## Poster Presentations:

Posters will be presented at the following poster sessions:

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| Poster Session 1 | 11:45am – 12:30pm | Odd Number Posters |
| Poster Session 2 | 2:45pm – 3:30pm | Even Number Posters |

1. Dogs about the Strait: Using dog genomes to assess human interactions

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Dog-human relationships have been an important part of many cultures since the Pleistocene. These relationships often mirror human-human relationships, with dogs and humans travelling together on long migratory journeys, interacting with new people and dogs, and aiding each other in subsistence pursuits. Dogs accompanied people into the Americas on numerous occasions. The most recent joint venture began around AD 1000, with new populations of dogs and humans, specifically the Thule Inuit, spreading across the Canadian and Greenlandic Arctic. However, the Thule Inuit were not the first people in the region carrying the larger Inuit cultural tradition. We recently showed that the Birnirk (AD 800-1200), a potential cultural predecessor of the Thule, carried the same mitochondrial haplotype gene pool as the Thule Inuit. We set out to investigate if Birnirk dogs were similarly related to Thule dogs, by attempting to capture and sequence whole mitochondrial genomes from ancient dog remains at a Birnirk site, Cape Espenberg, on the Bering Strait in Alaska. We compare these ancient dog mitochondrial genomes to Siberian and Alaskan wolves to see if there is any geographic or temporal affinity. Our results help to identify if Birnirk people were migrants to the area or were long-term residents and push back in time our record of the last pre-colonial migration of dogs into the Americas. Our work on dog genomes from the Birnirk will continue to clarify relationships between dogs and humans, and among human groups, without the need for destructive analysis on ancestral human remains.

1. ZWYX: Software for detecting sex-linked regions and chimeras in genome assemblies

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I present a new R-language software package “ZWYX” which employs sex-specific sequencing coverage data to delineate regions of a genome assembly corresponding to sex chromosomes. The X or Z chromosomes should show a two-fold differences between sexes, while the sex-specific Y or W chromosomes should show an opposing and far more biased pattern of coverage; autosomes should show no differences. Using sex-specific sequencing coverage is an established and increasingly common method for identifying scaffolds derived from X, Y, W, and Z chromosomes. However, such analyses are typically bespoke, highlighting the need for software to streamline and standardize this approach. ZWYX aims to meet this need, and is implemented in the context of the Bioconductor Project, to take advantage of existing R-language infrastructure for genome analysis. ZWYX provides a structure and functions for identifying and visualizing scaffolds with sex-biased coverage on average as a whole, but also via windows along each scaffold. Importantly, ZWYX incorporates “changepoint” algorithms for detecting shifts in sex-specific coverage that occur within a scaffold, which often indicate a mis-assembly that erroneously joins autosomal and sex-linked regions. Thus, in conjunction with relevant sex-specific sequencing data, ZWYX offers a valuable method for assessing the quality of genome assemblies. For demonstration, I apply ZWYX to a version of the Drosophila melanogaster genome computationally rearranged to include Z-Autosome and Y-Autosome chimeric scaffolds.

1. Single-cell RNA Sequencing of Oligodendrocytes to Map how Nuclear Hormone Receptor Ligands Regulate Differentiation

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Oligodendrocyte-derived myelin is the foundation of a properly functioning central nervous system (CNS), while demyelination – the loss of the myelin sheath – occurs in many neurodegenerative diseases, such as multiple sclerosis, Parkinson’s diseases, and Alzheimer’s disease. In the CNS, oligodendrocyte progenitor cells (OPCs) differentiate into mature oligodendrocytes, which form myelin. Historically, thyroid hormones and other nuclear hormone receptor ligands (NR ligands) have a well described role in regulating oligodendrocyte differentiation and myelination during development, however, it remains unclear which NR ligands are required to drive and promote these effects. In our project, we aim to apply both phenotyping and genotyping to OPCs treated with NR ligands to evaluate the effects on the differentiation of OPCs. We will evaluate Triiodothyronine (T3), Progesterone, Vitamin D3, 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), 9-cis-Retinoic acid, β-estradiol, and Testosterone). The OPCs were isolated from P7-8 Sprague-Dawley rats and were differentiated in the presence of the NR ligands for 3-6 days. We performed single-cell RNA sequencing on day 4 of differentiation and analyzed the transcriptomic profiles using Partek Flow software. Immunophenotyping was performed with neuron-glial antigen 2 (NG2) to identify OPCs, and myelin basic protein (MBP) to label mature oligodendrocytes. qPCR assays were performed using cells differentiated for 3 or 5 days to investigate the expression level of three genes: cgt, mag (both are early oligodendrocyte markers), and mbp (encodes the myelin sheath). The single-cell RNA sequencing data in combination with other analyses offers a high-resolution view that could reveal the regulatory relationships between NR ligands and OPC differentiation.

1. Analyzing the Cellulolytic Capabilities of a Bacterial Subcommunity Isolated from Soil

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Cellulose is a major energy source for many microbial soil communities, but the community interactions between cellulolytic and non-cellulolytic species during cellulose degradation is poorly understood. We grew ten subcommunities from a cultivated soil community from east central Kansas on carboxymethylcellulose (CMC) plates. Metagenomic sequencing revealed a high level of variability in species diversity and composition between the ten randomly established bacterial assemblages on CMC even though they were from the same temperate soil sample. We then isolated eight of the dominant species onto CMC plates, sequenced them with Illumina NextSeq and Oxford Nanopore MinION platforms, assembled the genomes with the Galaxy-based Unicycler algorithm and annotated them using RAST and KEGG servers. In hopes of correlating the growth capabilities on CMC with the genomic features we have identified the genes most closely associated with cellulose utilization, namely the exoglucanase, endoglucanase, and beta-glucosidase classes of cellulase enzymes. The number of identified cellulase genes is highly variable, ranging from zero in the gram-negative Pseudomonas sp. strain ES-006 and Achromobacter sp ES-001 to 34 cellulases in both *Kitasatospora albolonga* ES-004 and *Gordonia sp* ES-007, each of which are gram-positive Actinobacteria. We have also quantified CMC digestion for each species using the Congo Red Analysis of Cellulose Concentration (CRACC) assay and found a general positive correlation with the number of cellulase genes. Perhaps of greater importance is the potential synergy in cellulose degradation observed between cellulosic and non-cellulosic species. RNA-seq will be employed to determine which genes in the non-cellulosic species are most highly regulated in the presence of cellulose and/or cellulose-degrading species. This study indicates that integrated waste management practices and cellulose-based biofuels industries would benefit by optimizing cellulolytic species composition

1. Evidence for advanced generation hybridization between two prairie milkweeds.

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Two species of milkweeds, *Asclepias speciosa* and *A. syriaca*, meet on the Great Plains, and the contact zone spreads from Kansas to Minnesota. Several researchers have observed multiple individuals of intermediate morphology in this area, and they proposed introgressive hybridization as the explanation for the observed pattern of morphological variation. However, there are alternative explanations, which include a preservation of ancestral polymorphism (i.e. Incomplete Lineage Sorting) or the action of selective pressure imposed by environmental clines. The last explanation seems especially plausible, since the species meet on a strong precipitation gradient that may have resulted in a geographical cline in plants’ morphology. The goals of this research were to characterize *A. speciosa,* *A. syriaca* and the intermediates morphologically and genetically and validate the hybrid origin of the individuals of intermediate morphology. We analyzed a range-wide sample of 552 individuals obtained from herbarium and field-collected samples. Our morphological analyses were based on measurements of 15 floral and vegetative traits, and genetic analyses were based on 7480 genome-wide SNPs. The analyses showed that the morphological variation between *A. speciosa* and *A. syriaca* is not correlated with geographic distance, which allow us to rule out the environmental clines as the explanation for the morphology of the intermediates. At the same time, these individuals are genetically and morphologically intermediate between *A. speciosa* and *A. syriaca*. There is also an evidence of interspecific gene flow in sympatric populations of these species. Individuals in the zone of sympatry demonstrate various degrees of admixture, and the pattern of shared genetic variation is consistent with that of advanced generation hybridization. Our results demonstrate that the individuals of intermediate morphology are indeed hybrids, and their existence cannot be explained by environmental clines or incomplete lineage sorting. This research helps us to understand the spatial distribution of morphological and genetic variation in prairie plants and the processes that shape this variation.

1. The influence of the oral microbiome on head and neck squamous cell carcinoma

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The oral microbiome is an emerging field with high potential for uncovering new avenues for cancer therapy. Over 700 microbial species have been identified within the human aerodigestive tract, however the impact of many of these is still unknown. Head and neck squamous cell carcinoma (HNSCC) is the most frequently occurring malignancy of oral cavity cancers with an incidence of over 90%. Treatment options for HNSCC include surgery, chemotherapy, and radiotherapy; all of which still provide a low 5-year survival rate which indicates a need for additional treatment options. The overall goal of this research is to provide information on how *Fusobacterium nucleatum*, and other significant microorganisms, influence HNSCC, as well as increasing the scientific knowledge of their role within the oral microbiome. We hypothesized that the oral microbiome is distinct in patients from paired cancer-free controls. We have interrogated the profile of the oral microbiota by use of 16S rRNA amplicon sequencing of saliva samples from HNSCC patients and their significant others. The patients showed a higher relative abundance in *Prevotella*, *Neisseria*, *Fusobacterium*, and *Campylobacter* when compared to their significant others. *Fusobacterium nucleatum* is one of the most common cultivable microorganisms found within the oral cavity that has been shown to exhibit pathogenic properties. Here, we observed significantly higher relative abundance of *Fusobacterium nucleatum* when comparing patients to their significant others. In this research, we detailed the key properties involved with the anaerobic cultivation of *Fusobacterium nucleatum*, including optimized growth conditions and medium, as well as increased *in-vivo* proliferative properties in conjunction with metagenomic and metabolomic data. Further studies will provide information on the biological mechanisms *Fusobacterium nucleatum*, in conjunction with other microorganisms, utilizes to alter the progression and therapeutic response of HNSCC.

1. Role of maternal RNA decay during zebrafish embryo development

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In animals, the earliest stages of development are driven by maternally deposited mRNAs. The decay of most of these molecules and their substitution by zygotic-transcribed mRNA during a process known as the Maternal-to-Zygotic transition (MZT) has been extensively studied in many bulk RNA-seq experiments. However, it has been shown that some mRNAs during the MZT are distributed in specific cells of the early embryo. Here, we are focused on the maternally deposited and specific-localized mRNAs, hypothesizing that these RNAs play an important role in the early developmental process through their specific localization. To address this question, we took the advantage of CRISPR-Cas13d tool, which was recently developed to degrade RNA. Using the CRISPR-Cas13d system, we have previously shown that CRISPR-RfxCas13d is an effective and precise system to deplete specific mRNA transcripts in zebrafish and other animal embryos. We demonstrate that zygotically-expressed and maternally provided transcripts are efficiently targeted. We have shortlisted a set of maternally deposited cell-specific RNA in the zebrafish embryo. Our preliminary data suggest that perturbation of the expression levels of cell-specific maternal RNA using the CRISPR-Cas13d system leads to abnormal early embryo development. Together with these results, we aim to dive deep to decipher the mechanism of cell-specific maternal RNA decay and their role in the regulation of early embryo development in zebrafish.

1. Next Generation Sequencing at KU Genome Sequencing Core

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The Genome Sequencing Core (GSC) is one of three research service core labs in the NIH COBRE Center for Molecular Analysis of Disease Pathways (CMADP) at the University of Kansas (KU). The major mission of the GSC is to provide researchers with next-generation sequencing (NGS) technologies. NGS, carried out in a massively parallel fashion, has been revolutionizing bio-medical research and used in a growing list of applications. Projects supported by the GSC include de novo genome assembly, genome re-sequencing for identification of mutations and polymorphisms, transcriptome analysis (RNA-seq), and epigenomic and gene regulation studies such as ChIP-seq, Methyl-seq, and small RNA analysis. The GSC enhances the genomics infrastructure already at KU by providing a range of Illumina sequencing platforms including the NextSeq 2000 and NextSeq 550 (mid-sized genome re-sequencing or transcriptome projects) and the MiSeq (metagenomic or targeted amplicon sequencing projects) to researchers at KU-Lawrence and across the region. To capture the full power of NGS, we provide a range of project support, including project consultation, sample quality check, sequencing library construction, Illumina sequencing, and FASTQ generation and demultiplexing. For latest pricing, current sequencing queue, or other information, visit the Genome Sequencing Core’s website: https://gsc.ku.edu/.

1. iCodon: Harnessing mRNA stability to customize gene expression

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The codon composition of messenger RNA (mRNA) conveys regulatory information that strongly affects transcript stability, allowing cells to fine-tune gene expression. Further, specific codons are enriched in stable mRNAs, whereas others occur in unstable mRNAs. Yet, current codon optimization methods revolve around codon usage metrics that omit these observations, and therefore weakly correlate with mRNA stability. In addition, these methods only work in one direction, which means that they only seek to increase expression, limiting their applications for RNA biology research. To address these challenges, we hypothesized that by using our knowledge of mRNA stability, we could create a tool that customizes gene expression. Hence, we trained a machine learning model using stability profiles generated after blocking transcription and measuring mRNA decay over time. These profiles from several vertebrate species allowed our model to predict mRNA stability based on codon composition. This led us to develop iCodon, a tool that predicts mRNA stability and produces a list of synonymous sequences covering a range of expression levels by introducing synonymous codon substitutions. First, using a massive reporter library, we showed in a transcription-independent manner that the stability of more than 2500 mRNAs significantly correlates with the stability predicted by iCodon. Next, we validated that our predictions correlated with gene expression by testing 18 constructs designed by iCodon encoding different fluorescent proteins and endogenous genes in human cells and zebrafish embryos. Additionally, iCodon synonymous variants had higher expression levels than sequences designed by codon usage based methods. In conclusion, iCodon provides a powerful tool to interrogate mRNA stability and design strategies to modulate gene expression in vertebrates, for a wide range of applications for research, and for the potential optimization of RNA-based therapeutics and vaccines.

1. Ancient genomes from the Plains: Preliminary evidence for long-distance population continuity

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The genetic diversity of past peoples within the Central and Southern Plains, a region that is crucial for understanding the population histories of the Americas, is poorly characterized. The Plains served as a place of extensive migration and trade from the dispersal of the First Peoples through present day. In a community-led collaboration with a Plains tribe we performed human genome-wide targeted SNP capture from two individuals from different archaeological sites: one in the Central Plains (Kansas, direct radiocarbon dated between AD 1280 and 1390) and one in the Southern Plains (Texas, likely lived around AD 1150-1450). We successfully genotyped 346,199 SNPs from the Kansas individual and 164,390 SNPs from the Texas individual. Preliminary analyses using principal component analysis show that these two individuals cluster with the “Southern Native American” (SNA) lineage. Maximum likelihood estimates of ancestry components supports this observation. Furthermore, genetic kinship modeling indicates that these two individuals are second-degree relatives (e.g. grandparent-grandchild, half-sibling, or aunt/uncle-niece/nephew). We show the potential for modeling long-distance population continuity between the Central and Southern Plains during the Plains Village Tradition (AD 1100 to 1700). Beyond its contribution to micro-scale population modeling and the specific interests of the collaborating tribe, this project fills a critical gap as there have been no ancient genomes published from individuals living in the Plains prior to European contact. These individuals, and others, will inform larger research aims investigating mortuary practices, dietary shifts, and population migrations throughout the Plains.

1. What is *Salvinia molesta*? Determining the Genetic Composition and Number of Origins of the Invasive Giant Salvinia

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The occurrence of polyploidy, having multiple complete genomes, is now recognized as a major influence on the evolution, genetic composition, and diversification of many plant lineages. Polyploidy is widely viewed as following two pathways. An autopolyploid is one that is the result of genome doubling involving a single species. In contrast, an allopolyploid is a polyploid that is the result of hybridization between two or more species. This can involve multiple independent hybridization events, a process which creates allopolyploid species that have several independently constructed genomes. The aquatic fern *Salvinia molesta* D.S. Mitch. belongs to a clade of closely related species known as the “*Salvinia auriculata* complex,” and is an allopentaploid hybrid with unknown parentage. *Salvinia molesta* is an invasive that can have devastating ecological effects on the freshwater ecosystems it colonizes. The lack of clarity surrounding the genomic composition of *S. molesta* complicates current eradication methods, as it is not clear how many genotypes are present and what these genotypes are. This research focuses on identifying the maternal genome of *S. molesta* and determining if this species consists of a single or multiple independently derived lineages. To answer these questions chloroplast genomes (plastomes) will be sequenced from field and herbarium samples of *S. molesta* and several closely related species. These samples will be used to identify the maternal genome of S. molesta, as well as to construct a phylogeny that delineates species level relationships within the genus. Plastome diversity within *S. molesta* will also be examined, and the presence of multiple divergent genotypes will strongly suggest multiple origins of this hybrid.

1. RNA Sequencing Reveals Donor Variability in Sex- and Age-Matched Human Meniscal Fibrochondrocyte Response to Estrogen

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INTRODUCTION: More than 650 million people worldwide have knee osteoarthritis (OA). Meniscal tears pose significant risk in the development of OA. Recent epidemiological data suggests differences in meniscal injury rates, repair, and correlation to subsequent OA between males and females. These data and others indicate estrogen could be fundamental for knee joint health. However, the cell response to estrogen, and differences in response of cells from different donors, is unknown. Thus, the purpose of this study is to investigate the role of pulsed and continuous dosing of estrogen on gene expression of meniscal cells harvested from 2 female patients of similar age and injury. We hypothesized that cells from both patients would respond similarly to treatment and that cell response would vary based on estrogen dose and dosing kinetics. METHODS: Cells were harvested from tissue resected from the right medial meniscus of 2 female patients aged 16 and 17 at Children’s Mercy Hospital (IRB Exemption #STUDY00000746). RNA was isolated using a Qiagen RNeasy Mini Kit. Libraries were generated for full transcriptome sequencing using an NEBNext Ultra II Directional RNA Library Prep Kit and sequenced utilizing Illumina NextSeq 550 at the KU Genome Sequencing Center. RESULTS: Contrary to our hypothesis, there were few DEGs that overlapped between the two donors within the same treatment group. Further, there were marked differences in the number of DEGs between the donors. DAVID analysis revealed that diverse groups of genes related aspects of basic cellular functions were differentially expressed between the donors even without estrogen treatment. DISCUSSION: Elucidating the role of estrogen signaling on knee meniscal fibrocartilage could provide starting points for the development of new patient-centered therapies to reduce the onset of knee osteoarthritis. This study serves as an important reminder of the inherent differences in cells sourced from different donors and the importance in considering donor source when designing studies.

1. Tracking SARS-CoV-2 variants through municipal wastewater

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The COVID-19 pandemic has highlighted the potential role that wastewater-based epidemiology can play in assessing aggregate community health. The University of Kansas (KU) has been monitoring the prevalence of SARS-CoV-2 variants in wastewater for the state of Kansas, covering 95 counties. However, efforts to translate Sars-CoV-2 information obtained from wastewater samples into meaningful community health indicators are nascent. KU has undertaken two approaches to uses this data to protect public health. The first measured quantities of Sars-CoV-2 nucleocapsid (N) genes (N1 and N2). Four biomarkers (human mitochondrial gene NADH dehydrogenase subunit 5 (mit5), creatinine, ammonia, and biological oxygen demand (BOD)) were quantified and used to normalize Sars-CoV-2 gene copy numbers to account for variations in sewershed conditions. The normalized values were correlated to daily new case data and one-, two-, and three-week cumulative case data. For early stages of the pandemic, the wastewater samples may have indicated active COVID-19 cases before clinical indications. In addition to prevalence, wastewater measurements can be used to assess the emergence of novel variants in communities. KU has been sequencing the spike (S) gene to detect variants including Delta and Omicron.

1. Fishing with CRISPR/Cas13d: Elucidating micropeptide functions in zebrafish development

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‘Omics-based techniques have revealed messenger RNA (mRNA) sequences once defined as non-coding that produce short proteins (&lt; 100 amino acids) called micropeptides. Emerging studies have shown the importance of micropeptides in diverse biological processes. For example, the micropeptide APELA maintains pluripotency in human embryonic stem cells and is critical for heart patterning in zebrafish and mice. Additional micropeptide functions during vertebrate development, however, remain largely unexplored. Zebrafish is an outstanding model for interrogating developmental vertebrate gene function(s) due to its genetic tractability and synchronous, external embryogenesis. In fact, zebrafish express over 400 micropeptides during early development whose characterization is hindered by three key barriers: 1) many early mRNAs are maternally provided and can mask the effects of a targeted gene disruption, 2) conventional RNAi is ineffective in zebrafish, and 3) established, morpholino-based mRNA targeting has been questioned over toxicity and off-target effects. To overcome these barriers, our lab has established the CRISPR/Cas13d system in zebrafish that targets and degrades mRNA, including maternally provided mRNA. In this study, we selected five maternally provided micropeptide mRNAs with high abundance across the first eight hours of zebrafish development to assess for function with CRISPR/Cas13d. RT-qPCR revealed that CRISPR/Cas13d significantly reduced mRNA levels in 4 out of 5 targeted micropeptide mRNAs at 6 hours post-fertilization (hpf). Notably, CRISPR/Cas13d targeting of candidate “micropeptide 4” mRNA produced over 98% knockdown and developmental delay at 6 hpf. RNA-seq in knockdown embryos at 6 hpf revealed specific targeting of “micropeptide 4” mRNA and suggests a loss-of-function defect in zygotic transcriptional activation. Ongoing work will determine the localization of “micropeptide 4” mRNA and protein and determine its molecular function. Micropeptides that are critical for zebrafish development are expected to provide novel insights into vertebrate, and ultimately human, biological processes.

1. Family-based whole exome sequencing identifies *BUD13* variants involved in specific language impairment

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Specific language impairment (SLI) is a neurodevelopmental disorder that displays high heritability estimates and aggregation in families. While the language development of individuals with SLI has been well-documented, the genetic architecture underlying this disorder remains unclear. We performed whole-exome sequencing (WES) in multiple members of one family with SLI (family 489; N = 11) and prioritized co-segregating rare variants (MAF ≤ 0.005 in the 1000 Genomes Project) in three genes: *BUD13*, *APLP2* and *NDRG2*. To determine the significance of rare variants, we Sanger sequenced all the coding regions of these three genes in unrelated individuals with SLI (N= 175). We observed a total of 13 additional variants in 18 unrelated individuals with SLI. Gene level burden analysis revealed *BUD13* is involved in SLI. Additionally, variant level multiple in silico analyses predicted the pathogenicity of five *BUD13* variants. Bud13 is a component of the retention and splicing (RES) complex, an important mechanism of RNA splicing, which was found to be important for neural phenotypes observed in an animal model. Recent report of a copy number variant spanning *BUD13* was observed in an individual with a neurodevelopmental phenotype. Previous reports and our family-based investigation suggest *BUD13* could be a new target for the neural basis of language.

1. A workflow for the identification of novel transposable element insertions in *Drosophila virilis* pooled long reads

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Transposable elements, first discovered by Barbara McClintock in the 1940s, are known to jump around the genome. Currently, there is a lack of robust tools to discover where these elements insert. This can be attributed to the limitations of short sequencing reads and the diverse nature and characteristics of each transposable element. Recent advancements in long-read sequencing technology have now made it possible to identify new insertions without ambiguity. I have created a workflow that enables the detection of de novo transposable element insertions using nanopore sequencing. The workflow begins with running RepeatMasker on available genome assemblies and long nanopore reads collected from pooled individuals. Nanopore reads masked for transposable elements are then mapped to the masked assemblies to provide a normalized genomic coordinate system for later comparison. bedtools is then used to compare coordinates of known transposable element insertions in the genome assemblies to the location of insertions identified within the nanopore reads. Using this approach, we have validated the mobilization of transposable element families that are known to become activated in a syndrome of hybrid dysgenesis in *Drosophila virilis*. This workflow could be influential in future transposon research by providing a singular tool for the annotation of de novo transposable element insertions in pooled long-read sequencing experiments.

1. Digging up DNA: Sedimentary ancient DNA as an interdisciplinary tool to reconstruct past biodiversity

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Sediments and paleosols are known repositories of both micro- and macrofossils commonly utilized to reconstruct past environments. Recent advances in DNA extraction methodologies and high-throughput sequencing technologies have allowed for the isolation and sequencing of genetic material directly from ancient sediments (sedaDNA). SedaDNA complements traditional methods of paleoenvironmental reconstruction by providing greater taxonomic resolution as well as by generating data from sources with no visible fossil evidence. To date, sedaDNA from a variety of depositional settings has been successfully analyzed to survey local and regional biodiversity, to detect the presence of specific plants and animals (including humans), and to track vegetation changes as a response to climatic shifts. The rapid growth of the field has garnered broad interest to use sedaDNA to address their own disciplinary questions. However, working with genetic material from ancient sources is notoriously difficult as the molecules are degraded, often extracted in low concentrations, and very susceptible to contamination from multiple sources. Additionally, the taphonomy of sedaDNA molecules is vastly understudied--especially in terrestrial sedimentary deposits from lower latitudes. In response, we present a guide outlining what considerations must be taken when working with ancient sedimentary DNA, best practices for sample collection and storage from a variety of sedimentary deposits, and recommendations of different sampling strategies to collect the most relevant data to address specific research questions.

1. Membrane-anchored UNC-6/Netrin reveals roles of both close- and long-range interactions in regulating VD growth cone dorsal outgrowth

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UNC-6/Netrin directs dorsal-ventral axon pathfinding and is expressed in ventral cord neurons. Classically, UNC-6 was thought to form a ventral-to-dorsal gradient that was interpreted by growth cones. Our previous studies on VD growth cones indicated a more complex mechanism, involving discrete aspects of growth cone polarity coupled with regulation of protrusion. This polarity/protrusion model parts from the classical gradient model in important ways. First, growth cone polarity is separable from growth cone protrusion. Second, UNC-40/DCC and UNC-5 receptors both have roles in growth cones that grow away from UNC-6. To further test our model, we constructed a membrane-anchored UNC-6 called *unc-6(lq154).* Diffusible UNC-6 is predicted to be absent in this mutant. During development, the AVM neuron extends an axon ventrally toward UNC-6. As there is no contact between AVM and UNC-6-expressing cells, diffusible UNC-6 is predicted to guide the axon. unc-6(lq154) animals display axon guidance defects to the same extent as *unc-6(ev400)* null mutants, consistent with the idea that diffusible UNC-6 is absent. In contrast, VD/DD axon guidance defects of *unc-6(lq154)* were less severe than those of *unc-6(ev400). unc-6(ev400)* VD growth cones were unpolarized, whereas polarity in VD growth cones near the ventral surface in *unc-6(lq154)* was normal. Growth cones further from the ventral surface, in the dorsal half of the animal, were unpolarized in *unc-6(lq154)*. This result suggests that initial polarity is normal in *unc-6(lq154),* but polarity is lost as growth cones migrate dorsally. Possibly, a close-range or contact-mediated interaction of UNC-6 and UNC-5 polarizes the growth cone, but longer-range diffusible UNC-6 is required to maintain polarity. Preliminary studies using unc-5 hypomorphs, which affect only the long isoforms of UNC-5, show a similar phenotype. This indicates the short unc-5B isoform mediates close-range polarity, possibly through a contact-mediated event, whereas the long isoforms might mediate maintenance of polarity requiring diffusible UNC-6.

1. Protein Quality Control in Early Vertebrate Development

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Maternal-to-zygotic transition (MZT) is a crucial step during embryogenesis where the zygotic genome assumes developmental control from maternally provided gene products. During this step, embryos must activate their genome after clearing maternally deposited products, failure to do so prevents development and is consequently lethal. Maternal RNA degradation has been extensively studied during MZT, yet maternal protein dynamics have remained largely unexplored. Recently, one of the protein degradation pathways, the ubiquitin-proteasome pathway, was implicated in MZT in *Drosophila*, but a conserved mechanism in vertebrates has yet to be described. Thus, we hypothesize that vertebrate genome activation requires degradation of maternal proteins. To test this, we employ zebrafish embryos to perform biochemical assays, proteomics, RNA sequencing, and genetic manipulations with the CRISPR-Cas13d system. The latter is an effective and fast system to deplete specific mRNA transcripts in vertebrates. First, we established a fluorescence-based assay to visualize proteasome degradation activity in zebrafish which is active since fertilization. Then, we employed the CRISPR-Cas13d system to knock down several proteasome subunits that are highly deposited as mRNA in zebrafish oocytes. These embryos are arrested at the onset of MZT and die right after. We also confirmed that knocked down embryos have lower proteasome activity that correlates with reduced protein expression seen by Western Blot. Further, knocked down embryos display downregulation of zygotic genes, whereas otherwise cleared maternal RNA targets are upregulated, supporting a failure to fully activate their genomes. Upon GO term analysis, we observe enrichment of differentially expressed genes related to apoptosis and protein stress. Next, we will analyze protein abundance by mass spectrometry to identify candidate maternal proteins to dissect for their roles during zebrafish genome activation. In conclusion, these results suggest that maternally deposited proteins require degradation and highlight the importance of looking beyond RNA clearance regulation during early development.

1. SNPfiltR: an R package for interactive and reproducible SNP filtering

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I have developed the R package SNPfiltR which can form the backbone of a customizable, reproducible single nucleotide polymorphism (SNP) filtering pipeline implemented exclusively via the widely adopted R programming language. SNPfiltR extends existing SNP filtering functionalities by automating the visualization of key parameters such as sequencing depth, quality, and missing data proportion, allowing users to visually optimize and implement filtering thresholds within a single, cohesive work session. All SNPfiltR functions require vcfR objects as input, which can be easily generated by reading a SNP dataset stored in standard variant call format (vcf) into an R working environment using the function read.vcfR() from the R package vcfR. Performance and accuracy benchmarking reveal that for moderately sized SNP datasets (up to 50M genotypes, plus associated quality information), SNPfiltR performs filtering with comparable accuracy and efficiency to current state of the art command-line-based programs. These results indicate that for most reduced-representation genomic datasets, SNPfiltR is an ideal choice for investigating, visualizing, and filtering SNPs as part of a user friendly bioinformatic pipeline. The SNPfiltR package can be downloaded from CRAN with the command install.packages(“SNPfiltR”), and the current development version is available from GitHub at: (github.com/DevonDeRaad/SNPfiltR). Thorough documentation for SNPfiltR, including multiple comprehensive vignettes detailing realistic use-cases, is available at the website: (devonderaad.github.io/SNPfiltR/).

1. Role of Epistasis in Differential Resistance Outcomes

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Microbes evolve resistance to antibiotics at an astonishing rate. Only 20 years after the discovery of penicillin in 1928, penicillin-resistant *Staphylococcus aureus* had become a global pandemic. By 2050, more than 20 million people are expected to die annually from antimicrobial resistant infections. Development of new antimicrobials does little to alleviate this problem; once a microbe is exposed to an antimicrobial, resistance follows shortly thereafter. Combatting antimicrobial resistance require that we adjust how we study the phenomenon itself. Rather than looking at the final outcome i.e., resistance to a set of antimicrobials, we need a better understanding of the evolutionary steps leading to resistant genotypes. Ultimately, this will enable us to proactively mitigate the occurrence of resistance. Accordingly, we want to investigate the importance of epistasis in adaptation against antimicrobials using empirically derived fitness landscapes. We hypothesize that antibiotics present more epistasis than antimicrobial peptides, as measured by global fitness landscape ruggedness. In addition, we expect epistasis to correlate with naïve resistance (resistance from standing genetic variation before new mutations). In contrast, antimicrobial peptides will present less epistasis and lower incidence of resistance from standing genetic variation. This approach will both enrich our understanding of genetic adaptation and provide a new way to approach the crisis of antibiotic resistance.

1. Corn-y Cultures: Maize Root Endophyte Culture Collection

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Maize (*Zea mays*) is a model plant system and an important staple crop grown around the world. Understanding the complex relationship between maize and its associated microbial community is vital to sustainably increase yield. Axenic microbial cultures are crucial tools for studying microbiome function, conducting synthetic community experiments, and investigating evolutionary-based questions, which cannot be interrogated with sequencing alone. There has not been an extensive collection of maize root endophytes reported. To increase the number and diversity of axenic microbial cultures, we collected soil from four pristine tallgrass prairie remnants along the natural Kansas precipitation gradient. Then, maize seedlings were grown in sterile clay inoculated with soil slurries. After one month of growth, endophytes were cultured from surface-sterilized roots. To date, over 900 bacterial strains have been isolated and taxonomically identified using full-length 16S Sanger Sequencing. Of the 27 unique genera in our collection, *Luteibacter spp*. (553 isolates) were most frequently isolated. Other frequently isolated bacterial genera include *Burkholderia spp.* (128 strains), *Pantoea spp.* (83 strains) , *Rhizobium spp.* (82 strains), and *Paraburkholderia spp*. (61 strains). These wild isolates will be screened for a myriad of traits including, antibiotic resistance and drought tolerance, and be used in synthetic community studies. Furthermore, this maize root endophyte culture collection will be made available to the maize-microbe research community.

1. The More You Watch, the Less You Know: How Genetic Literacy Relates to Consuming Media About Genetic Ancestry Tests

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This poster draws on data from two national U.S. surveys to discuss the role of popular media about genetic ancestry tests in individuals’ genetic literacy. First, the Health Information National Trends Survey V (n = 3,865), establishes media content about ancestry tests as the chief genetics topic that individuals see in the media. The public is significantly more familiar with genetic ancestry tests than with genetic screening tests for cancer or other health risks. The data also shows that significantly larger shares of the public identify television and the internet as sources of information about genetic tests than identify other sources (e.g., social media, family member). These results justify further research into genetic ancestry test media messages, and into the potential consequences of this content on genetic literacy. Second, data from an online survey of 413 U.S. adults identifies the factors that predict whether individuals pay attention to media messages about genetic ancestry tests, and how this relates to their genetic literacy. Individuals who are engaged in genealogy research, who express greater certainty in their ancestry, who have second-hand test experience, who are younger, and who are more religious, tend to pay more attention to media messages about genetic ancestry tests than their counterparts. In turn, those who pay greater attention to media about genetic ancestry tests know less about genetics than those who pay less attention to such media messages. Moreover, among those who pay attention to media about genetic ancestry tests, those with first-hand ancestry test experience know less about genetics than those without first-hand test experience. In all, these data suggest that while media messages about genetic ancestry tests are a key source for learning about genetics, consuming such media in higher quantities may contribute negatively to individuals’ understanding of genetics.

1. Computer-Aided, Resistance-Gene-Assisted Genome Mining for Proteasome and HMG-CoA Reductase Inhibitors

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Fungi produce biologically active small molecules, called secondary metabolites (SMs), many of which are medically valuable. The genes that encode SM biosynthetic pathways are usually clustered together in the genome, forming biosynthetic gene clusters (BGCs). Genome sequencing reveals that the number of SM BGCs vastly exceeds the number of known SMs, and, thus, that huge numbers of potentially valuable SMs are yet to be discovered. Resistance-gene-assisted genome mining is a strategy to exploit the greater fungal secondary metabolome efficiently, by identifying SM BGCs that are likely to make useful products. It takes advantage of the fact that some SM BGCs contain a gene encoding a resistant version of the protein targeted by the compound produced by the BGC. This allows the producing organism to survive while its competitors are inhibited. The bioinformatic signature of such SM BGCs is that they contain an allele of an essential gene with no SM biosynthetic function, and there is a second allele elsewhere in the genome. Manually searching thousands of sequenced genomes for this signature is daunting, so we have developed a computer-assisted approach that allows users to query large databases for SM BGCs that putatively make compounds that have particular targets of therapeutic interest. We have applied this approach to look for SM BGCs that target the proteasome β6 subunit, the target of the proteasome inhibitor fellutamide B, or HMG-CoA reductase (HMGCR), a key enzyme in sterol biosynthesis and the target of cholesterol reducing therapeutics such as lovastatin. Our approach proved effective, finding known fellutamide and lovastatin SM BGCs as well as fellutamide- and lovastatin-related BGCs with variations in the SM genes that suggest they may produce structural variants of fellutamides and lovastatin. Gratifyingly, we also found SM BGCs that are not closely related to lovastatin BGCs but putatively produce novel HMGCR inhibitors.

1. Maximizing the phylogenomic utility of formalin-fixed museum specimens

Kevin Chovanec

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Formalin-fixed natural history specimens preserve snapshots in time that are increasingly accessible to genomic investigation. A fundamental challenge of working with degraded DNA from such material is that rare or extinct taxa of interest typically lack suitable (i.e. phylogenetically close) reference genomes for validation and comparison. Following protocols optimized in the field of paleogenomics, we sequenced multiple single-stranded DNA libraries from a 50-year-old specimen of *Anolis distichus*, a common West Indian lizard within a genetically well-characterized genus. These libraries allow us to better quantify post-mortem DNA damage in formalin-fixed museum specimens and to explore the phylogenomic utility of resulting datasets. Using novel alignment and assembly strategies, we recover thousands of informative loci with sufficient depth and coverage for downstream phylogenetic analysis. Across multiple datasets, we reliably place the specimen within a clade of living relatives. Our results are replicable and robust to increasing degrees of reference divergence, suggesting these methods are appropriate for extinct taxa whose closest living relatives are unknown. Wet bench and bioinformatic strategies presented here contribute to a growing literature on how to appropriately analyze ancient DNA from formalin-fixed museum specimens.

1. Verifying insertion of a plasmid by non-homologous end joining using PCR and sequencing

Nicholas Lacy, Justin P. Blumenstiel, and Kelley Van Vaerenberghe

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CRISPR has revolutionized genetic modification. One method of inserting genes using CRISPR involves creating plasmids with homology arms, which requires significant expertise and time. An alternative method is to use a plasmid without homology arms and allow it to insert by non-homologous end joining. In our experiment, a pUASz plasmid was inserted into *Drosophila melanogaster* lines using CRISPR-Cas9 and NHEJ. Here we evaluate this method. The presence of the insertion was confirmed and the direction determined in forty-four lines using PCR and gel electrophoresis of the junction between the existing genomic DNA and inserted plasmid. Four lines had double insertions of the plasmid with two copies back-to-back in opposite directions. The exact location of the insertions in 16 lines was determined using Sanger sequencing. Unique patterns of genetic damage around the insertion site act as signatures that can be used to distinguish between different insertions using Sanger sequencing.

1. Identifying DNA transposable element excisions in a Drosophila virilis syndrome of hybrid dysgenesis using long-read sequencing

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DNA transposons are sequences that are capable of moving in the genome resulting in profligate DNA damage and genome instability. Estimating transposon mobilization rates and the damage they cause is important for understanding genome evolution and genome stability. *Drosophila virilis* has several strains with varying copy number of DNA transposons and differences in germline piRNAs profiles. Transposons become activated when females lacking the requisite piRNAs that silence transposons inherited paternally, resulting in hybrid dysgenesis. With these strains we can study global transposon mobilization to determine the rates of excision and the overall change in copy number. In this study, we use pooled long-read DNA sequencing to identify excision events for DNA transposons. This excision analysis will be performed to estimate an excision rate using a likelihood model for estimating global excision rates for DNA transposons. This model will incorporate specific DNA transposon insertions, family, and piRNA profiles as parameters. We predict that differences in piRNA profiles, DNA transposon family identity, location, and internal deletion status will jointly determine the excision rate.

1. Efficacy of CRISPR-assisted insertion tagging

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CRISPR has revolutionized genetic analysis by allowing targeted break formation at any DNA sequence. Using CRISPR, one can target transgene insertion to specified sites within the genome. One method of targeted insertion using CRISPR employs homology-dependent insertion. This is achieved by generating homology arms that flank the transgene and also the target cut site. However, this requires specialized constructs to be made for each insertion location. CRISPR-assisted insertion tagging serves as an alternative method to homology-dependent CRISPR/Cas9 transgene insertion. Instead, this approach uses non-homologous end joining (NHEJ) to repair the DSB made by the Cas9-sgRNA machinery. Using *Drosophila melanogaster*, CRISPR-assisted insertion tagging was used to target three genes for disruption. These genes were *p53*, *chk2*, and *CG6325*. Here we evaluate the efficacy of this method.

1. CRISPR-Cas13d induces efficient mRNA knock-down in animal embryos

Gopal Kushawah

Stowers Institute for Medical Research

Early embryonic development is driven exclusively by maternal gene products deposited into the oocyte. Although critical in establishing early developmental programs, maternal gene functions have remained elusive due to a paucity of techniques for their systematic disruption and assessment. CRISPR-Cas13 systems have recently been employed to degrade RNA in yeast, plants and mammalian cell lines. However, no systematic study of the potential of Cas13 has been carried out in an animal system. Here, we show that CRISPR-RfxCas13d is an effective and precise system to deplete specific mRNA transcripts in zebrafish embryos. We demonstrate that zygotically-expressed and maternally-provided transcripts are efficiently targeted, resulting in a 75% average decrease in transcript levels and the recapitulation of well-known embryonic phenotypes. Moreover, we show that this system can be used in medaka, killifish and mouse embryos. Altogether our results demonstrate that CRISPR-RfxCas13d is an efficient knock-down platform to interrogate gene function in animal embryos.

1. Understanding the features of highly diverged Wtf proteins to elucidate their mechanism of action

Sam Campbell

Stowers Institute for Biomedical Research

Meiotic drivers are selfish genes that unfairly influence gametogenesis to increase their transmission into the offspring. The wtf meiotic driver was first discovered in the recently diverged fission yeast species *Schizosaccharomyces pombe* and *S. kambucha*. Since then, wtf drivers have been identified across diverse fission yeast species including *S. octosporus*, *S. osmophilus*, and *S. cryophilus*. These gamete-killing *wtf* genes drive by encoding both a poison protein and an antidote protein. The Wtfpoison targets all fission yeast spores during gametogenesis, while only the spores carrying the wtf+ allele are rescued by the Wtfantidote. First, the Wtfpoison forms punctae throughout the ascus. The Wtfantidote then colocalizes with the Wtfpoison in wtf+ spores. The meiotic driver is maintained in the population as only wtf+ gametes survive. In this work, we aim to compare features of other highly diverged Wtf proteins to identify key properties that are relevant to their function. The results of this work will elucidate if the highly diverged wtf genes function similarly, and further the understanding of the mechanism of wtf drive in fission yeast.

1. The *tom-1/tomosyn* locus encodes different isoforms with opposing roles in growth cone protrusion

Snehal Mahadik

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Previous work from the Lundquist lab showed that the UNC-6/Netrin receptors UNC-40 and UNC-5 regulate growth cone protrusion. UNC-40 stimulates protrusion whereas UNC-5 inhibits protrusion, and asymmetric distribution of protrusive activity across the growth cone results in directed growth cone migration away from UNC-6/Netrin (the Polarity/Protrusion model). *unc-5* mutant VD growth cones display unpolarized and excessive protrusion. To explore the role of vesicle fusion in growth cone protrusion, we analyzed *tom-1/tomosyn* mutants. Tomosyn normally occludes formation of the SNARE complex by interacting with and inhibiting *syntaxin-1*. VD growth cones of tom-1 null mutants were similar to wild-type. However, tom-1 null mutants suppressed the effects of constitutively-activated MYR::UNC-5, which alone causes small growth cones with little protrusion. This suggests that TOM-1 is normally required for the inhibitory effects of MYR::UNC-5 on growth cone protrusion. Mutations specifically affecting tom-1 long isoforms showed small and non-protrusive growth cones, and did not suppress MYR::UNC-5. This suggests that TOM-1 short and long isoforms might have opposing roles, with TOM-1 short normally inhibiting protrusion, and TOM-1 long stimulating protrusion. A short isoform specific mutation was generated by CRISPR/Cas9 genome editing, and suppressed MYR::UNC-5, consistent with this idea. Similarly, *tom-1* null and short isoform specific mutation did not suppress protrusive phenotype of *unc-5* mutants, but mutation specifically affecting tom-1 long isoform did. This is consistent with differential role of two isoforms of TOM-1. Finally, transgenic expression of full-length tom-1 short(+) in tom-1 null background resulted in small and non-protrusive growth cones, consistent with a role of TOM-1 short in inhibiting protrusion downstream of UNC-5. In sum, these studies show that the genomic organization of the *tom-1* locus produces isoforms with opposing roles in growth cone protrusion. It is possible that the long isoform inhibits the activity of the short isoform in a potential autoregulatory manner.

1. Annotation of isomiR dynamics across the *C. elegans* developmental stages  
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microRNAs (miRNAs) are short, ~22 nucleotides, small RNAs that repress genes through translational repression and mRNA destabilization. During canonical miRNA biogenesis, several miRNA species, or isoforms, known as isomiRs, are produced from a single precursor miRNA. Templated isomiRs are generated through Drosha or Dicer cleavage at alternate positions on either the primary or the precursor miRNAs, generating truncated or extended 5’ or 3’ miRNA ends. Because mature miRNA sequence modifications can alter their gene target repertoire, we wished to investigate the extent of isomiR prevalence and dynamics across *C. elegans* developmental stages. We performed small RNAseq from staged animals to assess isomiR variability across developmental stages. Using isomiR-SEA (Urgese et al 2016) for isomiR identification and quantification, we provide an isomiR profiling map against the miRBase annotated miRNAs at all stages of *C. elegans* development. We found that many miRNAs display isomiR level variability at different developmental stages, suggesting that the functional specificity of isomiRs to the developmental stage may exist. Not surprisingly, 3’ end miRNA alterations were more frequent than the potentially seed-altering 5’ end extensions or truncations. Some miRNA loci produced templated isomiRs that were just as, or more abundant than their annotated canonical mature miRNAs. These isomiRs included those with 5’ end truncations and extensions, predicted to target new, potentially distinct sets of genes. Overall, we will present annotation of isomiR dynamics across *C. elegans* developmental stages, which we hope can provide us with insights into miRNA biogenesis and the intriguing potential of isomiR function.

1. Incorporating bioinformatics and genomics in undergraduate curriculum

Anuradha Ghosh

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This poster is a reflection on the efforts made to incorporate advanced topics in undergraduate curriculum over a span of 5 years. The goal was to engage undergraduate students in scientific discovery using modern tools of genomics and bioinformatics. The specific experience involved isolating and culturing of microbes from various sources and their characterization by whole genome sequencing. This process engaged the students in core principles of microbiology, genetics, and cell biology while providing a substantial introduction to core skills in bioinformatics. This talk also elaborates on how graduate research was benefited from this new curriculum component.

1. Oral administration of water extract from *Euglena gracilis* alters the intestinal microbiota and metabolites and prevents lung carcinoma growth in mice

Deepa Upreti

Anatomy and Physiology, Kansas State University

*Euglena gracilis*, a single-celled alga used as a nutritional dietary supplement, possesses a broad range of medicinal properties including anticancer activity against a few types of cancers. The antitumor effects of a partially purified water extract from *Euglena gracilis* (EWE) and EWE treated by boiling (bEWE) were evaluated using orthotopic lung cancer syngeneic mouse models with Lewis lung carcinoma (LLC) cells. Daily oral administration of either EWE or bEWE started three weeks prior to the inoculation of LLC cells significantly attenuated tumor growth. The intestinal microbiota compositions in both extract-treated groups were more diverse than that in the PBS group. Fecal microbiota transplantation using bEWE-treated mouse feces attenuated tumor growth to an extent equivalent to bEWE treatment. Further, *Euglena* water extract’s anti-cancer properties and effects on the gut metabolic landscape were investigated in a tobacco smoke carcinogen: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung carcinoma mouse model. Oral administration of bEWE 2 weeks prior (pre-bEWE) or 10 weeks post (post-bEWE) NNK injection greatly attenuated NNK-induced tumorigenesis in the mouse lungs. A clear difference in fecal metabolites between the PBS and bEWE treated groups was observed. High-throughput metabolic profiling identified succinate, malate, triethanolamine, acetylserine, glyceric acid, aspartic acid, glutamic acid, and threonine, which can be metabolized to short-chain fatty acids (SCFAs), were increased with the bEWE treatment. Furthermore, a significant increase in SCFAs, such as acetic acid, butyric acid, and propionic acid, was observed in the feces collected from either pre- or post-bEWE treated mice. Moreover, treatment with SCFAs significantly suppressed the proliferation of both human and murine lung cancer cell lines in cell culture via induction of apoptosis. Our studies strongly suggest that daily oral administration of partially purified water extracts from *Euglena gracilis* attenuates lung carcinoma growth via the alteration of the intestinal microbiota and altering the gut metabolites to increase SCFA production.

1. A telomere-associated system of paramutation in *Drosophila virilis* mediated by maternally provisioned piRNAs

Ana Dorador

Department of Ecology and Evolutionary Biology, University of Kansas

Paramutation is the phenomenon by which a silent alle can turn off a normal allele in trans in an epigenetic manner. The silenced state of the wildtype allele can persist through generations even in the absence of the original paramutagenic allele. The mechanisms underlying paramutation are poorly understood. Further, little is known about how paramutation shapes gene expression under natural conditions. In this study, we investigate a system of genic paramutation in *Drosophila virilis*. Previous studies have shown that maternally transmitted piRNAs that target the center divider (*cdi*) gene in *D. virilis* have the capacity to silence expression of *cdi* in the next generation. In addition, it has been shown that piRNAs that target cdi can be maintained in subsequent generations in the absence of the original silencing allele. However, it is not known whether this pattern of piRNA biogenesis and maternal transmission coincides with epigenetic repression of cdi expression across multiple generations. To determine if cdi piRNA biogenesis mediates paramutation, we measured the expression of cdi in the ovaries of females heterozygous for the silencing allele, as well as their daughters that lack the silencing allele. In two independent experiments, cdi expression was quantified in 20 F1 heterozygous mothers and 20 first-generation backcross daughters, lacking the original silent allele, using RT-qPCR. Confirming previous studies, we found that heterozygous females that maternally inherited the piRNA producing allele had low expression of cdi in ovaries. We further found that the first-generation backcross daughters - lacking the paramutagenic allele – had significantly lower cdi expression in the ovaries compared to a genotypically identical strain with no piRNAs mapping to cdi. This study thus describes a new system of paramuation in gene expression of *Drosophila virilis,* which can serve as a baseline for future studies that seek to understand how paramutation is regulated.