The prediction model validated robustly, with EFS and OS hazard ratios of 2.15 and 2.53 (p-value: 0.0019, 0.0027) respectively, which confirms its prognostic potential in multiple pediatric-only trials. In summary, we developed and validated a DNA-methylation-based prognostic risk-score predictive of clinical outcome in pediatric AML. Our pipeline will be further integrated with nanopore real-time sequencing technology for rapid and affordable prognostication of patients.

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A Genome-Wide Survey on the Host Factors that Modulate Intestine Permeability

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The gastrointestinal (GI) system plays a key role in pathogen defense and nutritional homeostasis. Disruptions of the GI tract contribute to a variety of conditions such as inflammaging, leaky gut, irritable bowel diseases, and even nervous system conditions including Alzheimer's Disease and autism. The intestine of *Caenorhabditis elegans* can be used as an analogue to study these diseases, particularly that of leaky gut through the use of the SMURF assay. The SMURF assay describes a method for visualizing leaky gut in live animals through feeding of a non-toxic blue food dye that leaks from the intestine and stains the body blue; leakage is quantified as the ratio of 'stained' worms to the total worm number. Treatment of wildtype (N2) worms with the SMURF assay reveals an age-dependent increase in intestine permeability. This trend is recapitulated when tested with two known lifespan modulators: the long-lived insulin/IGF-1 receptor (*daf-2*) mutant shows a large decrease in staining compared to N2 at the same timepoint, whereas the short-lived FOXO transcription factor (*daf-16)* mutant shows an increase in staining compared to N2. Understanding this trend, the entire *C. elegans* genome (~20,000 genes) was screened using the RNA interference (RNAi) technique combined with the SMURF assay. We aim to identify novel genes involved in intestine physiology and investigate how they might regulate aging and intestinal health. This ongoing project has provided a large amount of data on potential regulators of aging and will also help researchers further understand how the gut integrity is established and maintained.

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Genome-wide Association Study Identifies Variants Associated with Clinical Outcomes in Pediatric Patients with Newly Diagnosed Acute Myeloid Leukemia

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The role of inherited genetic variants with regards to variation in AML treatment outcome remains obscure. To that end, we conducted a genome wide association study (GWAS) on 400 newly diagnosed pediatric AML patients to identify single nucleotide polymorphisms (SNPs) that may be associated with minimal residual disease (MRD1) after induction 1 chemotherapy, event-free survival (EFS) and overall survival (OS). We utilized whole-genome genotyping via Illumina's Omni2.5Exome-8 array and imputation of untyped SNPs to investigate association with clinical outcome in 2 pediatric AML cohorts: the multi-site AML02 [NCT00136084, n=167] and AML08 [NCT00703820, n=233] trials. For EFS, a genome-wide significant association was observed for a SNP in *CSMD1* and associated with reduced EFS (HR=3.29, 95% CI (2.177-4.975), $P=1.61x10^{-8}$). CSMD1 is a membrane-bound complement inhibitor that has been shown to act as a putative tumor suppressor gene in other malignancies. Another significant marker was an intronic SNP in *CDKN2B-AS1* that associated with poor outcome (EFS: HR=2.37, 95% CI (1.700-3.317), $P=4.40x10^{-7}$ and OS: HR=2.87, 95% CI (1.954-4.225), $P=8.06x10^{-8}$). *CDKN2B-AS1* has been reported to play a role in tumorigenesis and chemoresistance in pediatric T-cell acute lymphoblastic leukemia. Though limited by numbers, this is one of the largest GWAS conducted in pediatric AML to identify SNPs associated with clinical outcomes. Our top results show that genetic variation may impact MRD1, EFS and OS. Future studies are aimed at looking at SNP/gene and association with gene expression collected from leukemic cells at time of diagnosis, functional validation of most promising SNPs, and replication in larger cohorts.

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Poster Number: 27 Day 1

Title: A seed-size gene in maize: *Sorbitol dehydrogenase 1*

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Maize kernels rapidly synthesize, transport, and metabolize their own sorbitol via sorbitol dehydrogenase (SDH, EC 1.1.1.14). The physiological roles of sorbitol synthesis over developmental time remain elusive. Maize SDH catalyzes the reversible interconversion of fructose + NADH ← → sorbitol + NAD. However, the reaction favors sorbitol synthesis in the high-sugar, low-oxygen interior of the endosperm and possibly the reverse in other locales. Maize SDH is encoded by a single copy *Sdh1* gene, which is strongly expressed early in the endosperm-filling stage. Preliminary analysis of a new, *Ac*/*Ds*-induced *sdh1* mutation indicates that it confers a small-kernel phenotype. Since availability of NAD is typically limited under the low-O_2 conditions in maize endosperm, the capacity for SDH to generate NAD provides a mechanism for protecting redox balance and sustaining kernel growth. To address this hypothesis, we are characterizing the *sdh1* mutant and generating *Sdh1* over-expression lines for biochemical and molecular genetic analysis. Meanwhile, to study the physiological role of SDH in high-sugar environments, double mutants that combine *sdh1* with the high-sugar endosperms of sweetcorn mutants are being grown for morphological and biochemical analysis. Outcomes will determine the suitability of *Sdh1* and its regulators as possible targets for genetic manipulation or metabolic engineering to alter quality and/or quantity of maize kernels.
Research support by NSF-IOS-PGRP-1748105

Splicing in genetic disease: the problem AND a potential solution

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RNA splicing can play a significant role in rare genetic disease through a variety of different mechanisms. Here, we describe our work with two families living with rare diseases to investigate how alterations in splicing might contribute to pathogenesis. First, a family member of a patient with idiopathic peripheral neuropathy came to us with whole genome sequencing results that implicated two mutations in the PHGDH gene. We generated a minigene construct containing one of the mutations to study its impact on PHGDH splicing; the mutation resulted in skipping of a constitutive exon. The second rare disease we studied was creatine transporter deficiency (CTD), caused by dysfunction of the SLC6A8 creatine membrane transporter. The patient we worked with had a deletion spanning intronic and exonic sequences of this gene, and we characterized the mis-splicing and protein expression of SLC6A8 in their fibroblasts. A reasonable therapeutic strategy that could be applied in both of these disorders, and in other diseases caused by loss-of-function mutations, is gene replacement. However, overexpression of gene therapies could have detrimental long-term effects in patients. Incorporation of alternative splicing as a regulatory strategy to control gene therapies could be a way to formulate more efficient and less toxic gene therapies, through autoregulation. We are developing knockdown-and-replace gene therapy approaches for various genetic diseases, using RNA-binding proteins that can auto-regulate, thus avoiding potential deleterious effects of over-expression. In summary, while changes in splicing can contribute to genetic disease, regulated splicing can also be utilized to develop smarter gene therapies.

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Poster Number: 29 Day 1

The ice plant genome and single-cell transcriptome provide insight into the mechanisms underlying the C_3 to CAM transition

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A major goal for the coming years will be to develop crops capable of coping with abiotic stresses due to declining freshwater resources and changing temperatures. A number of plant species from different lineages have evolved the Crassulacean acid metabolism (CAM), a water-conserving photosynthetic pathway that supports growth and development under arid conditions. Some plants known as facultative CAM preferentially use C_3 or C_4 photosynthesis under well-watered conditions, but have the ability to reversibly induce CAM under water shortage. Since the overwhelming majority of food plants perform C_3 or C_4 photosynthesis, transferring this ability to agricultural crops and tree species may be the key to improving their drought resistance. However, efforts to achieve this goal have been hampered by limitations in available genomic resources for facultative CAM species.

Thus, we report here the genome sequence of the facultative CAM species *Mesembryanthemum crystallinum*, the common ice plant. High molecular weight DNA was sequenced on the ONT PromethION platform. The assembled genome has a size of 367.91 Mb divided into 9 chromosomes, a contig N50 of 7.18 Mb, and a BUSCO score of 97.8%. Full-length root, stem, and leaf transcriptomes were sequenced using the Iso-Seq method to support the genome annotation and investigate alternative splicing events during CAM induction. Finally, we present the early results of the first single-cell transcriptome study of the C_3 to CAM transition in the ice plant, and provide the first single-cell transcriptome atlas of a CAM-performing plant.

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Two are Better Than One: ZccR and Smu1790c Control the Expression of the ZccE Metal Exporter in *Streptococcus mutans*

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A resident of dental biofilms, *Streptococcus mutans* is a keystone pathogen of dental caries, one of the most prevalent and overlooked diseases globally. Zinc is an essential trace metal to all forms of life that becomes poisonous at high concentrations. Because of its antimicrobial nature, anti-inflammatory properties, and relatively low toxicity to mammalian cells, zinc is used therapeutically for various infectious and noninfectious conditions, including through incorporation in toothpastes and mouthwashes. Zinc efficacy in preventing dental caries remains controversial and the mechanisms allowing zinc survival in oral bacteria like *S. mutans* are largely unknown. Recently, we discovered *S. mutans* tolerates higher zinc concentrations compared to other streptococci because of a metal-translocating P1B-type ATPase (termed ZccE) unique to the species. Additionally, *zccE* is positively regulated by a MerR-type regulator immediately upstream, named ZccR (*zccE* regulator). Gel mobility shift assays revealed that ZccR directly and specifically binds the *zccE-zccR* intergenic region (IGR) in a zinc-dependent manner. While Δ*zccE* and Δ*zccR* strains are hyper-sensitive to zinc salts, stable suppressor strains can arise in the Δ*zccR* background. qRT-PCR analysis revealed basal *zccE* transcription is much higher in the Δ*zccR* suppressors than the parent strain. Whole genome sequencing identified a second MerR-type regulator, *smu1790*, with an early truncation all suppressor strains shared. Deletion or truncation of *smu1790* partially restored zinc sensitivity in Δ*zccR*. Thus, *zccE* is regulated by two MerR regulators that likely interfere with each other's capacity to bind the *zccE-zccR* IGR. Studies investigating this and defining the scope of each regulon are ongoing.

Preliminary Identification of a Quantitative Trait Locus for Body Size Proportion and head length in the American Quarter Horse

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The action of artificial selection during domestication produced diverse morphological traits in the horse. Skeletal development and growth phenotypes impact the desirability and function of horses in diverse disciplines, often key characteristics in the development of breeds. We utilize body measurements taken at two years of age from 91 American Quarter Horses bred and owned by the University of Florida. To investigate variations in body size and head shape within this population, we summarized the 32 body measurements collected from each horse using a principal component analysis followed by a varimax rotation. Body Factor 1 explains 19.5% and Factor 2 explains 16.2% of overall variation across the 32 measures describing body size proportions and head length. The Body Factor 1 trait describes body proportions, heavily loading on the measures of height at the dock and croup. DNA was sampled from blood and hair through previously published methods and genotyped at GeneSeek using the Affymetrix Axiom Equine HD 670k array. We then conducted a genome wide association study with 311,085 high quality SNPs (poly-high resolution, and a MAF > 0.05). This preliminary analysis suggests a quantitative trait locus for the Body Factor 1 trait on chromosome 6 (P = 1.356e-09 and Bonferroni = 4.51e-07) and Body Factor 2 suggests a quantitative trait locus on chromosome 7 (P=4.721e-07 and Bonferroni=0.0635) Further investigation will aim to identify functional variants within these QTL's, identify additional relevant loci and develop predictive models for these phenotypes in the horse.

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Machine learning prediction and phyloanatomic modeling of viral neuroadaptive signatures in the macaque model of HIV-mediated neuropathology

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In human immunodeficiency virus (HIV) infection, virus replication in and adaptation to the central nervous system (CNS) can result in neurocognitive deficits in approximately 25% of patients with unsuppressed viremia. While no single mutation can be agreed upon as distinguishing the neuroadapted population, earlier studies have demonstrated that a machine learning (ML) approach could be applied to identify a collection of mutational signatures within the envelope glycoprotein (Gp120) predictive of disease. The S[imian]IV-infected macaque is a widely used animal model of HIV neuropathology, allowing in-depth tissue sampling infeasible for human patients. Yet, translational impact of the ML approach within the context of the macaque model has not been tested, much less the capacity for early prediction in other, non-invasive tissues. We applied the previously described ML approach to prediction of SIV-mediated encephalitis (SIVE) using *gp120* sequences obtained from the CNS of animals with and without SIVE with 73% accuracy. The presence of SIVE signatures at earlier time points of infection in non-CNS tissues indicated these signatures cannot be used in a clinical setting; however, combined with protein structural mapping and statistical phylogenetic inference, results revealed common denominators associated with these signatures, including 2-acetamido-2-deoxy-beta-D-glucopyranose structural interactions and the infection of alveolar macrophages (AMs) at a rate of 35-100%. AMs were also determined to be the phyloanatomic source of cranial virus in SIVE (but not SIVnoE) animals, implicating a role for these cells in the evolution of the signatures identified as predictive of both HIV and SIV neuropathology.

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Correlates, Anticorrelates of COG Pathways Explore the Diversity of tRNA Modifications Across Benchmark Microbial Assemblies.

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The modifications of tRNA molecules have been found to be impressively diverse between taxonomic clades, in addition to having demonstrated significant influence on cell viability and virility. Such modifications and their genes have even been linked to various human conditions, including but not limited to aging and chronic/progressive disorders. Since its establishment, the Clustered Orthologous Genes Database has proven an essential resource to comparative genomics and genome annotation. Despite its ubiquity in use, there are lapses in its coverage of COGs related to tRNA modification pathways. Here, this list of tRNA modification pathway-relevant COGs was reviewed, pruned of errors, and expanded to better reflect published data. In this, novel patterns of absence-presence could be explored across a set of benchmark microbial genomes. Focusing on a single thiolation pathway of tRNA base 34, it was discovered that many Actinobacteria were absent the major genes of this pathway, if not the pathway in entirety. One taxonomic range demonstrating the latter was found to be Bifidobacteriaceae, many of which are well-known commensal bacterial species of humans. With further data exploration, it may be possible to highlight classes of absence-presence of tRNA modifications across bacterial species and potentially recognize patterns in other biological traits, such as virulence or symbiotic benevolence.

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Genetic Engineering of Plant Polysaccharide Synthesis and Detection

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Polysaccharides such as hemicelluloses play essential roles in plants and serve as biomaterials in many industries. Golgi-localized glycosyltransferases (GTs) from the cellulose synthase-like A (CSLA) family synthesize heteromannans, an important subclass of hemicelluloses, which are found throughout the plant kingdom.

To unveil the genes and their encoded protein motifs for modulating (gluco)mannan production, we have heterologously expressed various native and chimeric *CSLA* genes in the yeast *Pichia pastoris*. Detailed carbohydrate analyses of the *Pichia* cell walls revealed that some protein domain swaps influenced the quantity and biological effects of the plant β-mannans in yeast cells.

To reveal the distribution and dynamics of heteromannans *in vivo*, I am developing genetically encoded probes to non-invasively label heteromannans *in vivo*. The basis of these probes are non-catalytic Carbohydrate-Binding Modules (CBM). Their native flexible attachment to polysaccharide-degrading glycosyl hydrolases makes CBMs excellent candidates for designing innovative protein-based heteromannan-labelling tools, as they fold and act independently. Genetically engineered to bear a red fluorescent protein, a self-cleaving ratiometric turquoise fluorescent protein, and a signal peptide for secretion, CBM-hybrids were heterologously expressed in the yeast *Pichia pastoris*. Compared to wild-type *Pichia* strains, the CBM probes secreted less from heteromannan-producing *Pichia* strains due to co-localization with the heteromannan-producing CSLAs in the endomembrane system. The new CBM-based biosensors will enable high-throughput screening of new GT variants and should additionally facilitate the live imaging of cell wall dynamics *in planta*.

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Cloning, expression, and purification of a glycerol kinase from an extremophilic archaeon isolated from the Dead Sea

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Extremophilic microorganisms are useful as biocatalysts and hold promise to convert glycerol waste into high-value bioproducts. The archaeon *Haloferax volcanii*, originally isolated from the Dead Sea, is not only useful for its halophilic and thermophilic properties but also displays a preference for glycerol over glucose as a carbon source. *H. volcanii* channels glycerol to central metabolism using the glycerol kinase GlpK. Recently, the *H. volcanii* lysine acetylome was mapped using an LC−MS/MS-based proteomic approach, and GlpK was found acetylated at lysine 153. This finding opens a wide range of possibilities related to the post-translational regulation of GlpK in central metabolism. While glycerol kinase enzymes are known to be regulated by post-translational modifications and allosteric effectors, the role of lysine acetylation in regulating GlpK remains to be determined. Clarifying the regulation mechanisms is essential for basic science and for optimizing their use in biotechnology applications. The first objective of this project is to purify the *H. volcanii* GlpK in an active form. Here we show His- and StrepII-tags are useful in the purification of GlpK by affinity chromatography, with the yield of GlpK more efficient with His-tag. Additionally, His- and StrepII-tagged GlpK showed phenotypic complementation *in vivo*, and the His-tagged GlpK purified from *H. volcanii* grown in rich and glycerol minimal media exhibited a monomeric structural conformation. Furthermore, His-tagged GlpK purified from rich medium shows an acetylated state. Future work is needed to ensure the tags do not affect GlpK functionality and to determine the influence of lysine acetylation on GlpK activity.

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Single-administration of a cyclic peptide-conjugated CUG-repeat steric blocker rescues myotonia and molecular phenotypes in *HSA*^{LR} mice

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In Myotonic Dystrophy type 1 (DM1), expanded CUG repeats in the *DMPK* transcript sequester MBNL splicing proteins. This causes molecular phenotypes including nuclear foci accumulation and aberrant splicing, leading to symptoms such as myotonia and muscle weakness. Multiple oligonucleotide therapeutics have been developed to either cleave *DMPK* mRNA or displace MBNL from the CUG repeats, but a major barrier to successful implementation of these approaches is efficient delivery to muscle tissue. Conjugation of oligonucleotides to antibodies, antibody fragments, and peptides to enhance delivery efficiency are currently in development by several groups. Here, we show that cyclic peptide oligonucleotide conjugates efficiently displace MBNL from expanded CUG repeats in cell and mouse models of DM1. Nuclear CUG repeat foci were reduced in a CUG-repeat knock-in cell line (HeLa480), and the Mbnl1 exon 5 splicing event was also assayed and rescued in HeLa480. A single intravenous administration into HSALR mice eliminated myotonia one week after injection. Aberrant splicing patterns of Atp2a1 exon 22, Mbnl1 exon 5, and Nfix exon 7 were nearly completely rescued in gastrocnemius, quadriceps, and tibialis anterior, as assayed by RT-PCR, and preliminary RNAseq analyses of gastrocnemius also confirmed splicing correction in these events. This study demonstrates the promise and potential of cyclic peptide oligonucleotide conjugates for the treatment of DM1.

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Quantifying Locomotor Phenotypes in Fragile Foal Syndrome Carriers Using Artificial Intelligence

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The domestic horse has unmatched within-species polymorphism in locomotor pattern and provides an advantageous model to study genetic collagen diseases and their effect on locomotion. Fragile Foal Syndrome (FFS) was chosen for this investigation due to its point missense mutation in the *PLOD1* gene that causes disorganization in collagen fibers. We determined a FFS carrier frequency higher than the reported rate of previous studies. To further understand the effect of the carrier genotype on gait quality, first a critical need for tools that can reliably and accurately quantify various locomotor parameters was addressed. We utilized the software package, DeepLabCut (DLC) to apply anatomical landmarks to video recordings of trotting horses. Geometric parameters calculated within a custom gait analysis pipeline written in MatLab processed the raw output files produced by DLC. Preliminary work investigating sport horses demonstrated the effectiveness of this approach in detecting gait parameters like limb extension and fetlock angle. On-going studies will apply this new phenotyping approach to investigate the joint range of motion in a population of horses carrying the variant for the heritable connective tissue disorder FFS. Improved quantification of locomotor phenotypes, and our understanding of the physiology of this and other known connective tissue disorders will aid in creating a biomechanical model for analogous human diseases like Ehlers Danlos Syndrome.

SIGNATURE OF MUTATIONAL PROCESS UNDER MINIMIZED SELECTION IN Caenorhabditis elegans

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Abstract

Mutation is the fuel of evolution, and as well as the underlying cause of many diseases, including cancer. Particular sources of mutation often have a characteristic signature, defined as the unique combination of mutation types that are related to a specific mutagenesis process. We previously demonstrated that the frequency distribution of type-specific mutations (the "mutational spectrum") differs between mutations accumulated in laboratory "mutation accumulation" (MA) lines and wild isolates of the nematode *C. elegans*, but that the difference is largely restricted to mononucleotide repeats. By using The Catalogue Of Somatic Mutations In Cancer (COSMIC) database, we reanalyze the sequence data to characterize the mutational signature of 7,053 base-substitution variants accumulated in three different sets of mutation accumulation lines under relaxed selection and also the signature of rare variants in a set of wild isolates, toward the goal of understanding the underlying sources of variation in the mutational process.

The majority of COSMIC signature in non-mononucleotide sequence is SBS40 in both MA lines and wild isolates. In contrast, SBS90 is the primary signature in mononucleotide regions SBS90 MA lines, whereas it was not identified in rare standing variants. The proposed COSMIC etiology for SBS90 is Duocarmycin exposure, which is a natural product first isolated from Streptomyces bacteria. Duocarmycin alkylates DNA, and although the MA lines did not encounter Streptomyces, many DNA damaging agents may give a similar spectrum of mutations.

Microbiome characterization of archival bat gut tissues of museum specimens preserved in ethanol and formalin or frozen

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The Florida Natural History Museum maintains a collection of preserved whole bat specimens which could serve as valuable sources of genetic information for insight into the history of Florida's bat populations and ecologically connected species. Specifically, the preservation of the microbiome in these samples will provide direct insight into the diet and disease state of these populations of bats which may contribute to zoonotic pathogen transmission. To analyze the gut microbiome of these samples, rectum tissue was resected from frozen preserved, ethanol preserved, and formalin preserved museum bat samples. Resected tissue underwent DNA and RNA extractions which were tailored to optimally collect genetic material in accordance with their respective preservation method. To identify presence of bacterial DNA corresponding to sample tissue microbiome, amplification of the V3/V4 region of the r16S gene was preformed utilizing total DNA extracted from each sample. Next Generation Sequencing libraries for each sample were then prepared from DNA and RNA reverse transcribed with either random hexamers or Oligo(dT), these libraries were then run on an Illumina NovaSeq machine. The resulting reads were then analyzed by multiple metagenomic profiling tools. Reads were analyzed with Kraken 2, metaphlan 4, and diamond to holistically profile the microbiome for viral and bacterial population abundances. Abundances of microbiome populations were graphically visualized to compare microbiome compositions between preservation methods. The results of this study provide an important platform for future work with preserved bat microbiome samples and their metagenomic analysis.

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Neural Network Predicts Allen Brain Map Cell Type From Single-Cell RNA-Sequencing Transcriptome.

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A neural network was created using TensorFlow that classifies cells within the cortex and hippocampus according to cell types established by the Allen Brain Map transcriptomic taxonomy. The Allen Brain Map dataset includes a collection of single-cell RNA-sequencing data from whole mouse cortex and hippocampus. 1.1 million cells were sequenced then clustered into 379 cell types based on differentially expressed genes. A TensorFlow neural network was trained on a large subset of sequencing data and then tested on another set. The model was able to predict the correct cell type with 83% accuracy with the correct type being in the top five ranked types 99% of the time. A TensorFlow neural network performs well categorizing single-cell RNA-seq data in an extraordinarily complex dataset.

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Initiator methionine retention in the proteome reduces global translation fidelity

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Gene expression should be accurate and precise. However, mRNA translation is highly error prone. Approximately 10% of nascent proteins have at least one error, yet how translation fidelity is regulated is largely unknown.

Our previous work identified the function of the eukaryote specific rRNA domain named expansion segment 27L (ES27L). ES27L recruits the nascent protein N-terminal processing factor Methionine aminopeptidase (MetAP) to increase translation fidelity. All protein synthesis begins from initiator methionine (iMet) and these iMet will be co-translationally cleaved off from ~1/3 of proteins. We also investigated how MetAP affects the translation fidelity of both MetAP targets and nontargets. Our data clearly showed that MetAP deletion reduces translation fidelity in both cases. This suggests that a specific target of MetAP has a role in global translation fidelity. We also noticed that 80% of ribosomal proteins (RPs) are substrates of MetAP. We hypothesize that iMet retention of RPs on the ribosome distorts the ribosomal structure, which causes inaccurate mRNA translation. To test this hypothesis, we are developing an in-vitro translation system to place WT and Δ*map1*-derived ribosomes in a cytoplasmic lysate, with WT translation factors (TFs).

Further, we are trying to develop a method to identify iMet retained proteins using the methionine analog L-Homopropargylglycine *(*HPG) to label methionine containing proteins in WT and *ΔES27L* yeast. Preliminary data show the different amount of HPG retention in the RP fraction between WT and *ΔES27L* ES27L mutant. Our research will be important in understanding fundamental mechanisms to maintain the functional proteome.

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Drug Discovery: Targeting Nuclear Receptors to treat metabolic diseases

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Nuclear Receptors (NRs) superfamily constitutes a group of 48 proteins. They are ligand regulated transcription factors that have proven to be a rich source of targets for the development of drugs to treat a myriad of human diseases including diabetes, Alzheimer's disease, and Duchenne Muscular Dystrophy (DMD).
We are working in close collaboration with medicinal chemists, who design compounds to be potential new regulators for NRs. The Burris lab develops new techniques that allow for screening of these compounds. The screening helps us understand their Structure/Activity Relationship (SAR). Each newly synthesized molecule enters a flow scheme consisting of different drug screening assays. Once we have a hit, the compound moves forward to the next assay. Our panel of assays start with *in-vitro* biochemical assays (Scintillation Proximity Assay, ALPHA screen, FRET… etc) leading into cell-based assays (Transcription reporter assay, cytotoxicity assay, gene expression, *in-vitro* PK…etc) and then to *in-vivo* deep characterization studies (PK/PD, studies on specific disease animal models, histology, tissue gene expression…etc).
The assays developed in the Burris lab have raised the interest of multiple pharmaceutical companies which contracted us to screen part of their libraries. Our work also led to the creation of several pharmaceutical companies.

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Using spatial transcriptomics to reveal the responsive genes in sugarcane infected by orange rust disease

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250 word maximum

Sugarcane (*Saccharum spp*.) is an important economic crop widely used as a source of sugar and biofuel. Orange rust disease, caused by *Puccinia kuehnii*, is one of the major diseases threatening sugarcane production in the United States. Breeding for disease-resistant cultivars is a challenge because research on sugarcane orange rust resistance is lagging, and disease resistance genes have yet to be discovered. Two sister clone lines with opposite orange rust resistance phenotype (resistant line: 540 and susceptible line: 664) were identified from an F1 hybrid population derived from a cross between CP95-1039 and CP88-1762 clones. Spatial transcriptomics was used to investigate the molecular dynamics of orange rust disease resistance of the two lines. Sugarcane plants were inoculated with orange rust spores. Leaf tissues were collected for analysis at 13 days after inoculations. The gene expression from the leaf sections was analyzed to identify the differentially expressed genes in the two contrastive lines in responding to orange rust disease. Genes associated with rust resistance will be potentially identified and used to facilitate marker-assisted selection and breeding.

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An anchored experimental design and meta-analysis approach to address batch effects in large-scale metabolomics

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Untargeted metabolomics studies are unbiased but identifying the same feature across studies is complicated by environmental variation, batch effects, and instrument variability. Ideally, several studies that assay the same set of metabolic features would be used to select recurring features to pursue for identification. Here, we developed an anchored experimental design. This generalizable approach enabled us to integrate three genetic studies consisting of 14 test strains of *Caenorhabditis elegans* prior to the compound identification process. An anchor strain, PD1074, was included in every sample collection, resulting in a large set of biological replicates of a genetically identical strain that anchored each study. Pairing this anchored design with meta-analytic approaches provides a method to identify stable spectral features and determine differences in features between and among test strains without the need for complex normalization strategies. We collected 104 test samples for three genetic studies across six batches to produce five analytical datasets from two complementary technologies commonly used in untargeted metabolomics. Here, we use the model system *C. elegans* to demonstrate that an augmented design combined with experimental blocks can be used to anchor studies and enable comparisons of stable spectral features across time without the need for compound identification. This approach is generalizable to systems where the same genotype can be assayed in multiple environments and provides biologically relevant features for downstream compound identification efforts. All methods are included in the newest release of the publicly available SECIMTools based on the open-source Galaxy platform.

Characterizing Stem Cell Heterogeneity in *Hydractinia symbiolongicarpus* with Methanol-Fixed 10X Single-Cell RNA-sequencing

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Studying stem cells from a wide range of taxa holds promise for ultimately developing stem-cell-based regenerative medicine therapies for humans. Recent studies in planarians and acoels reported adult stem cell heterogeneity based on single-cell transcriptomic profiles, suggesting a previously unappreciated complexity involved in this population of cells. *Hydractinia symbiologicarpus*, a colonial marine hydroid, has a population of pluripotent adult stem cells called i-cells. Similar to planarians and acoels, we hypothesized that the i-cells of *Hydractinia* are transcriptionally heterogeneous and could even be classified into subtypes. To test this hypothesis and the feasibility of using methanol-fixed *Hydractinia* cells for single-cell RNA-sequencing (scRNAseq), 20 polyps were mechanically dissociated and fixed with 80% methanol and the cells were processed with standard 10X scRNAseq protocols. The quality of the sequencing reads was comparable to a previous dataset using live cells. After integrating the two datasets with CCA, we identified all previously annotated cell types, including two clusters of i-cells. We discovered that mechanical dissociation resulted in high levels of ambient RNA in the resulting dataset whereas enzymatic dissociation did not, thus we recommend enzyme-based dissociation for *H. symbiolongicarpus*. Future research will include adding more replicates of fixed cells for scRNAseq to increase the number of i-cells to better characterize heterogeneity in this population.

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Genetic and Metabolic Signature of Butyrate and Tumor Necrosis Factor in Colonocytes: Implications for Hypertension

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Hypertension is a complex condition involving both altered metabolism and increased low grade inflammation. Recently, colonic butyrate transport has been shown to be decreased in the spontaneously hypertensive rodent model of hypertension. Simultaneously, the colonic epithelium of hypertensive rodents is known to have increased tumor necrosis factor alpha (TNFα) levels. Despite this concurrence, relatively little is known about how TNFα influences butyrate metabolism in the colonic epithelium. To address this knowledge gap, we treated primary rat colonocytes to 500 μM butyrate, 500 pg/mL TNFα, the combination, and media alone for 24 or 72 hours and assessed differential gene expression of transcripts related to mitochondrial oxidative respiration, glycolysis, lipid metabolism, and cell differentiation. To further differentiate effects of TNFα and butyrate on metabolism, a Seahorse Flux Analyzer-based assessment of OxPhos and glycolysis was conducted, as well as untargeted UHPLC-MS/MS-based Lipidomics and metabolomics. Our results support a shift away from OxPhos and glycolysis toward lipid metabolism in cells treated with butyrate and TNFα. Specifically, transcripts related to lipid metabolism and cell differentiation were differentially abundant. Simultaneously there was a significant increase in Pdk4 expression in the cotreated group, indicating this kinase may serve an important role in the shift toward lipid metabolism. Lipidomics assessment predominantly revealed a global increase in triglycerides. As increased serum triglycerides are positively associated with increased systolic and diastolic blood pressure, as well as increased risk of cardiovascular disease, altered butyrate metabolism secondary to increased TNFα signaling may serve an etiological role in the progression of hypertension.

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RecJ3/4-RNase J nuclease complex of the Ubl interactome and DNA repair in Archaea

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To avoid uncontrolled degradation of nucleic acids, nucleases are regulated by various mechanisms including the formation of multisubunit complexes. Here a new type of nuclease complex composed of RecJ3, RecJ4, and RNase J was isolated in ATP-dependent association with the ubiquitin-like protein (Ubl) SAMP1 and AAA-ATPase Cdc48a from the archaeon *Haloferax volcanii*. Genetic analysis revealed RNase J to be essential, while RecJ3 and RecJ4 (along with Cdc48a) were found to function in the recovery of cells from DNA damaging agents. Tandem affinity purification enabled isolation of a complex composed of RecJ3:RecJ4:RNase J proteins at 2:2:1 ratio that functioned in the 3'-5' hydrolysis of RNA and ssDNA (with the latter non-processive). Separation of RNase J from RecJ3/4, by use of a *ΔrecJ3* mutant, revealed RNase J to be highly active in the hydrolysis of ssDNA and RNA in 3'-5' and 5'-3' directions. Further genetic modifications enabled purification of a RecJ3/4 subcomplex with uclease activities comparable to the RecJ3/4-RNase J complex. This prompted assay of RNase J with increasing amounts of RecJ3/4 that revealed RecJ3/4 to inhibit RNase J activity. As RNase J is found essential and RecJ3 and RecJ4 are found important in recovery from DNA damaging agents in *H. volcanii*, this shift in nuclease activity may promote DNA repair. The Ubl interactome of RecJ3/4-RNase J and Cdc48a is suggested to be related to these responses.

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Mouse lungs are sexually dimorphic at the single-cell level

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Previous studies have shown significant sexual dimorphisms in the lungs. For example, female mice, which have smaller alveoli and greater surface area for gas exchange compared to males, are known to be more resistant to inflammatory lung disease in response to ozone while males are more susceptible to pulmonary fibrosis. Neonatal human lungs express estrogen and androgen receptors, suggesting that differences between male and females could be mediated by sex steroids. Genetic differences may also contribute to sex differences in lung disease, as the Y chromosome confers protection against pulmonary hypertension in hypoxic conditions. The relative contributions of sex-linked genes and sex steroids to sex differences lung anatomy and function, however, are unclear, and even less is known at the single-cell level. To address this question, we used single-cell RNA sequencing (scRNA-seq) data from male and female mouse lungs and analyzed the results using Seurat to identify sexual dimorphisms in normal, male and female wild-type mice. We first identified specific cell-type populations based on expression of known marker genes. We then separated male and female cells to test for differentially expressed genes using the FindMarkers function in Seurat. We found that a number of genes show significant differences in expression between male and female cells, some of which are known to have significant effects on the physiology of cells in the lung. We also identified differential expression of genes known to play roles the growth and functions of capillary and vascular cells. We then drilled down to investigate the molecular mechanism that underie single-cell sex differences in the lungs, and we examined the effects of sex-linked genes and sex hormonal changes on lung structure. Together, these results identify novel single-cell sexual dimorphisms in mouse lungs and raise new questions about the relationship between fine-grained transcriptomic differences and sex-specific physiology and the function of the lung.

Impact of CYP3A5, CYP3A4, and ABCB1 genotypes on tacrolimus dosing and monitoring in liver transplant patients

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Background: Blood levels of the immunosuppressant drug, tacrolimus, are affected by a transmembrane efflux pump (ABCB1) and cytochrome (CYP) P450 3A4 and 3A5, enzymes that oxidize small organic molecules. We sought to determine how single nucleotide polymorphisms (SNPs) in these genes affect tacrolimus blood trough level variability immediately after liver transplantation.

Methods: Donor and recipient genotypes for CYP3A4, CYP3A5, and ABCB1 in 56 adult liver transplant recipients were analyzed. Outcomes compared included percentage of each patient's hospital stay in which tacrolimus trough level was greater than 2 ng/ml out of range, average tacrolimus dose per kilogram, and tacrolimus dose and level at discharge.

Results: Recipient ABCB1 SNPs significantly affected the percentage of each patient's hospital stay in which tacrolimus trough level was greater than 2 ng/ml out of range. Donor ABCB1 SNPs affected the average tacrolimus dose per kilogram, as well as tacrolimus level at discharge. Recipient CYP3A4*1B SNPs affected average tacrolimus dose per kilogram and tacrolimus dose at discharge. Recipient CYP3A5*3 SNPs affected average tacrolimus dose per kilogram and tacrolimus dose at discharge.

Conclusions: This study demonstrates that achieving and maintaining tacrolimus levels within therapeutic range in the immediate post-transplant period is influenced by genetic polymorphisms in drug metabolizing enzymes and drug transporters. These SNPs determine the ultimate immunosuppression dose and impact the ease and predictability of post-transplant dosing.

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Phylogenetic analyses of spiralian innexins reveals potential loss in ribbon worms and extensive independent evolution in eight other lineages

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Innexins are transmembrane proteins that form multisubunit channels which are permeable to ions and small molecules. These channels either form gap junctions that connect the cytoplasms of two cells or hemichannels that connect cytoplasm with extracellular environment. Innexins are only found in animals and were likely present in the last common animal ancestor. Despite their seemingly critical role in cellular communication, innexins have been lost in Porifera (sponges), Placozoa, and Echinodermata (sea stars, sea cucumbers, etc.). To date, phylogenetic characterization of innexin genes have been performed in only a small subset of major animal lineages within the large group Spiralia. Here, we phylogenetically analyze the innexin complement of species from the following spiralian lineages: Mollusca, Annelida, Rotifera, Platyhelminthes, Ectoprocta, Nemertea, Brachiopoda, and Phoronida. We extracted and aligned innexin complements from protein models and translated transcriptomes. We analyzed these aligned sequences using both maximum likelihood and Bayesian phylogenetics. As in analyses of other lineages, our data show many examples independent evolution, but also reveal some conserved families. Surprisingly, we found no evidence of innexins in the ribbon worm (Nemertea) transcriptome nor did we see evidence in the only publicly available ribbon worm genome. Our results provide increased resolution to the remarkable evolutionary dynamics of innexins. These channel-coding genes, which play critical roles in cell communication, have experienced distinct periods of conservation, loss, and extreme diversification over evolutionary time, and as such, have likely played an influential role in shaping animal diversity.

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Modification of a Vegetative Index Imaging System Utilizing a Polarizer In Arabidopsis

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Long term space inhabitation will require plant cultivation on both large- and small-scale growth systems, for its uses as bioregenerative life support systems, the psychological benefits of gardening, and as food resources. Since extraterrestrial environments provide an array of stressors, systems to monitor plant health in real time would be beneficial for successful cultivation of plants in these environments. Remote plant health imaging systems that utilize multiple wavebands, such as Normalized Difference Vegetative Index (NDVI), could benefit from further development. This study examines the addition of a polarizing lens to a plant health imaging system to determine its impact for potential spaceflight implementations. The imaging system was composed of two parts with one being two FLIR Blackfly series monochrome cameras, one having a pixelated polarizer, and the second being a filter wheel with band pass filters for far-red, red, green, and blue wavelengths. *Arabidopsis thaliana* grown on media plates was treated with ammonium nitrate or sodium chloride solutions to test the cameras. Both cameras revealed significant differences in the mean reflective values between the experimental and control samples for both treatments, this being consistent with a decline in health of the experimentally treated plants. RNA sequencing was conducted to evaluate the transcriptomic responses of the treated plants to determine its consistency with the applied stress. This illustrating the camera with the added polarizing lens could detect the change in reflectance consistent with a change in plant health, despite providing no additional benefits to the remote plant health imaging system.

CLASHBase: a comprehensive database of miRNA-target in animals

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microRNAs (miRNA) is ~22 nucleotides single strand RNA expressed in various animals that are involved in the posttranscriptional regulation of gene expression by guiding the association between the RNA-induced silencing complex (RISC) and target RNAs. Despite extensive research, our ability to predict miRNA-target RNA interactions using computational algorithms is still limited by the complexity of atypical interaction models and the large number of false-positive results. To comprehensively identify endogenous miRNA-target RNA interaction, we used the modified CLASH (crosslinking, ligation and sequencing of hybrids) technique in 4 model animal species, including human, mouse, D. *melanogaster* and C. *elegans*. Next, miRNA-seq and RNA-seq were used to detect the abundance of miRNA and target RNA. CLASHBase provides a user-friendly interface for searching, browsing and analyzing miRNA and target RNA in various cell lines or animals.

Validation of a genome-wide association study for sorghum aerial root formation through the optimization of allele-specific SNP primers

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Sorghum is the fifth most widely produced cereal in the world and is used for a variety of purposes, from serving as a food source to being used in the production of renewable fuels and chemicals. Some sorghum accessions have the ability to develop aerial roots that produce mucilage. The mucilage provides a low-oxygen environment for bacteria that are able to fix atmospheric nitrogen and provide it in a bioavailable form to the host. This process can be utilized to reduce the amount of nitrogenous fertilizer used in commercial settings, leading to more sustainably produced sorghum. However, the loci controlling aerial root formation are unknown. Two diverse sorghum collections, the minicore and Sorghum Association Panel (SAP), were evaluated for the presence and absence of aerial root formation to be used in a genome-wide association study (GWAS). We identified five single nucleotide polymorphisms (SNPs) in the GWAS that are associated with the aerial root phenotype. Allele-specific primers were designed to differentiate genotypes. Polymerase chain reactions were optimized by altering the concentrations of magnesium and deoxyribonucleotide triphosphates (dNTPs) following a Taguchi design and optimized for annealing temperature. These markers will be used for validation studies using an F2 population that was developed by crossing UF15 and UF20 sweet sorghums with the landrace IS 23992, which produces extensive aerial roots. The ultimate goal is to use these populations to breed sweet sorghums with aerial roots to enable more sustainable production of renewable chemicals and fuels.

Understanding the genetic and environmental factors controlling biological nitrogen-fixation on the aerial roots of sorghum

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Biological nitrogen fixation (BNF) by crop plants via microbial symbiosis is an effective approach to improving the economic and environmental costs of crop production by decreasing fertilizer dependence. Some sorghum accessions have demonstrated BNF by hosting nitrogen-fixing bacteria in the mucilage produced by their aerial roots. Sorghum is a climate-resilient crop due to its ability to produce high biomass and grain yields with limited inputs and its tolerance to biotic and abiotic stresses. Determining the genetic and environmental factors controlling BNF in the mucilage of sorghum's aerial roots can contribute to more sustainable sorghum production. We are performing a genome-wide association study of two panels of genetically diverse genotypes, the sorghum minicore, a collection of 242 landraces, and the sorghum association panel (SAP), a collection of 388 sorghum genotypes representing major cultivated races and important U.S. breeding lines and their progenitors. We are also investigating the effect of the nitrogen fertilization level and location (Florida vs. Wisconsin) on aerial root traits. Based on initial screening in Florida, aerial roots were observed in 114 accessions of the minicore (47%), but only 18 accessions of the SAP (5%), suggesting aerial root formation is a trait that has been under negative selection in modern breeding programs. Additionally, we have initiated breeding populations from which bioenergy sorghums supporting BNF will be selected, and we are developing near-isogenic lines with BNF traits for transgenic validation experiments. Together, these studies will enhance our understanding of BNF in sorghum and enable the development of nitrogen-fixing bioenergy sorghums.

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Elucidating the molecular mechanism of translational errors by applying in vitro technique to in vivo systems

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Given the increasing complexity of the proteome from yeast to mammalian systems, the accuracy of protein synthesis should also increase across evolution. Additionally, a recent study revealed that interferon stimulates translation error in cancer cells, increasing the production of error containing peptides recognized by immune cells to promote cancer immunity. As such, translation fidelity and error rate could vary across evolution and among conditions within the same species. However, how mRNA translation systems modulate their accuracy and precision is largely unknown. My strategy to address this outstanding question is developing an *in vitro* translation system to monitor translation fidelity and identify the factors which are critical for accurate translation. In principle, we can switch ribosomes and cytoplasmic lysate between yeast and mammalian systems or between healthy and cancer cells to identify whether the ribosome itself or other translation factors change translation fidelity.

To begin establishing the ribosome switching system, our previous research found that co-translational initiator methionine (iMet) processing by methionine aminopeptidase (MetAP) is critical for translation fidelity. We aim to identify the substrates of MetAP involved in maintaining translation fidelity either in ribosomal proteins or translation factors. Once we establish this system, the outcome may not only enhance our understanding of the molecular role of translational error, but also reveal the physiological and pathological mechanisms of diseases caused by translational error and guide the development of novel therapeutics.

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The Role of snaR-A non-coding RNA in Breast Cancer

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Non-coding RNAs are RNAs that do not code for a protein but play important roles in gene regulation and cancer. The snaR (small NF90-associated RNA) family of non-coding RNAs are highly structured with conserved sequences. snaR-A is the most abundant of this family and has been shown to interact with the RNA binding protein NF90. snaR-A is upregulated in most immortalized cell lines, including cancer cells. The function of snaR-A is ambiguous, as well as the biological function of its interaction with NF90. We will study their interaction by generating conditional knockouts of the endogenous NF90 in both the MCF7 and MDA-MB-231 breast cancer cell lines. These cell lines will be used due to their differing levels of snaR-A expression. MCF7 has high expression of snaR-A, while MDA-MB-231 has relatively low expression of snaR-A. The goal of this study is to identify the role snaR-A and NF90 interactions may play in breast cancer. Previously published research indicates that snaR-A expression may be downregulated after the NF90 knockdown. Our preliminary data suggest that snaR-A knockdown inhibits cell proliferation. To probe this phenotype, we will be examining the NF90 knockout's effect on two cell lines that differentially express snaR-A. This project seeks to identify the role of NF90/snaR-A interactions in breast cancers and provide a better understanding of the pathological mechanisms.

Virus Sequence Discovery in the Small Hive Beetle

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The small hive beetle (SHB), *Aethina tumida*, causes significant damage to apiaries. We aimed to characterize the virome of SHB to 1) address the potential role of SHB in honey bee virus transmission, and 2) identify SHB viruses with potential for use in biological control of this pest. Insects from multiple regions in the U.S. were either field caught or lab-reared prior to freezing. RNA was extracted and sequenced, and the transcriptome assembled using Trinity. Contigs were annotated by BLASTx. Sequences from multiple honey bee viruses were found in field-caught SHB but not in lab-maintained insects. This result suggests that honey bee viruses were eliminated from beetles maintained in the lab, supporting the hypothesis that honey bee viruses do not replicate in SHB. We will test this hypothesis by infecting SHB cell lines with these viruses and monitoring viral replication. The near-complete sequence of a novel, putative phasmavirus (order: *Bunyavirales*) was discovered from field-caught adult beetles. In addition, a partial glycoprotein sequence from a different phasmavirus was integrated into the genome of SHB in all samples. We plan to sequence the small RNAs of SHB to determine whether there is active replication of the putative phasmavirus. Whether the putative SHB phasmavirus has potential for biological control of SHB remains to be determined.

The evolution of sex-biased expression and sex-limited chromatin in *D. melanogaster* and *D. simulans*

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Increased fitness in one sex may come at a cost in the other sex, such conflicting evolutionary dynamics are sexually antagonistic. Antagonistic coevolution drives evolution of reproductive-related functions and is expected to contribute to sex-bias and sex-limited expression. In this study of *D. melanogaster* and *D. simulans* head tissue, a majority of sex-biased gene expression patterns were conserved and balanced between male- and female-bias. Genes that are sex-biased in only one of the two species are more likely to be male-biased than female-biased, suggesting rapid evolution favoring male-biased expression. *D. melanogaster* shows more male-bias than *D. simulans*. A model for *cis*-regulation of sex-biased expression where genes with male-biased expression have H3K4me3 marks (open chromatin) in males, and genes with female-biased expression have open marks in females is supported by our data. In addition, genes with male-biased expression are likely to have H3K27me2me3 (closed chromatin) in females, in both *D. melanogaster* and *D. simulans* on the autosomes and in *D. melanogaster* on the X. These results are consistent with intralocus sexual conflict, where the excess of male-bias in expression (attributable in part to open chromatin) is resolved via silencing (with closed chromatin marks) in females, with divergence of this resolution on the X chromosome between the species. Genes with female-beneficial alleles are associated with activation and open chromatin marks in females but are not associated with silencing and closed chromatin marks in males, representing an asymmetry in sexual-conflict resolution. These findings are consistent with the faster-male hypothesis in XY systems.

Exposure to the antineoplastic ifosfamide alters molecular pathways related to cardiovascular function, increases heart rate, and induces hyperactivity in zebrafish (*Danio rerio*)

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Alkylating antineoplastics are employed in various chemotherapeutic treatments. With cancer prevalence increasing globally, exigency for increased administration of chemotherapeutic pharmaceuticals within a narrow therapeutic index expands, further necessitating a toxicological understanding of the agents. These chemicals enter the environment through hospital effluent and are detectable in surface waters. Ifosfamide is one such agent, yet few studies have assessed its threats to aquatic organisms. To address this gap, the three major objectives of this investigation concerning the zebrafish embryo and larvae model are as follows: (1) characterize the acute toxicological, teratogenic, and mutagenic potential of ifosfamide as manifested in morphological deformities, (2) determine if there exist acute behavioral and anxiogenic potential of the agent, and (3) assess changes in mRNA levels associated with oxidative stress via transcriptomics following acute environmentally-relevant ifosfamide exposure. Zebrafish larvae were exposed over seven days to ifosfamide at environmentally relevant concentrations, and morphological deformities such as pericardial edema, swim bladder abnormalities, axial malformations, and encephalic irregularities were assessed. Significant changes in deformities, morality rates, and reactive oxygen species induction were not observed at low exposure concentrations. However, the Visual Motor Response (VMR) assay revealed evidence for larval hyperactivity at low, environmental levels (0.1 µg/L). Non-mitochondrial respiration was depressed at 1000 µg/L and 2500 µ/L ifosfamide. Molecular mechanisms underlying responses to ifosfamide exposure will be discussed. Experimental conclusions will permit extension to the larger class of alkylating antineoplastics employing ifosfamide as a model to guide future investigations into the toxicological, teratogenic, and mutagenic potential of the drug class.

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Revealing adult stem cell heterogeneity in *Hydractinia* using a combination of single-cell transcriptomics and experimental molecular techniques

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Adult stem cells are known to confer incredible abilities, such as unlimited regeneration and immortality, to animal taxa such as planarians and *Hydra*. The cnidarian *Hydractinia symbiolongicarpus* has remarkable abilities for infinite growth, lack of aging, and unlimited regeneration, due to its population of adult stem cells, known as interstitial stem cells or 'i-cells'. Our goal is to characterize the i-cell population in *Hydractinia* to better understand the vast potential these cells have in generating all cell types within the adult animal. **It is unknown if all i-cells have the same potential to generate all cell types, or if there are subpopulations of i-cells with differing or more restricted capabilities in the animal**. Using single-cell transcriptomics, we created a cell-type atlas for adult *Hydractinia* and found two separate cell clusters with known stem cell marker expression. One cluster was connected to a germ cell fate while the other was linked to a somatic fate. We obtained highly expressed marker genes from each cluster, as well as markers expressed in both, to generate a list of ~700 potential i-cell candidate genes. We are spatially characterizing the expression pattern of eight of these top i-cell markers, with some exclusive to one cluster and some present in both, using *in situ* hybridization. Results indicate heterogeneity in expression patterns among markers in different polyp types and life stages. We have also started to generate fluorescent transgenic reporter lines for a subset of the i-cell markers to further understand their contribution to different cell lineages and to regenerating tissues. This work will provide a foundation for understanding adult stem cell heterogeneity in *Hydractinia* and bring further insights into their incredible regenerative abilities.

Probing the Role of Alternative Splicing of *IKZF1* in Disease Pathogenesis

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Abstract (*250 words maximum)*
Genome-wide association studies suggest that the *IKZF1* gene is associated with risk for Type 1 Diabetes (T1D). *IKZF1* encodes Ikaros, a transcription factor and critical regulator of lymphocyte development. The *IKZF1* gene includes 9 exons that produce at least 10 distinct transcripts. Exons 4-6 encode 4 zinc finger motifs that mediate DNA binding, while the last exon encodes 2 zinc fingers that allow protein dimerization. We have observed differential exon usage of *IKZF1* between T1D cases and controls.
To explore the role of alternative splicing of *IKZF1* in T1D, CRISPR/Cas9 was used to generate Jurkat T-cell lines with truncating mutations in either *IKZF1* exon 4, exon 6 or both. Clones were isolated by serial dilution and characterized by PCR amplification and DNA sequencing. Transcriptome and chromatin structure analysis was performed by RNA-seq and ATAC-seq and protein analysis by immunoblotting.
Twenty clones with *IKZF1* induced mutations were selected and compared to four wild-type clones. Protein and transcriptomic analyses revealed shifts in Ikaros isoform abundance consistent with the exon targeting but with considerable heterogeneity between individual clones. Based on differences in gene expression and chromatin accessibility, clones with single-targeted events in exon 4 or 6 were interspersed but clustered separately from double-targeted clones. Manipulation of alternative splicing of *IKZF1* also resulted in differential expression of other T1D-associated genes and elicited compensatory changes among other IkZF family members. Our results suggest that the number of zinc finger domains in Ikaros, rather than their identities, is critical to its function in T cells.

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Evaluating pigmentation levels in *Drosophila melanogaster* tergites using machine learning methods

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There is abundant quantitative natural variation in pigmentation traits of *Drosophila melanogaster*. Some of these traits are qualitative (e.g. presence/absence of a thorax trident or middle stripe) while others are quantitative (e.g. percent of abdominal segment melanized). *D. melanogaster* displays seasonal fluctuations in tergite pigmentation with lighter phenotypes in the summer and darker in the winter. These shifts occur rapidly, completing a cucle within 5-10 generations. This has led to widespread interest in pigmentation pattern variation and its adaptive evolutionary implications. Typically, scoring the proportion of melanization is done manually using human raters who score each individual tergite on an ordinal scale of 0 to 10. This process is time intensive and opens the door to potential rater bias. To facilitate comprehensive quantitative scoring of color variation, we have developed a novel algorithm that segments *Drosophila melanogaster* dorsal images into the following body component annotations:(1) head, (2) thorax, (3) scutellum, (4-9) 6 abdominal tergites, and (10) other fly tissues (e.g. wings). Abdominal tergite melanization is computationally determined from the image annotations as the proportion of pixels in a given component that are darker that a set grayscale threshold. Computational scores are evaluated relative to the ordinal human rater scale for the same component annotations.

The role of rhodanese domain and ubiquitin-like proteins in sulfur mobilization and protein conjugation in Archaea

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Archaea have a system of ubiquitin-like modification (SAMPylation) that regulates enzyme activity and protein degradation, especially during oxidative stress. Their ubiquitin-like proteins (small archaeal modifier proteins or SAMPs) also have a role in the mobilization of sulfur for tRNA thiolation and molybdenum cofactor (MoCo) biosynthesis. Rhodanese domain proteins (RHDs) are known for their role in persulfide chemistry. Importantly, these domains have been connected to ubiquitin-like modification and sulfur mobilization in other organisms. The halophilic archaeon *Haloferax volcanii* encodes two standalone RHDs, UbaC and UbaB. Here, UbaC is found to reproducibly co-purify with the E1-like activator protein UbaA in an apparent complex. UbaC is also found to be required for $tRNA_{Lys}^{UUU}$ thiolation and can reconstitute SAMPylation *in vitro* when compared to the UbaC C64S active site variant. A potential role is also discovered for RHD UbaB in SAMP1ylation via the generation of a *ΔubaCΔubaB* double mutant. This study provides further evidence that RHDs are playing a significant role in the ubiquitin-like modification and sulfur mobilization system in Archaea.

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Molecular and behavioral responses to the anti-depressant citalopram in zebrafish (*Danio rerio*) embryo/larvae

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Over the past several years, pharmaceutical remnants leaving treatment facilities and entering bodies of water have been increasing with a growing and concentrated human population in urban areas. Citalopram, a selective serot1onin reuptake inhibitor (SSRI) used to treat depression, is a pharmaceutical often detected in aquatic environments. High concentrations of citalopram have been detected in treated wastewater effluent, at levels up to 426.6 ng/L in freshwater systems in the United States. The objective of this study is to assess the acute toxicity of citalopram at environmentally relevant concentrations to zebrafish embryos by measuring mortality, morphological deformities, gene expression, and behavioral outcomes, specifically locomotor activity via the Visual Motor Response test and anxiolytic behavior through the Light/Dark preference test. Zebrafish embryos at 0 days post-fertilization (dpf) were exposed to either embryo rearing medium (ERM), or one dose of 0.1, 1, 10, 100, and 1000 µg/L citalopram for 7 days. Deformities such as spinal curvature, pericardial edema, and yolk sac edema were quantified following citalopram exposure with low mortality observed. Inflated swim bladders were present following citalopram exposure. A Visual Motor Response test revealed citalopram at environmental concentrations did not alter locomotor activity but there was a reduction in activity with 1000 µg/L. Molecular data are currently being collected to understand mechanisms underlying exposure and will be discussed. The results of this study aim to improve understanding of the potential harm citalopram may cause fish and will help contribute to environmental risk assessments for citalopram to aquatic organisms.

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Examining electron flow and post-translational regulation of an archaeal 2Fe-2S ferredoxin and flavin-based ferredoxin reductase

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Due to the ability of haloarchaea to survive and adapt to extreme hypersaline conditions, their genetics and enzymes are ideal for applications in biotechnology. *Haloferax volcanii* has adapted and developed response mechanisms to survive these stressors, although many of these stress response mechanisms are still unknown. Currently, findings show that in response to oxidative stress there is an upregulation of post-translational modifications (PTM) such as ubiquitin-like (Ubl) modifications and lysine acetylation, suggesting regulation as an oxidative stress response. Associated with oxidative stress, 2Fe-2S ferredoxin is identified to be the most impacted molecular target of lysine acetylation. Though ferredoxins are distributed among all domains of life, it is unknown how lysine acetylation may impact the function of ferredoxins. We hypothesize that *H. volcanii* 2Fe-2S ferredoxin is regulated by lysine acetylation to regulate electron flow for redox balance during oxidative stress, which is caused by an imbalance of oxygen reactive species (ROS) and antioxidants. The overall aim is to understand how *H. volcanii* utilizes and post-translationally modifies ferredoxin for redox balance during oxidative stress. The first objective of this study is to characterize potential protein partners of 2Fe-2S ferredoxin (HVO_2995) and ferredoxin reductase (HVO_2345) that serve in mediating electron transfer during an oxidative stress response. By using His_6-tag overexpression plasmid and affinity chromatography, SDS-PAGE and immunoblotting analysis show successful protein purification, leading to future work of characterizing these putative protein partners. This work will provide an important perspective on post-translational modification of the metalloprotein ferredoxin and oxidative stress response in the organism.

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Microbial Associations of Abyssal Gorgonians and Anemones (>4,000m Depth) at the Clarion-Clipperton Fracture Zone

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Deep coral-dominated communities play paramount roles in benthic environments by increasing their complexity and biodiversity. Coral-associated microbes are crucial to maintain fitness and homeostasis at the holobiont level. However, deep-sea coral biology and their associated microbiomes remain largely understudied, and less from remote and abyssal environments such as those in the Clarion-Clipperton Fracture Zone (CCZ) in the tropical Northeast (NE) Pacific Ocean. Here, we study microbialassociated communities of abyssal gorgonian corals and anemones (>4,000 m depth) in the CCZ; an area harboring the largest known global reserve of polymetallic nodules that are commercially interesting for the deep-sea nodule mining. Coral samples (n = 25) belonged to Isididae and Primnoidae families, while anemones (n = 4) to Actinostolidae family. Significant differences in bacterial community compositions were obtained between these three families, despite sharing similar habitats. Anemones harbored bacterial microbiomes composed mainly of Hyphomicrobiaceae, Parvibaculales, and *Pelagibius* members. Core microbiomes of corals were mainly dominated by different Spongiibacteraceae and Terasakiellaceae bacterial members, depending on corals' taxonomy. Moreover, the predicted functional profiling suggests that deep-sea coralsharbor bacterial communities that allow obtaining additional energy due to the scarce availability of nutrients. This study presents the first report of microbiomes associated with abyssal gorgonians and anemones and will serve as baseline data and crucial insights to evaluate and provide guidance on the impacts of deep-sea mining on these key abyssal communities.

Molecular and behavioral toxicity assessment of tiafenacil, a new PPO-inhibiting herbicide, in zebrafish embryo/larvae

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Tiafenacil is a newly registered contact herbicide within the pyrimidinedione chemical family classified as a protoporphyrinogen IX oxidase (PPO) inhibitor. Studies are lacking that investigate the potential for sub-lethal effects of PPO-inhibitors in aquatic species. As such, we conducted a series of toxicity assays using tiafenacil in zebrafish and measured molecular, biochemical, and behavioral endpoints in embryonic and larval fish. We hypothesized that tiafenacil induces apoptosis, oxidative stress, and behavioral toxicity in zebrafish. Embryos and larvae were exposed to tiafenacil at concentrations ranging from 0.1 µg/L up to 10 mg/L depending on the assay for 7-days post-fertilization. Decreased survival in about 50% of the population were noted at exposure concentrations >1 mg/L. Heartbeat frequency at 3 dpf was decreased in zebrafish exposed to >10 µg/L tiafenacil. This coincided with an increase in reactive oxygen species in larvae treated with 10 µg/L. Oxygen conception consumption rates of zebrafish were not affected by tiafenacil, nor did we detect differences in apoptotic events in larvae (acridine orange). We also measured eighteen transcripts related to oxidative stress and mitochondrial complexes I through V in larval fish but did not detect any change in steady state transcript abundance. Hypoactivity was noted in the light-dark preference test in larvae exposed to 100 µg/L. These data contribute to risk assessment evaluations for a new class of herbicide and suggest the presence of abnormal cardiac function, ROS induction, and behavioral abnormalities in zebrafish exposed to tiafenacil.

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Elucidating the molecular function of anthracnose resistance genes in *Sorghum bicolor*

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Sorghum is the fifth most cultivated cereal crop in the world, grown for feed, food, fodder, and the production of renewable chemicals and fuels. Sorghum is adapted to a wide variety of environments, but its productivity and sustainability can be hindered by fungal pathogens. *Colletotrichum sublineola* is the causal agent of anthracnose, resulting in leaf blight, stalk rot, and head blight in susceptible sorghums. The spread of anthracnose has led to a reliance on the application of fungicides for disease control. Alternatively, the development of anthracnose-resistant cultivars can act as a more sustainable and economical means for disease management. Previously, a genome-wide association study (GWAS) of the sorghum association panel (SAP) identified two loci on chromosome 5 that appear to be associated with anthracnose resistance: *Sobic.005G182400* from the accession SC1330 annotated to encode a receptor-like kinase, and *Sobic.005G172300* from SC110, that encodes an F-box protein overlapping with an ascorbic acid mannose regulator 1 domain. Sequence analysis of the resistance alleles revealed amino acid variations in the predicted protein encoded by *Sobic.005G172300* relative to the susceptible reference BTx623, but the impact on protein function remains unclear. Gene expression analysis of *Sobic.005G182400* and *Sobic.005G172300* revealed a significant increase in expression at 1-day post-inoculation (dpi) with *C. sublineola* in the sorghum accessions that harbor the resistance alleles. A subsequent transcriptome analysis of inoculated and mock-inoculated seedlings of resistant and susceptible accessions sampled at 1, 3, and 5 dpi suggests *Sobic.005G182400* and *Sobic.005G172300* regulate programmed cell death and accumulation of reactive oxygen species, respectively.

The mutational spectrum of prostate tumors from African American men

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Abstract

African American (AA) men have the highest PCa incidence and death rates for of any racial or ethnic group. The wide variation observed in the group is multifactorial, with varying effects arising from socio-economic status and access to healthcare among others. However, even after adjusting for socio-economic, disparities in incidence and mortality remain significant, suggestive of a role for molecular and genetic factors in driving disease outcomes. Moreover, given the dearth of data, there is an urgent need to characterize the molecular profile of tumors derived from AA PCa patients, which would provide relevant insight into the drivers of PCa progression in AA men. The objective herein is to determine the association between the somatic mutational spectrum, specifically base-substitution (BS) variants, and adverse outcomes (eg stage, grade, survival). DNA and RNA was extracted from 55 paired normal-tumor tissues from AA men which underwent subsequent NG sequencing. De novo somatic mutations will be identified by applying Mutect2 in GATK4 (v4.1.9.0). The signature of BS variants will also be compared among other populations, including Caucasians and other men of African descent, using The Cancer Genome Atlas. Further, de novo signature for each population will be extracted using SigProfilerExtractor and the GRCh38 references genome. The identified de novo signatures would be matched to the known COSMIC (v94) mutational signatures. Improving the characterization of the PCa tumors in AA men will improve risk stratification and treatment of PCa in AA men according to the genomic composition unique to each individual, potentially reducing PCa disparities.

Isolation of microRNA from deer serum exosomes for potential early detection of chronic wasting disease

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Chronic wasting disease (CWD) is a lethal, degenerative neuronal disease that affects a range of cervid species in the United States. Current diagnostic testing for CWD is highly invasive, often requiring brain or lymph tissue. To improve early detection of CWD and provide accurate diagnosis before symptoms are present and shedding of prions occurs, we are developing new methods to screen for CWD biomarkers in cervid blood. Changes in microRNA (miRNA) expression profiles have been associated with other prion diseases such as scrapie and CWD in elk serum. Here we describe a methodology for isolating and extracting miRNA specifically from white-tailed deer serum exosomes, . This will enable managers and farmers to surveil wild and domestic cervid populations for CWD using minimally invasive blood sampling, and thus reduce the reliance on postmortem diagnostics. Effective detection of CWD during early disease will improve the quality of wild deer populations, an important natural resource, and reduce livestock loss from farmed herds.

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Characterization of the Functions of Oxylipins in Stomatal Immunity

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There is a tremendous yield loss of crops worldwide due to diseases caused by microbial pathogens. Many pathogens are inhabitants of the phyllosphere, using stomata as their primary route of entry. Stomatal pores formed by pairs of specialized guard cells in the leaf epidermis open and close to regulate CO_2 intake and transpirational water loss. Plant pathogens have long been known to exploit stomatal pores as major entry points to the intracellular leaf space. Conversely, plants have evolved immune mechanisms to limit pathogen entry into the plant body. Efficient detection of pathogens and mounting of timely defense responses are essential for plant survival and crop viability.

Lipoxygenases (LOX) are non-heme iron containing dioxygenases, which catalyze the incorporation of molecular oxygen in polyunsaturated fatty acids (PUFA) such as linoleic acid forming hydroperoxides or hydroperoxyl fatty acids. To date the role of oxylipin regulation on the stomatal immunity is not well-characterized. Linolenic acid and linoleic acid were not altered much in wild type Arabidopsis, while both decreased dramatically in *lox1* mutant upon pathogen infection. In *lox1* upon pathogen infection, the lipoxygenase products RES oxylipin 13HPODE was increased, while 9HPODE was decreased. In addition, several metabolites e.g., coumaric acid, ferulic acid, pipecolic acid and phytohormone melatonin were regulated in oxylipins-mediated stomatal immunity. We hypothesize that LOX pathway regulates downstream of melatonin and COR hijack signal cascades are regulated by LOX1 oxidized RES oxylipins. Our results suggest that exogenous melatonin improves stomatal immune responses by directly reduce ROS and alternating expression of genes involved in the metabolisms of phytohormones jasmonic acid and salicylic acid.

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Optimizing Gene Editing in Canine Cells Using CRISPR/Cas9 for Pyruvate Dehydrogenase Kinase 4

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Genetic mutations cause dilated cardiomyopathy in canines, one of which is a 16-base pair deletion in the intron of the pyruvate dehydrogenase kinase 4 (PDK4) gene. The mutation leads to altered splicing and decreased metabolism of the heart. The objectives of this study were to determine the efficacy of homology-independent targeted insertion (HITI) gene editing canine cells**.** We hypothesized that clustered regularly interspaced short palindromic repeats (CRISPR) and its associated protein-9 (Cas9) would be effective in genetically editing PDK4. Guide RNAs (sgRNA) were designed to target the mutant region and enable replacement of the deleted region using the homology-independent targeted insertion approach. First, sgRNAs were tested with *in vitro* cleavage reactions. Then candidate sgRNAs were nucleofected into canine cells. Cleavage efficiency was assessed by sequencing and Tracking of Indels by Decomposition (TIDE) and Interference of CRISPR Edits (ICE) analysis. Four sgRNAs showed evidence of cutting *in vitro* in heterozygote and homozygote DNA templates. Post-nucleofection TIDE and ICE analysis surprisingly showed different results. We observed total editing efficiency of up to 9.5% with SaCas9-61for and KKH-71rev. TIDE and ICE analysis were not able to detect editing efficiency in sgRNA designed to cut only in the presence of the mutation, KKH-123for and KKH-Drop, indicating the need for further optimization for *in vivo* experiments. This study investigates a novel approach to gene editing in canine cells is expected to create treatment options for genetic diseases in canines.

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Mitochondrial and behavioral toxicity assessment of perfluorotetradecanoic acid (PFTeDA) in zebrafish (*Danio rerio*) embryos/larvae

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Industrial and consumer products, such as pesticides, lubricants, and cosmetics, can contain perfluorinated compounds (PFCs). Although some PFCs have been linked to physiological and behavioral changes, there are limited data on longer-chained PFCs. The objective of this study was to determine the effect of perfluorotetradecanoic acid (PFTeDA) exposure on zebrafish (*Danio rerio*) during early developmental stages. We measured several endpoints, which included gene expression, mitochondrial bioenergetics, and behavioral activity in zebrafish. We hypothesized that oxygen consumption would be impaired *in vivo*, leading to mitochondrial dysfunction and impaired locomotor activity. Survival, timing of hatching, and deformity frequency were unaffected by PFTeDA at each concentration tested (0.01, 0.1, 1, and 10 μM) following 7 days of exposure. The expression levels of mitochondrial-related genes (*cox1* and *mt-nd3*) and oxidative stress-related genes (*cat, hsp70,* and *hsp90a*) were increased in larval fish after 10 μM PFTeDA exposure; however, there was no change in oxidative respiration (i.e., basal respiration and oligomycin-induced ATP-linked respiration). Intriguingly, reactive oxygen species were significantly reduced in fish treated with 10 μM PFTeDA, which corresponds to the genomic response of antioxidant defense mechanisms. Both the visual motor response test and light-dark preference test were conducted on 7 dpf larvae and yielded no significant findings. This study improves knowledge of toxicity mechanisms for PFTeDA in the early stages of fish development.

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Dendrimer-base lipid nanoparticles for *in situ* transfection of tumor-infiltrating monocytes

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The rapid clinical progression of mRNA-lipid nanoparticles (LNPs) highlighted the critical need to expand their applications beyond vaccine. *In situ* engineering of immune cells, such as tumor-infiltrating monocytes (TIMs), using LNPs has shown great potential towards clinical translation. However, innate immune cells are difficult to transfect. Even the most efficient LNP formulations can only mediate 1-4% of RNA release into the cytoplasm – a critical step in mediating RNA transfection. Developing mRNA formulations that efficiently transfect monocytes *in vivo* with low toxicity is still needed. Here, we proposed to construct and screen a library of dendrimer-based mRNA formulations for efficient mRNA delivery to TIMs *in vivo* with low toxicity. Compared with conventional lipids that lack the structure flexibility for diverse library construction, dendrimer has broad structural-tuning flexibility and high degree of molecular uniformity, allowing us to examine the influence of dendrimer structural properties on transfection efficiency. Specifically, we will construct 216 dendrimer-based ionizable lipids from 6 initiators and 4 lipid functional groups. These lipids will then be formulated with Luc-mRNAs to form nanoparticles for *in vitro* and *in vivo* screening to identify candidate(s) that efficiently transfect TIMs with low toxicity. Our pilot *in vitro* experiment has identified two candidates with higher in vitro transfection efficiency than FDA-approved LNP formulation. Successful accomplishment of this study will establish the structure-function relationships between dendrimer-based formulation and mRNA delivery. Implemented in clinics, the delivery platform from this study can be used for gene therapy focuses on *in situ* engineering of TIMs to treat cancer.

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A large genetic screen identifies modifiers of combined Aβ42 and tau toxicity in flies

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Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by dementia and cognitive decline due to progressive cerebral cortical atrophy. Brains of AD patients are characterized by the accumulation of microscopic extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau. The deposition of Aβ42, which is one of the fragments of amyloid precursor protein (APP), has been known to play a role in initiating the events leading to the formation of amyloid and subsequently hyperphosphorylation of tau. However, animal models expressing either Aβ42 or tau individually do not mimic the complexity of the human condition. Indeed, recent evidence suggests that Aβ42 and pathological tau interact synergistically to modulate neurotoxicity in AD. To shed light on their concerted roles in AD pathogenesis and to discover pathways mediating Aβ42 and tau interactions, we generated transgenic flies co-expressing human Aβ42 fused to a signal peptide along with the longest wild-type tau isoform. Overexpression of Aβ42 or tau in Drosophila using the UAS-Gal4 system causes mild to the moderate rough eye. In comparison, co-expression of Aβ42 with tau causes severe roughening and reduction of the eye size. The level of neuronal cell death in eye tissues was also significantly enhanced in flies co-expressing Aβ42 and tau. To identify pathways mediating Aβ42+tau interactions, we are currently using the Aβ42+tau eye phenotype as platform to screen 1,500 UAS lines expressing a variety of human genes. We have identified few enhancers and suppressors not previously known to be involved in AD pathogenesis, which will be helpful to uncover new molecular pathways and potential therapeutic targets. This work is supported by NIH grant R21AG069050 to DERL.

Genetic and Epigenetic Control of Sexual Dimorphic Inflammaging

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Sex is an important determinant for innate immunity and inflammation. Woman account for about 80% of autoimmune diseases patients in the USA. Inflammaging, a hallmark of aging, is characterized by chronic inflammation in the absence of infection. Recent RNA-Seq and ATAC-Seq studies revealed that the aging process of the human immune system varies significantly between men and women. Using genomic data generated by ModENCODE, we discovered that *Drosophila* aging is characterized by an elevated expression of innate immune genes. Though there is a pattern of inflammaging shared by both sexes, there is a set of innate immune genes that are exclusively elevated in old females, but not in males. The genetic accessibility of the fruit fly model allows us to investigate the genetic and epigenetic mechanisms that underlie this sexual dimorphism of inflammaging.

Macrophages of *DNMT3A*-mutant Clonal Hematopoiesis Infiltrate Colorectal Cancer Microenvironment: How Blood Chats with Solid Tumors?

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Clonal hematopoiesis (CH) is defined as overrepresentation of mature blood cells derived from a single hematopoietic clone that often carries leukemia-associated mutations. CH is also known to contribute to non-hematologic diseases with a prominent immune component including atherosclerosis and COPD. In solid tumors, presence of CH is associated with poor prognosis. Despite the clinical relevance, CH-solid tumor interaction is poorly understood. We investigated whether CH driven by heterozygous *Dnmt3a* loss, the most common CH-related genetic alteration, contributes to the severity of colorectal cancer by altering its tumor immune microenvironment. We have previously found that in AOM/DSS model of colitis-associated colon cancer (CAC), mice engrafted with *Dnmt3a*(+/-) bone marrow to mimic CH had increased tumor burden and heightened colon tumor pathology compared to wild-type-engrafted control animals. To profile CH-related changes in the tumor immune microenvironment, we subjected purified tumor-infiltrating donor-derived hematopoietic cells to single-cell RNA-sequencing. Unsupervised clustering followed by lineage marker analysis to assign cell identities found numeric overrepresentation and changes in polarization of macrophages in tumors from CH mice. *Ex vivo* assays confirmed impaired phagocytic activity in *Dnmt3a*(+/-) bone marrow-derived macrophages (BMDMs). Using mouse (BMDMs + MC38 syngeneic colon cancer cell line) and human (THP-1 derived macrophages + Caco2 colon cancer cells) systems, we are currently investigating the functional, transcriptomic, and epigenetic mechanisms perturbed in CH macrophages. The results of this study will deepen our understanding how mutations in the hematological system characteristic of CH promote solid tumor progression, with the goal to inform novel immunotherapy approaches for colorectal cancer.

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N6 methyladenosine modification of the 7SK non-coding RNA Regulates Global RNA Polymerase II Transcription and Tumorigenesis

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Numerous studies suggest that RNA N^6-methyladenosine (m^6A) modification play crucial roles in RNA Polymerase II (Pol II) transcription regulation. A major step in Pol II transcription regulation is the promoter-proximal pausing, which is essential to regulating both cell transcription and normal cellular activities. However, the intrinsic mechanism for the regulation of m^6A on RNAP II pausing remains unclear. Here, we find that 7SK snRNA is highly m^6A modified in non-small cell lung cancer (NSCLC) cells . We identified METTL3 and ALKBH5 as the major m^6A “writer” and “eraser” enzymes for 7SK. To investigate the functional importance of m6A modifications on 7SK, we engineered NSCLC cell lines expressing a dCasRx fused to wild-type or catalytically inactive ALKBH5 . By co-expression of a 7SK targeting small guide RNA, we specifically modified the m^6A methylation status of 7SK while leaving m^6A on other RNAs intact. Strikingly, we observed that demethylation of 7SK significantly inhibits NSCLC cell proliferation. Meanwhile, removal of 7SK m^6A methylation leads to the attenuation of promoter-proximal Pol II, thereby decreasing global transcription levels. Moreover, we observed that the levels of 7SK m^6A methylation, RNAP II CTD Ser 2P and global transcription are higher in non-small cell lung cancer cells (NSCLC) as compared to the non-malignant lung epithelial cells, suggesting that aberrant 7SK m^6A methylation correlates with NSCLC tumorigenesis. Taken together, our study uncovers a previously unrecognized role of m^6A methylation on 7SK in regulating global transcription and NSCLC tumorigenesis by controlling RNAP II pausing releasing.

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Sexual dimorphism in *Caenorhabditis elegans* stress resistance

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Sexual dimorphism in stress responses in the model *Caenorhabditis elegans*
Biological sex has broad and well-documented effects on disease, aging, and longevity, but molecular mechanisms of dimorphism are poorly understood. *C. elegans* has well-understood mechanisms of stress resistance and longevity, but studies are largely limited to one sex, self-mating hermaphrodites; males have been largely ignored. Here, we found that males are more resistant to heat shock and pro-oxidant stress than hermaphrodites in four genetically and geographically diverse *C. elegans* strains. Males were more resistant when the two sexes were cultured together in groups and when keep as individuals indicating that the dimorphism is not dependent in interacts between worms. Males induced canonical stress response genes in the same tissues and at comparable levels relative to hermaphrodites suggesting the importance of other mechanisms. TRA-1 is a transcriptional master regulator of sexual differentiation. We found that TRA-1 strongly influences stress resistance, suggesting that downstream organ differentiation pathways affect resistance to stress. Future studies will investigate organ-specific mechanisms that may have broad relevance to stress resistance, aging, and longevity differences found in other species.

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Single-cell sexual dimorphisms in a non-sexual organ

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Differences between the sexes, known as sexual dimorphisms, have been reported for virtually every major organ system. The most obvious sexual dimorphisms occur in organs associated with reproduction. However, sexual dimorphisms also occur in non-sexual organs, but little is known about the underlying mechanisms. Here we investigate the urinary bladder, a non-sexual, genitourinary organ that has no known structural sex differences but shows striking differences in disease susceptibility. Single-cell RNA sequencing (scRNA-seq) of male and female mouse bladders revealed previously known sexual dimorphisms at the single cell level. We identified both quantitative and qualitative sex differences in a variety of bladder cell types. To determine the mechanisms responsible for these single-cell sexual dimorphisms, we used a transgenic mouse model to decouple effects of genes on sex chromosomes from the influence of gonadal sex hormones. Our results revealed subsets of cells that respond exclusively to sex hormones or to sex chromosomes, and others that are influenced by both factors. Taken together, these results provide a deeper understanding of structural and functional sexual dimorphisms of the bladder at a single-cell level, and provide new insights into molecular mechanisms that establish and maintenance sex differences in the genitourinary system. The results have direct implications for understanding sex differences in bladder health and disease.

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A barrier extracellular matrix sensor for environmental stress is active throughout larval development in *Caenorhabditis elegans*

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Epidermal tissues have extracellular matrices (ECMs) as structural support and permeability barriers. Stressors that penetrate barrier ECMs and enter cells activate stress response genes that promote survival, slow aging, and delay degenerative diseases. Stress response signaling within cells is well-studied, but almost nothing is known about the role of ECMs in direct contact with the environment. The model nematode *C. elegans* has a cuticle barrier ECM of collagen. We recently used genetic screening to identify 'furrow' collagen structures in the cuticle that are required for regulation of stress responses and for wild type body shape. Cuticles are replaced during development *via* molting but ECM signaling has only been studied in adults. We recently demonstrated that furrow organization and body shape are resistant to furrow collagen mutations in early larvae and become progressively disrupted in late stages. Here, we investigated furrow structure, body shape, and stress response gene expression during development in worms with mutations in a non-furrow collagen and non-collagen ECM proteins OSM-7 and OSM-8 that are thought to function downstream from furrows. The non-furrow collagen mutation changed body shape but did not disrupt furrows or activate stress responses. Loss of OSM-7 and OSM-8 had no effect on furrows or body shape but strongly activated stress response genes at all stages. These results support furrow organization as a signal for stress responses that is independent of body shape and suggest that the signaling mechanism is functional at all stages.

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A CIBERSORTx Signature Predicts Outcome in Pediatric AML Patients

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Acute Myeloid Leukemia (AML) is a devastating disease associated with high morbidity and mortality. The role of supportive immune cells such as NK cells and mast cells in AML tumorigenesis and relapse has been increasingly scrutinized, however studies to evaluate impact of varying cell type fraction on the clinical outcome are lacking.
In this study, we leveraged the CIBERSORTx algorithm to develop a cell type score (CiberScore) using the gene-expression data from bone marrow specimen obtained at diagnosis (using Affymetrix U133A microarray) from pediatric AML patients treated on the multi-site AML02 clinical trial (NCT00136084). 163 patients with both gene expression and outcome data were included in a machine-learning approach consisting of 1,000 iterations of 10-fold cross-validation of Cox Proportional Hazard regression. Cell-type fractions passing at least in 800 of 1000 models (9 hematopoietic cell types) were selected to create *CiberScore*: an equation that multiplies average coefficients obtained from 1000 models. With respect to clinical outcome, patients within High-Ciberscore group had greater MRD1 positivity (p=0.010), poor EFS (HR 2.96, p<0.0001), and OS (HR 4.78, <0.0001) as compared to patients within Low-Ciberscore group. In multivariable analysis, CiberScore remained significantly associated with EFS and OS after adjusting for induction 1-MRD1 status, risk-group, FLT3-status, WBC-count at diagnosis, and age, implying it to be an independent prognostic factor. In conclusion, we used CIBERSORTx to deconvolute cell type fractions using bulk gene-expression data from pediatric AML patients and further utilized machine learning algorithms to develop a prognostic model based on 9 hematopoietic lineages.

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SARS-CoV-2 cDNA library construction using a reduced reaction volume protocol on the Mosquito HV Genomics and Dragonfly Discovery nanoliter liquid handling instruments

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The identification of SARS-CoV-2 viral variants remains a critical step in controlling spread of infection. Using genome sequencing from isolated viral RNA, it is possible to detect and quantify circulating viral lineages (including novel, potentially more transmissible and/or pathogenic) in the human population. This has important implications regarding the surveillance of viral variants and the ability to proactively implement specific public health measures. The ICBR miniaturized a SARS-CoV-2 genome library construction using the SPTLabtech **Mosquito® HV Genomics** and **Dragonfly® Discovery** nanoliter liquid handling platforms using the Illumina COVIDSeq emergency use authorized test protocol.

We manually tested a library synthesis reaction volume at half and quarter volume reduction with varying total RNA inputs. Results showed that DNA library of sufficient quality and quantity were consistently generated with 10ng RNA input at 1/4 reduced reaction volume (RRV). Adapting the COVIDSeq library prep onto the SPTLabtech **Mosquito®** allowed further volume reduction to 1/5 scale.

To assess the effects the RRV protocol has on DNA sequencing, 384 RNA samples obtained from SARS-CoV-2 positive patients were separated into two groups. One group was processed manually at full-scale reaction volume and the second group was processed on the Mosquito at 1/5 reaction volume. DNA sequencing was performed on the Illumina NovaSeq6000 on the S1 flow cell using the 2 x 100 configuration. The sequencing results showed no difference in SARS-CoV-2 genome coverage or in variant identification rate. The RRV protocol facilitates the generation of 384 high quality COVIDSeq libraries per day with significant reagent cost savings.

A rapid CRISPR-based biosensor for the detection of *Pseudogymnoascus destructans*, the causative fungal agent of white-nose syndrome

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White-nose syndrome (WNS) is an infectious disease that has killed millions of bats in North America and is further threatening imperiled bat species. To aid in the early detection of *Pseudogymnoascus destructans* (*Pd*), the causative fungal agent of WNS, we propose the development of a novel, user-friendly, qualitative, CRISPR-based biosensor.

Early detection of *Pd* is crucial in providing avenues for isolation and containment to limit the unintended spread of the fungus to additional bat hibernacula. Current *Pd* screening methods often face limitations related to the use of expensive instruments not suitable for the field, long processing periods, technical expertise needs, and the invasive capture of bats. Our proposed approach addresses these limitations by enabling accurate DNA-based *Pd* identification using a sensitive, portable platform that combines all necessary reagents for sample processing into three reagent tubes. The assay only requires a portable incubator and a handheld mid-wave UV lamp to provide a rapid (~1hr) detection readout.

Our objective is to provide a reliable diagnostic tool for managers to independently conduct accurate and cost-effective *Pd* molecular screens. In future research efforts, we hope to translate this portable assay for the detection of other wildlife diseases, such as amphibian chytrid fungus disease, and the monitoring of environmental DNA of invasive species (*e.g.*, zebra mussels).

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Validation of a Primary Thyroid Feline Cell Line and Treatment with Methimazole

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Hyperthyroidism is the most common endocrine disease in cats, with approximately 10% of cats over 10 years of age affected. It can lead to widespread systemic effects such as cardiomyopathy and hypertension and may be fatal if left untreated. In vitro assays are useful for assessing the efficacy of new potential compounds for treating hyperthyroidism. The objective of this study was to validate primary cat thyrocyte cell cultures for in vitro studies and to test the effects of anti-thyroid methimazole (MMI). Thyroid tissue was excised from 10 cat cadavers to develop primary feline thyrocyte cultures. The purity of cell cultures was verified by conducting histology and immunohistochemistry. Immunohistochemistry showed positive reactivity for thyroid peroxidase and thyroid stimulating hormone receptor, confirming purity of the thyrocytes. Thyrocytes were exposed to 20 ng/mL and 2,000 ng/mL MMI, with the former mimicking a realistic therapeutic dosage. The mRNA steady state levels of transcripts related to thyroid hormone production and transport were measured in thyrocytes after a 72-hour treatment. MMI did not alter the expression levels of receptors (*thra*, *thrb*), enzymes (*dio1*, deiodinases) nor transporters. Thyroid hormones (T3 and T4) were also measured in the media using a competitive ELISA. T3 was below detection limits and efforts are currently focused on improving detection. Intriguingly, T4 levels did not show any difference among groups with MMI treatment, suggesting that the therapeutic effects of MMI may require additional feedback mechanisms *in vivo*. Data demonstrates that thyrocytes can be isolated and cultured to study treatments related to hyperthyroidism.

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Global identify target-directed microRNA degradation (TDMD) triggers by Cross-Linking, Ligation, and Sequencing of Hybrids (CLASH) in Drosophila

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MicroRNAs (miRNAs) associate with Argonaute (AGO) proteins to direct widespread posttranscriptional gene repression. However, extensive base-pairing between miRNAs and target RNAs can induce miRNA degradation, a phenomenon termed target RNA-directed miRNA degradation (TDMD). Here, we performed AGO1-CLASH in Drosophila S2 cells, with Dora (ortholog of vertebrate ZSWIM8) knockout mediated by CRISPR-Cas9 to identify TDMD pairs in Drosophila. In AGO1-CLASH experiments, target RNA/miRNA pairs are captured by UV-crosslinking and intermolecular ligation, with resulting target RNA/miRNA hybrid reads obtained from Illumina sequencing. Based the base-pairing pattern, conservation of target sequence, abundance (>100 FPKM) and enrichment of RNA/miRNA hybrid in Dora-KO, we identified numerous candidate TDMD triggers. When knockout candidate TDMD triggers in S2 cells by CRISPR, five triggers induce degradation of corresponding miRNAs. In addition, blocking TDMD trigger with Morpholinos in S2 cells and trigger deletion Drosophila flies can also effectively inhibit the degradation of corresponding miRNA. So far, no endogenous TDMD trigger has been reported in Drosophila. Here, we uncovered widespread TDMD triggers in Drosophila by AGO1-CLASH, accelerating research in the TDMD field.

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Development of an environmental (e)DNA metabarcoding assay for the surveillance and detection of reptiles in the Florida Everglades

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The collection and next-generation sequencing of environmental DNA (eDNA) allows for non-invasive surveys of aquatic ecosystems, providing managers with species' detection and identifications based on species-specific DNA sequences. Established invasive reptile species in south Florida, such as the Burmese python, are severely reducing native mammal populations and threaten at-risk species. Here, we develop 16 novel assays using the 12S and 16S ribosomal genes and screen each for efficiency and accuracy to detect reptile DNA in water samples. In addition to a comprehensive survey for known native and invasive species, metabarcoding can provide early detections for newly introduced species, increasing the likelihood of eradication prior to establishment in critical habitats, such as the Florida Everglades. The assay will also provide detections for at-risk native reptile species, such as the American crocodile, Eastern indigo snake, and green sea turtle, and inform the judicious allocation of field resources for monitoring of these species.

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Identifying the Mechanism of Integrator Complex Hijacking by Kaposi's Sarcoma Herpesvirus

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The Integrator protein complex has been implicated in transcription regulation and is critical in the biogenesis of small nuclear RNAs and enhancer RNAs through 3' end processing. More recently, it has been demonstrated that host cell Integrator can be used by *Herpesvirus samiri* (HVS) to process viral microRNAs. Integrator has also been shown to hijacked by another γ-herpesvirus, Kaposi's Sarcoma Herpesvirus (HSHV), where it has a demonstrated impact on RNA polymerase II-mediated transcription. Knockdown of Integrator in KSHV-infected cells resulted in decreased lytic activation of KSHV, as compared to scrambled knockdown controls. However, the mechanism of how host cell Integrator is recruited to, and interacts with, the KSHV episome is largely unknown. In this study, we used transient transcriptome sequencing (TT-seq) to identify specific motifs enriched in nascent transcripts processed by Integrator. To examine precise locations within these nascent transcripts that Integrator interacts, we performed crosslinking and immunoprecipitation sequencing (CLIP-seq). To identify what potential viral cofactors help to facilitate the interaction of Integrator with the KSHV episome, a proximity biotin labeling approach was used to enrich for the proteins that interact closely with Integrator throughout the process of lytic reactivation. This approach enabled enrichment of both KSHV and other host cell transcriptional control components that were then characterized by tandem mass spectrometry (LC-MS/MS). This study demonstrated new approaches to studying novel mechanisms of Integrator function and may be critical in understanding the regulation of KSHV lytic reactivation.

Archaeal Ubiquitin-like-Proteasome System and its Protein Partners Characterized Through *In Vivo* Crosslinking

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Protein turnover and regulation play important roles in the survival of organisms from all domains of life. Ubiquitination in eukaryotes and the related ubiquitin-like modification system in archaea are important regulators of protein turnover by proteasomes. *Haloferax volcanii* serves as a model system for the study of the archaeal ubiquitin-like proteasome system. In this organism, the ubiquitin-like SAMPs are ligated to protein substrates by a process known as sampylation. This process is presumed to be tightly regulated by deubiquitinase homologs of the JAMM/MPN+ metalloprotease family, namely HvJAMM1 and HvJAMM2. Requiring a zinc ion to be catalytically active, HvJAMM1 hydrolyzes the bond between the SAMP and a protein substrate. From here, the protein substate can be degraded by proteasomes while SAMP is recycled to maintain a consistent level of free SAMP to be used in subsequent reactions.

While the catalytic function of HvJAMM1 has been demonstrated *in vitro*, HvJAMM2 still remains a protein of interest to be characterized. A previous study reveals HvJAMM2 mutants are deficient in the turnover of SAMP conjugates, yet when HvJAMM2 is purified, it does not catalyze desampylase activity. We hypothesize that to be catalytically active HvJAMM2 may require association with protein partners and propose a protocol to capture said partners. Using crosslinking, proteins that associate in proximity to HvJAMM2 and proteasomes will be covalently linked, purified and identified by LC-MS/MS-based proteomics. By doing so, we hope to better understand how HvJAMM2 may function in promoting the turnover of proteins by the SAMP-proteasome system.

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A Bayesian hierarchical model to integrate a mechanistic growth model into genomic prediction

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Genomic prediction can improve the accuracy of estimated breeding values for traits driven by additive genetic effects within common settings but prediction of traits affected by non-additive genetic effects and GxE remains a challenge. Mechanistic growth models express growth performances as nonlinear functional interactions between underlying latent traits and nutritional environmental effects. With the latent traits assumed to be less affected by non-additive genetic effects and GxE, these models can capture certain non-additive genetic effects and GxE at the phenotype level and allow prediction at unobserved ages for longitudinal data, e.g. mature weight and mature feed intake. In this study, we developed a Bayesian hierarchical model to integrate a nonlinear Gompertz model for body weight and feed intake into genomic prediction models for pigs. By predicting breeding values for biologically relevant underlying latent traits, these models have the potential to advance genetic improvement across populations and environments.

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Temporal and Geographic Assessment of Python eDNA Occurrence North of the Greater Everglades Ecosystem

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Environmental DNA (eDNA) has been employed as a genetic tool to estimate widespread detection of invasive Burmese pythons (*Python bivittatus*) across the Greater Everglades Ecosystem area of southern Florida. Traditional survey methods to evaluate population size and geographic range have limited success (<1% detection rate), but eDNA can be collected from a wider area and shows significant increase in detection rates (38-70%). Expansion of the python population into more developed areas and their impact on native biodiversity prioritizes pythons for invasive species management efforts. As invasive species move throughout the environment, eDNA monitoring can provide early detections outside of known established areas and allows for rapid detection to inform control or management efforts. Although Burmese pythons are established in the Everglades of South Florida, their northern extent into central Florida is unknown. We aimed to assess the extent of Burmese python occurrence in South Florida with 1,500+ water samples collected in 2014-2016 and 2018-2022. The geographic extent of the samples includes the southern edge of Lake Okeechobee, east and west along the Caloosahatchee and St. Lucie River watersheds, and north from Lake Okeechobee through the Kissimmee Slough. We assessed the temporal and geographic changes of Burmese python eDNA occurrence rates using three level eDNA occupancy models. Additionally, we evaluated the sampling design throughout the years and its putative influence on detection rates. The use of eDNA as a widespread monitoring tool can help detect initial introductions of invasive species and monitor changes in occurrence over time.

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